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**Final Abstract Number: 23.001**

**Session: Antibiotic Resistance Gram-Negative**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Clinico epidemiologic and molecular characterization of metallo beta lactamases (MBLs) producing nosocomial *Pseudomonas aeruginosa* (PSA)

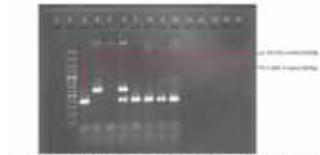
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**Background:** The high prevalence of co-resistance to betalactam, aminoglycoside and quinolone against PSA has necessitated increased use of carbapenems. MBL production among PSA is one of the several mechanisms causing carbapenem resistance (CARB-R) transferable by integrons. We surveyed MBL production among PSA isolates collected via multicentric surveillance study.

**Methods:** During March-September 2009 BMPLIII received 75 consecutively collected PSA causing infections of (skin and soft tissue-48, blood and respiratory tract 11 each, and other sites, 5) from four (50-AIMS, Kochi, 21-SGPGIMS, 2-MGIMS, 2-AIIMS, Delhi) Indian Medical centers. Antimicrobial susceptibility testing by Kirby Bauer method against ceftazidime (CZD), cefepime (FEP), piperacillin/tazobactam (TZP), ticarcillin/clavulanic acid (TIM), gentamicin (GEN), amikacin (AK), ciprofloxacin (CIP), imipenem (IMP), meropenem (MEM), aztreonam (ATM) and colistin (CL) was done. MBL was screened by the Combined Disk Diffusion Test (CDDT) method using 0.5M EDTA (930µg) as the inhibitor with IMP, positives (inhibition ≥7mm) confirmed by IMP+EDTA Etest and PCR (VIM and IMP genes).

**Results:** Of the 75 PSA isolates, 12 (16%) were MBL producers. Diabetes mellitus was found to be the major risk factor in PSA infections. Overall resistance to CIP 50.7% > CZD 37.3% > AK 36% > FEP 34.7%. Resistance pattern among MBL/NMBL was CZD (91.7%/23.8%), FEP (91.7%/20.6%), TZP (91.7%/15.9%), TIM (100%/27%), GEN (100%/23.8%), AK (100%/20.6%), CIP (100%/38.1%), IMP (100%/6.3%), MEM (75%/9.5%), ATM (91.7%/38.1%). Resistance to IMP and MEM was 22.7% and 21.3% respectively. All isolates were susceptible to CL. MDR (resistant to ≥2 classes of antimicrobials) was 34 (45.3%). Overall 14 (18.7%) were CDDT positive. IMP+EDTA Etest and PCR confirmed 12 to be MBL positives. Among the MBL isolates, one was also positive for IMP gene, which on sequencing was confirmed to be IMP 7. Sensitivity and specificity of CDDT was 100% and 96.8% respectively when compared to PCR. History of prior antibiotic usage of aminoglycosides (66.7%), 3rd generation cephalosporins (58.3%), quinolones (58.3%), carbapenems (50%), penicillins (25%), betalactam+betalactamase inhibitors (8.3%) was noted for the patients with MBL infection. Clinical improvement or cure with modification of initial antibiotics was found in 63.6% (7/11) patients with MBL PSA.



#### **PCR for VIM and IMP gene in PSA**

**Conclusion:** MBL production is an important mechanism of CARB-R among PSA. Spread should be further minimized by using carbapenems judiciously in the treatment of PSA infections. CDDT is a useful method for screening MBL. VIM type of MBL was seen among all MBL positive isolates.

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The resistance patterns of *Pseudomonas aeruginosa* in hospitals from Greece and Romania and its importance for the therapy of nosocomial infections and infection control practices

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**Background:** One of the most difficult problems in hospitals is the appearance of an increased number of *Pseudomonas* antibiotic resistant strains. The objective of our study is to describe the resistance pattern of *Pseudomonas aeruginosa* strains.

**Methods:** For isolating *Pseudomonas aeruginosa* strains, the usual nutritive media were used. Identification was made with Vitek2 system (BioMerieux). The susceptibility to the antibiotics of strains was performed by Kirby Bauer method as recommended by CLSI and Vitek2 system. Were tested 229 strains isolated from urine and surgical wounds, in Cluj Napoca, Romania, and 36 strains isolated from blood samples in Chania, Greece. The majority of the strains (86.1%) were isolated from patients in wards and a percentage of 13.8 from Intensive Care Unit.

**Results:** The strains isolated in Romania from urine showed a high resistance to beta-lactams, remaining susceptible to Carbenicillin, Carbapenems, Ceftazidime and Cefepime. Over 80% of the strains, were resistant to others of third generation cephalosporins. The strains isolated from blood, presented resistance to Aztreonam (30.43%) and Ceftazidime (13%). 13% were resistant to Imipenem and Meropenem. *Pseudomonas aeruginosa* isolated strains in Romania, showed resistance to Amikacin (71.4% isolated strains from urine and 29.7% from surgical wounds), and a low resistance to Gentamycin (18.6%), Tobramycin (25%). 29.6% were resistant to Colistin. The strains isolated in Chania, showed resistance to Gentamycin (21.4%). All strains isolated from urine, were resistant to Pefloxacin and a high resistance was detected to Nalidixic acid (75%), Ciprofloxacin (75%) and Ofloxacin (50%). The strains isolated from surgical wounds, presented a resistance to quinolones, oscillating between 33.1% and 51.9%. The isolated strains from blood showed resistance to Nalidixic acid (38.5%), Pefloxacin (38.5%) and Ciprofloxacin (38.5%)

**Conclusion:** Our results could reflect the implication of some hospital multi resistant *Pseudomonas aeruginosa* strains in nosocomial infections. In Greece, a decline in drug resistance of *Pseudomonas* strains has been noted. As a fact, it is rather encouraging, and probably we can attribute it to adherence to infection control practices and prudent chemotherapeutic agents use as proposed by Infectious Diseases Control Committee. The majority of the strains was detected in ward patients and owing to reduced morbidity factors compared with patients in ICU, the reduced resistance to antibiotics can be explained.

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Extended spectrum  $\beta$ -lactamase production at the Komfo Anokye Teaching Hospital, Kumasi, Ghana

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**Background:** In recent times enterobacteriaceae isolated at the Komfo Anokye Teaching Hospital (KATH) have shown significant resistance to 2nd and 3rd generation cephalosporins. In 2006, 18-32% of all enterobacteriaceae isolated from urine and blood were resistant to the cephalosporins cefuroxime, ceftriaxone, ceftazidime and cefotaxime. These antibiotics are the mainstay for the treatment of severe infections in the hospital. Microbial resistance to these antibiotics is due to the production of Extended spectrum  $\beta$ -lactamases (ESBL) may also indicate resistance to the fluoroquinolones, aminoglycosides and other antibiotics. This limits therapeutic options for the treatment of severe infections. We studied the extent of ESBL production amongst *Klebsiella sp* and *E. Coli* at KATH.

**Methods:** ESBL production in 300 non-selected, non-duplicate isolates of *Klebsiella sp* and *E.coli* obtained from blood, urine, wounds, and sputum of both in-patients and out-patients was determined by the combined disc method using ceftazidime, ceftriaxone and cefpodoxime discs singly and in combination with clavulanic acid.

**Results:** 44% and 55% respectively of *E.coli* and *Klebsiella sp* ( 57.8% *K.pneumo*, ) were ESBL producers. ESBL production in the organisms was commoner in in-patients (75.4%) than out-patients (24.6%), though not statistically significant (OR=1.40, 95% CI 0.79-2.46, p=0.31). In general, ESBL producing organisms apart from being resistant to cephalosporins were also resistant to gentamicin and ciprofloxacin. They were however susceptible to the carbapenems.

**Conclusion:** The high level of ESBL production found in these enterobacteriaceae with the resultant microbial resistance to available cephalosporins and other agents implies difficulties with the choice of therapeutic options for the treatment of severe infections. Carbapenems are expensive on the Ghanaian market and their use in non-severe infections (as may be suggested by these results) may be inappropriate. Both prescribers and pharmaceutical agents will need to reflect soberly on their contribution to this sordid state of affairs. We need to put an end to our practice of cracking nuts with sledge hammers!

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Antimicrobial susceptibility profile of *Pseudomonas aeruginosa* strains isolated at a tertiary-care University Hospital (S. Orsola Hospital, Bologna, Italy)

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University of Bologna, Bologna, Italy

**Background:** The increased rate of antimicrobial resistance among Gram-negative bacilli and Enterobacteriaceae is a general concern, especially in the hospital setting. A prospective microbiological monitoring including a continued surveillance of antimicrobial susceptibility rates of all relevant pathogens, is ongoing at our Hospital.

**Methods:** The temporal variations of *in vitro* antimicrobial sensitivity rates were registered at quarterly intervals for all suitable *Pseudomonas aeruginosa* strains, during the year 2008. The same pathogen cultured more than once from the same patient within one month, has been considered once.

**Results:** Among *Pseudomonas aeruginosa* isolates (494 strains tested on the whole), the best performance was obtained by the old colistin (colimycin), with a 100% susceptibility rate, followed by piperacillin-tazobactam (73.9-78.6.4% of tested strains), amikacin (71.7-84.5% of tested strains), imipenem (69.9-79.1% of tested strains), ceftazidime (from 68.0 to 82.5% of tested strains), tobramycin (from 63.0 to 76.4% of tested strains). On the other hand, significantly less effective sensitivity profiles were shown by gentamicin (57.5% to 71.3% of tested strains), ciprofloxacin (51.3-68.0% of tested strains), aztreonam (59.5-61.2% of tested strains), ticarcillin-clavulanate (54.2-66.9%), and mezlocillin (45.3-54.2% of tested strains). When examining temporal trends of antibiotic sensitivity figures in the examined period (January-December 2008), significantly favorable changes were observed only for ceftazidime and ciprofloxacin ( $p < .025$ ).

**Conclusion:** A prospective surveillance study of antimicrobial susceptibility rates of a major hospital-associated organism like *Pseudomonas aeruginosa* is of remarkable importance, to establish reliable guidelines of antibiotic treatment and prophylaxis, on local-regional basis. Piperacillin-tazobactam, amikacin, imipenem, and the same ceftazidime still maintain a reliable role in eventual, empiric regimens to be added pending microbial isolation and *in vitro* susceptibility studies, since they remained active in nearly 80% of hospital isolates of the last year 2008. Colistin maintained full *in vitro* activity against all *Pseudomonas* strains observed over time. An appreciable increase of sensitivity rates to ceftazidime and ciprofloxacin was also observed over the last year 2008.

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Characterization of ertapenem resistance in *Klebsiella pneumoniae* from Croatia

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**Background:** *Klebsiella pneumoniae* isolates with reduced susceptibility to carbapenems were recently reported in USA, UK, Turkey and some other countries of the world. Recently eight *Klebsiella pneumoniae* strains with reduced susceptibility to carbapenems were isolates in four different hospitals in Croatia. The aim of the study was to determine the mechanisms of ertapenem resistance in these strains.

**Methods:** Antibiotic susceptibilities were determined by broth microdilution method according to CLSI. Transferability of ertapenem resistance was determined by conjugation (broth mating method) using *E. coli* A15 R- as recipient. Production of metallo  $\beta$ -lactamases was detected by double-disk synergy test and E test.  $\beta$ -lactamases were characterized by PCR with primers specific for extended-spectrum  $\beta$ -lactamases, plasmid-mediated ampC  $\beta$ -lactamases, metallo  $\beta$ -lactamases of VIM and IMP series, KPC and OXA-48  $\beta$ -lactamases. Genotyping of the strains was performed by PFGE.

**Results:** All strains were resistant to ceftazidime, cefotaxime, ceftriaxone, piperacillin alone and combined with tazobactam, amoxicillin/clavulanate, gentamicin and ciprofloxacin. All except one strains showed resistance to ertapenem, intermediate susceptibility or resistance to meropenem and intermediate susceptibility or full susceptibility to imipenem. One strain was resistant to all three carbapenems. Ertapenem resistance was not transferable by conjugation to *E. coli* recipient in neither of our strains. PCR revealed the presence of blaSHV and blaCTX-M genes. Multiplex PCR was positive for group 1 CTX-M  $\beta$ -lactamases. Sequencing of representative blaCTX-M genes revealed the presence of CTX-M-15 beta-lactamase. The strain resistant to all three carbapenem was positive by E test for MBLs. However, PCR was negative for VIM and IMP  $\beta$ -lactamases. No KPC or plasmid-mediated ampC  $\beta$ -lactamases were found. The strains were not clonally related as shown by PFGE and displayed distinct PFGE fingerprints.

**Conclusion:** This is the first report of carbapenem resistant *Klebsiellae* in Croatia. Ertapenem resistance in *Klebsiella* was previously reported in UK, Turkey and Israel mainly due to the production of CTX-M  $\beta$ -lactamases of group 1 combined with porin loss (OmpK36 or OmpK35). The characterization of outer membrane porins needs to be done to clarify the mechanisms of ertapenem resistance in our strains. Further testing is necessary to determine the mechanism of carbapenem resistance in the strain with positive E test–MBL.

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Changing trends in antimicrobial resistance among salmonella serotypes in Southern India

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**Background:** Enteric fever caused by drug resistant *Salmonella enterica* serotype Typhi and *Salmonella enterica* serotype Paratyphi A has been the major public health concern in the Indian Subcontinent.

**Methods:** A Retrospective analysis of antibiogram and resistance pattern to Ciprofloxacin, Nalidixic acid, Ceftriaxone Azithromycin and other routine antibiotics to *Salmonella* isolates from PUO cases from blood cultures during 2005-2006 combined with a follow up during 2007-2008 represents the data presented in this study.

**Results:** Of the 2247 from PUO cases 198 salmonella species (65 were *Salmonella typhi*, 132 *Salmonella paratyphi* A and 1 *Salmonella enteritidis*. *Salmonella typhi* and *Salmonella paratyphi* A serotypes were sensitive to Chloramphenicol, Ampicillin, Cotrimoxazole, and Cefriaxone and sensitive /intermediate to Ciprofloxacin and resistant to Nalidixic acid (except one). MIC for Ciprofloxacin and Nalidixic acid resistance strains was 0.5/1 µg/ml except for 3 MDR salmonella strains which had MIC value of 16 µg/ml. This was reflected on disc diffusion test as intermediate zone of inhibition. Retrospective blood culture analysis of 2005-2006 has shown that MDR *Salmonella typhi* was common isolate then and the strains were sensitive for Ciprofloxacin (MIC being 0.125µg/ml). No antibiotic resistant *Salmonella paratyphi* A was isolated during this period.

**Conclusion:** Salmonella strains with Nalidixic acid resistance and reduced susceptibility and MIC to Ciprofloxacin have emerged as major cause of enteric fever in Indian Subcontinent. Nalidixic acid susceptibility (30µg disc) can be reliably used to monitor Ciprofloxacin resistance.

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Molecular epidemiology of aminoglycosides resistance in *Acinetobacter* spp. with emergence of multidrug-resistant strains in hospitalized patients in Iran

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**Background:** *Acinetobacter* spp. is emerging as an important nosocomial pathogen and is characterized by increasing antimicrobial resistance. Our aim was to evaluate antimicrobial susceptibility and aminoglycosides resistance genes of *Acinetobacter* spp. isolated from hospitalized patients.

**Methods:** Sixty isolates were identified as *Acinetobacter* species. The isolates were tested for antibiotic resistance by disc diffusion method for 12 antimicrobials. The presence of *aphA6*, *aacC1* *aadA1*, and *aadB* genes were detected using PCR.

**Results:** From the isolated *Acinetobacter* spp. the highest resistance rate showed against amikacin, tobramycin, and ceftazidim, respectively; while isolated bacteria were more sensitive to ampicillic/subactam. More than 66% of the isolates were resistant to at least three classes of antibiotics, and 27.5% of MDR strains were resistant to all seven tested classes of antimicrobials. The higher MDR rate presented in bacteria isolated from the ICU and blood samples. More than 60% of the MDR bacteria were resistance to amikacin, ceftazidim, ciprofloxacin, piperacillin/tazobactam, doxycycline, tobramycin and levofloxacin. Also, more than 60% of the isolates contained phosphotransferase *aphA6*, and acetyltransferase genes *aacC1*, but adenylyltransferase genes *aadA1* (41.7%), and *aadB* (3.3%) were less prominent. In this study 21.7% of the strains contain three aminoglycoside resistance genes (*aphA6*, *aacC1* and *aadA1*).

**Conclusion:** The rising trend of resistance to aminoglycosides poses an alarming threat to treatment of such infections. The findings showed that clinical isolates of *Acinetobacter* spp. in our hospital carrying various kinds of aminoglycoside resistance genes.

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Incidence of Carbapenemase Resistance Gene (KPC) among *Klebsiella pneumoniae* isolates and its Clinical Implications

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**Background:** Carbapenem antibiotics (Imipenem, Ertapenem, and Meropenem) indicated for infections caused by extended-spectrum  $\beta$ -lactamase (ESBL) carrying pathogens. Carbapenem resistance has been unusual in isolates of *Klebsiella pneumoniae*. The aim of this study is to identify the prevalence of KPC positive *Klebsiella pneumoniae*, and its clinical significance.

**Methods:** All isolates of *Klebsiella pneumoniae* species from October 1, 2007 to September 30, 2009 were tested for the presence of KPC gene using the modified Hodge test. Medical records of patients with KPC were studied.

**Results:** Over the period of two-years 40,309 samples were submitted for culture and sensitivity, out of which 7,836 were positive. Of the positives, there were 106 isolates of *K pneumoniae* and 11 were ESBL positive. Of the ESBL producing isolates, 8 carried the Carbapenem-hydrolyzing  $\beta$ -lactamase. Of the eight, three isolates were reported as being susceptible to Imipenem. Although all the eight isolates were resistant using the Hodge test. Piperacillin/Tazobactam (PT) and Vancomycin were the antibiotic used 7 of the 8 patients prior to isolation of *Klebsiella pneumoniae* resistant to Carbapenems. One patient was excluded in outcome as one patient's sample was clinically thought to be a contaminant was not treated. 3 patients in whom resistance to carbapenem was reported had their antibiotic was changed to Tigecycline and Polymyxin B resulting in clinical improvement. Of the remaining 4 patients who were reported as sensitive to carbapenem three patients had to undergo a repeat surgery due to clinical deterioration and one patient clinically died.

**Conclusion:** The incidence of KPC gene at our hospital was 7.5%. KPC positive isolates are rapidly emerging pathogens. It is very important to keep this organism in mind as if not treated there is a 100% probability of having a poor outcome. There is a complete cross resistance to all Carbapenems containing KPC, therefore if KPC is present, the *K pneumoniae* will be resistant to all Carbapenem regardless of the routine susceptibility testing as shown in three isolates that are KPC positive but susceptible to Imipenem. Current automated systems used for susceptibility testing may not accurately identify all these isolates. We must also control the use of antibiotics specially PT to prevent emergence of KPC positive organisms.

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*In vitro* activity of Tigecycline against molecularly defined Carbapenemase producing *Acinetobacter baumannii*

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**Background:** *Acinetobacter baumannii* are important opportunistic pathogens with increasing rates of multi-antibiotic resistance due to both intrinsic and acquired mechanisms. Carbapenems are often used to treat these infections, however carbapenem resistance is increasingly reported, leaving few therapeutic options. This resistance is most often associated with acquired or intrinsic OXA-group carbapenemase production. While *A. baumannii* carry the intrinsic OXA-51-like carbapenemase gene, carbapenem resistance has only been associated with these genes when the insertion sequence IS*Aba1* is upstream. In this study, we evaluated the *in vitro* activity of tigecycline against genetically defined *A. baumannii* from the Tigecycline Evaluation Surveillance Trial.

**Methods:** A total 352 imipenem resistant *Acinetobacter baumannii* from 35 countries (2004 to 2006) were evaluated. MICs were determined by broth microdilution and interpreted according to CLSI guidelines. Carbapenemase genes were detected by multiplex PCR.

**Results:** All isolates tested in this study contained an OXA-51-like gene. Additional genotypes are listed below.

	N	TIG MIC <sub>50</sub> (mcg/ml)	TIG MIC <sub>90</sub> (mcg/ml)	Range
OXA-23	161	1	2	0.12 - 4
OXA-40	39	0.5	1	0.25 - 4
OXA-58	107	0.5	1	0.12 - 4
IS <i>Aba1</i> -OXA-51	190	1	2	0.12 - 4

**Conclusion:** Tigecycline demonstrated excellent *in vitro* activity against carbapenem resistant *A. baumannii* regardless of carbapenemase type. Tigecycline's *in vitro* activity against isolates with either intrinsic IS*Aba1*-OXA-51 or the acquired oxacillinases (OXA-23, OXA-40 and OXA-58) suggests that it may be effective against resistant isolates of this clinically important pathogen.

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**Type: Poster Presentation**

Molecular typing of multi-drug resistant *Acinetobacter baumannii* from London hospitals  
**D. Wareham**, D. Bean  
Queen Mary University London, London, United Kingdom

**Background:** *Acinetobacter baumannii* has emerged as a major nosocomial pathogen causing outbreaks of infection in the immunosuppressed and critically ill worldwide. A number of molecular typing techniques have been developed for use in both local surveillance and global epidemiological studies. We applied a recently described multi-locus sequence typing (MLST) scheme to multi-drug resistant *A. baumannii* (MDRAB) recovered from London Hospitals in comparison with other molecular methods.

**Methods:** Sixteen MDRAB were identified using multiplex PCR for OXA-carbapenemases and included representatives previously assigned to the UK 'South East', OXA-23 (1) and OXA-23 (2) clones. Strains were typed using pulsed field gel electrophoresis (PFGE), random-amplified polymorphic DNA-PCR (RAPD), a multiplex PCR typing (MPT) scheme (*ompA*, *csuE* and *blaOXA-51* like genes) and an *A. baumannii* MLST scheme (*gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, *rpoD*).

**Results:** Five clearly distinguishable profiles were observed using PFGE and six with RAPD. MPT typing assigned all but one of the strains (OXA-23 (2) representative) to group 1. Four allelic profiles (sequence types ST) were obtained using MLST, two of which appeared to be novel. Comparison of results revealed isolates designated 'South East' clone by PFGE / RAPD belonged to the previously described ST22 and those designated OXA-23 (1) to ST53. The allelic profile of the OXA-23 clone 2 strain was novel and contained a new *gyrB* allele.

**Conclusion:** Molecular techniques were comparable for typing UK MDRAB. Isolates belonging to ST22, (found previously in Italy, Portugal, Czech Republic, China, Korea and Australia) and isolates belonging to ST53 (found previously in Italy) were the most frequently types, highlighting the widespread dissemination of these clones. Two novel sequence types were identified and are undergoing further investigation.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Emerging multi drug-resistant *Acinetobacter* in Iran: Study of 800 cases

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**Background:** *Acinetobacter baumannii* is a multi drug resistant organism associated with high morbidity and mortality. It is an emerging nosocomial pathogen in many parts of the world. The aim of the present study was to review the incidence of *Acinetobacter* in several hospitals in Tehran, Iran and also to find out pattern of antibiotic resistance of *Acinetobacter*. We published primary results of this study when it was consisted of 100 samples. It was accepted in 48th Annual ICAAC/ 46th Annual IDSA congress, Oct. 2008.

**Methods:** In a retrospective study we detected 800 positive cultures of *Acinetobacter* from 800 patients in different wards of several tertiary care hospitals in Tehran, Iran. Disk diffusion method was used to determine the resistance of isolated *Acinetobacter baumannii*. Antimicrobial sensitivity to 14 available antibiotics was analyzed. The following antibiotics were tested: ceftazidime, cefotaxime, ceftazidime, cefepime, trimethoprim-sulfamethoxazole, gentamicin, tobramycin, amikacin, ciprofloxacin, ofloxacin, imipenem, meropenem, piperacillin-tazobactam and tigecycline.

**Results:** The most frequently sites of infection were wound, respiratory tract, blood stream, and urinary tract. The most incriminated wards were burn and intensive care unit with a high prevalence of wound infection, pneumonia and septicemia due to *Acinetobacter*. *Acinetobacter* was isolated from wound in 58.9%, tracheal discharge 9.2%, sputum in 7.1%, urine in 6.6%, blood in 5.8%, CSF in 3.4%, pleural fluid in 2%, tip of catheter (CV line) in 0.6% and bronchoalveolar lavage (BAL) in 0.2%.

*Acinetobacter baumannii* showed resistance to ceftazidime, cefotaxime, ceftazidime and cefepime more than 90%. Resistance to gentamicin and trimethoprim-sulfamethoxazole were more than 80%. Resistance to amikacin, imipenem and ciprofloxacin were 72.7%, 70.5% and 45.7% respectively. Tigecycline, piperacillin-tazobactam, tobramycin and ofloxacin were evaluated in 100 samples. Sensitivity to tobramycin and ofloxacin were 28.9% and 25.3% respectively. Tigecycline and piperacillin-tazobactam were active against 100% and 74% of the strains respectively.

**Conclusion:** *Acinetobacter baumannii* is an increasingly isolated pathogen in Iran. Prevalence of carbapenem resistant *Acinetobacter* is high in our study. Newer antimicrobial compound is needed for treatment of this infection.

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**Type: Poster Presentation**

The prevalence of extended spectrum betalactamases at a tertiary hospital in South Africa

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**Background:**

The extent of extended spectrum beta lactamase (ESBL) producing clinical isolates is now a global concern. The prevalence of ESBLs varies between species, geographically and with time. Delays in commencing appropriate therapy have been shown to have an impact on morbidity, mortality and hospital associated costs. The availability of current South African data will provide the evidence required to implement appropriate health measures.

**Methods:** A retrospective descriptive study was performed for the period from May 2008 to April 2009. Consecutive clinical specimens were obtained from admitted patients and patients referred from primary health care clinics to Dr. George Mukhari tertiary hospital in Pretoria, South Africa. Isolates from blood, cerebrospinal fluid, urine, sputum, pus and stools were processed using conventional laboratory methods. Isolates were identified and susceptibility testing done on the Microscan® using the CLSI guidelines. Demographic, clinical and laboratory data were evaluated.

**Results:** ESBL production was identified in 454 (18%) of the enterobacteriaceae isolated this period. The clinical source was predominantly non-sterile sites, 386 (83%) compared to 78 (17%) from sterile sites. The majority of isolates were from pus swabs (170) 44%, while 134 (35%) were from urine. Of the isolates identified, 271 (29%) were *Klebsiella pneumoniae*, 89 (9%) *Escherichia coli*, 25 (3%) *Proteus mirabilis*, 25 (5%) *Enterobacter cloacae*, 12 (1%) *Klebsiella oxytoca* and the rest *Enterobacter aerogenes*, *Morganella morganii*, *Proteus vulgaris*, *Citrobacter freundii* and *Serratia marcescens*. The prevalence of ESBLs in the *Klebsiella pneumoniae* isolates was found to be 271 (46%) and that for *Escherichia coli* 89 (12%).

**Conclusion:** At our institution, the overall prevalence of ESBL producing enterobacteriaceae of 18%, reaffirms the global spread of ESBL producing bacteria. The presence of ESBLs in most of the common enterobacteriaceae is consistent with the horizontal spread of ESBL genetic material. Due to the significant epidemiological implications; detection and surveillance would benefit health interventions at our institution and the country.

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**Type: Poster Presentation**

First characterization of blaIMP and blaVIM cassette-containing Novel Integron in metallo- $\beta$ -lactamase producing *Pseudomonas aeruginosa* in Malaysia

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**Background:** Carbapenem play an important role in the treatment of infections caused by *Pseudomonas aeruginosa*. The increasing frequency of carbapenem-resistant *P. aeruginosa* isolates is of concern world wide. The carbapenemases can be class A, B (metallo- $\beta$ -lactamases) and D. The types of MBLs that have been described so far are, IMP, VIM, SPM, GIM, SIM, with three recently reported AIM, KHM and NDM. They are typically present as integrons, often in association with other resistance gene cassettes. The objective of this study is characterization of MBL cassette-containing integron from *P. aeruginosa* in Malaysian isolates.

**Methods:** A total of 90 IMP-resistant clinical isolates were randomly isolated from the patients admitted at University Malaya Medical Center (UMMC), during 2005-2008. MBL producing isolates were phenotypically identified using MBL Etest and double disk synergy tests. Duplex PCR and PCR-RFLP, were carried out to determine the presence of MBL and class of integron. The gene cassette regions for the class1 integrons were amplified using primer specific for the 5'CS and 3'CS combining by VIM-R/IMP-R and VIM-F/IMP-F respectively.

**Results:** Among 90 IMP-resistant isolates tested by the MBL Etest and double disk synergic test, 34 (37%) isolates were found to be MBL producers. Of these 34 MBL-positive strains, PCR analysis confirmed blaIMP and blaVIM genes in 32 isolates (94%). In the present study, DNA sequencing shows that the MBL-positive genes were present as class1 integrons. The gene cassette array of class 1 integrons containing MBL genes are listed as following: blaIMP-7, aacC1; single gene cassette of blaVIM-11; aacA7 and blaVIM-2. The blaIMP-4 gene was absent in the integrons of two strains (attI; aacA7). Amplicons with lengths of 1.1 kb to 3.3 kb were amplified using 5'-CS and 3'-CS primers for gene cassette regions and 4 types of PCR amplicons of gene cassette were identified in 32 isolates with class 1 integron-associated MBL genes.

**Conclusion:** We confirmed that the integrons were prevalent and may play an important role in multidrug resistance of *P. aeruginosa*. In addition, the class I was the most abundant type of integron in Malaysian isolates.

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Carbapenem hydrolyzing multidrug resistant *Acinetobacter baumannii*

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**Background:** Multidrug resistant (MDR) *Acinetobacter baumannii* has emerged as a major pathogen causing healthcare-associated infections. Carbapenem susceptibility in our local hospitals has declined from over 90% to less than 50% in past five years.

**Methods:** We analyzed *A. baumannii* isolated from various patient specimens which were identified using standard biochemical tests. Antibiotic susceptibility testing was done using standard disk diffusion method. Multidrug resistance was defined as resistance to 3 or more classes of antibiotics including aminoglycosides, antipseudomonal penicillins, carbapenems, cephalosporins, and quinolones. MDR isolates were tested for carbapenemase production using modified Hodge test. Phenotypic determination for metallo-beta-lactamase (MBL) production was done using combined disk (imipenem+EDTA) and two different types of double-disk synergy methods (imipenem+EDTA and ceftazidime+EDTA).

**Results:** We studied 60 non-duplicate isolates of *A. baumannii* that were grown from patient specimens including blood, bone, wound, sputum, and other sources, obtained over a 12-month period in 2008-2009. Among these isolates, 65% were resistant to 3 or more classes of antibiotics meeting criteria for MDR organisms, and 50% showed reduced susceptibility to carbapenems. Modified Hodge test was positive in 19 (83%) of carbapenem-resistant isolates showing carbapenemase production. All MDR isolates were negative for metallo-beta-lactamase (MBL) production when phenotypically tested with double-disk synergy method, using imipenem+EDTA and ceftazidime+EDTA. These findings were similar when we tested with combined-disk synergy test using imipenem+EDTA.

**Conclusion:** This study confirms that majority of the currently prevalent *A. baumannii* are multidrug-resistant organisms, and half of them are carbapenem resistant. Carbapenemase production appears to be the most common mechanism of carbapenem resistance by phenotype screening method. Since these isolates were negative for MBL phenotype, production of carbapenem hydrolyzing oxacillinase is the most likely mechanism of resistance.

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Antimicrobial resistance in non-typhoidal *Salmonellae*

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**Background:** Non-typhoidal salmonellae are among the primary causes of food-borne gastroenteritis worldwide. Treatment failures due to the in-vivo acquisition ESBL gene or fluoroquinolone resistance in these bacteria are well documented, particularly in India, though actual molecular information is currently lacking. In this study we investigated ESBL genes, and fluoroquinolone resistance in non-typhoidal salmonellae isolated.

**Methods:** 23 isolates of non-typhoidal salmonellae were collected. Isolates were identified biochemically and agglutination, with antibiotic profiles being obtained. PCR and sequencing were used to determine the genetic determinants of antibiotic resistance. Isolates were screened for qnr gene and integrons by PCR, and plasmid analysis was performed using PCR-based replicon typing. Crude extracts of  $\beta$ -lactamase were subjected to isoelectric focussing and the role of efflux pumps in fluoroquinolone resistance was also studied. Isolates were genotyped using PFGE and ERIC-PCR.

**Results:** Six different *Salmonella* serovars were identified, the majority being *S. Agona* (48%), with one of the *S. Agona* isolates being unusually positive for a combination of TEM-1, SHV-12, OXA-1-like and DHA-1 genes. Twelve isolates (49%) were phenotypically ESBL producers, with 12, 11, 3, 2 and 1 of these isolates being TEM-1, SHV-12, DHA-1, OXA-1-like and CTX-M-15 gene positive, respectively. Four out of these 12 ESBL positive isolates were also resistant to fluorquinolones with 3 out of 4 possessing double mutations in the *gyrA* gene. No mutations were detected in the *gyrB* and *parE* genes of these 12 isolates. Inc/rep typing detected the presence of FIIIS replicons in the majority of isolates. Phenotypic evidence for efflux pumps was detected in 4 isolates. Eight of the isolates were positive for integrons. Genotypic diversity was observed among the isolates, even within isolates from the same hospital.

**Conclusion:** Twelve of 23 (52.2%), a very high proportion of non-typhoidal salmonellae from India produced ESBLs. The SHV-12 ESBL is one of the most common non-CTX-M ESBLs and is identified in many Gram-negative species, including *Salmonella* species. Our study confirms the role of double mutation in *gyrA* combined with 1 or 2 mutations in the *parC* and efflux mechanisms in conferring high resistance to ciprofloxacin. In India, non-typhoidal salmonellae constitute approximately 20% of the *Salmonella* serovars and continued surveillance for the presence of new ESBLs and fluoroquinolone resistances is required in India.

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**Type: Poster Presentation**

First description of CTX-M extended-spectrum  $\beta$ -lactamase-producing clinical *Escherichia coli* strains from Macao, China

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**Background:** Extended-spectrum  $\beta$ -lactamases (ESBLs) producing *Escherichia coli* (*E.coli*) is an emerging major pathogen worldwide. It has the ability to hydrolyze and cause resistance to various types of newer  $\beta$ -lactamase antibiotics, including third generation cephalosporins and monobactams. Organisms that produce ESBLs remain an important reason for therapy failure with cephalosporin and have serious consequences for infection control. Those clinical microbiology laboratories detect and report ESBL-producing organism is therefore important. The objectives of this study were to determine the prevalence, genotype and clonal relationship of extended-spectrum  $\beta$ -lactamases (ESBLs) in 209 clinical *Escherichia coli* strains from Macao, China.

**Methods:** Phenotypic detection was used by the standard disk diffusion method, double-disk synergy test and E-test. The genotypic characterization was detected by isoelectric focusing analysis (IEF). The clonal relationship between the different isolates was studied by pulsed-field gel electrophoresis (PFGE)

**Results:** The prevalence rate of ESBLs was 30.1% according to the Clinical and Laboratory Standards Institute. By isoelectric focusing analysis, polymerase chain reaction and sequencing, we detected the major genotypic characterization of ESBLs was CTX-M-14 (76.2%). Two strains showed indistinguishable patterns by pulsed-field gel electrophoresis.

**Conclusion:** This study documented the CTX-M family as the predominant ESBL type among Macao population. The spread of CTX-M enzymes is concerning and deserves close monitoring in further investigation.

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**Type: Poster Presentation**

Utilizing hospital generated antibiograms to examine state trends in antibiotic resistance  
**S. Onofrey**, M. Morrison, M.-Y. Lin, B. Bolstorff, A. DeMaria  
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**Background:** Antibiograms are aggregated, hospital-generated reports on susceptibility of bacteria of interest to specific antibiotics. They are utilized within hospitals to assist in effective use of antibiotics. The Massachusetts Department of Public Health (MDPH) has been requesting voluntary submission of antibiograms from hospitals annually since 1999.

**Methods:** Susceptible proportions reported in antibiograms were analyzed to evaluate changes in levels of susceptibility over five years, while accounting for the effect of hospital characteristics. Trends were examined for specific antibiotic and bacteria combinations as well as antibiotic class susceptibility patterns. Data were analyzed using SAS software version 9.1 (SAS Institute Inc., USA).

**Results:** Significant trends in antibiotic resistance were seen with a strong decreasing trend in *E.coli* fluoroquinolone-susceptibility and a moderate decrease in *Klebsiella pneumoniae* and *Enterobacter cloacae*. Specifically, *E.coli* susceptibility to ciprofloxacin decreased substantially over five years, and this trend was more pronounced in specific regions of the state. Other hospital characteristics such as bed count and hospital type did not appear to have a significant association with antibiotic resistance trends.

**Conclusion:** Antibiograms may serve as useful tools in examining regional antibiotic resistance trends. Trends identified may be used to inform further studies and pinpoint areas of concern for hospitals.

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**Type: Poster Presentation**

A Comparative study on gram-negative bacterial infections in Mansoura University Hospitals, Egypt

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**Background:** Gram negative bacteria are responsible for numerous infectious diseases. These diseases can occur in and harm any part of the body, the skin, eyes and the nervous, cardiovascular, respiratory, gastrointestinal and urogenital systems.

**Methods:** In the present study, some phenotypic and molecular typing techniques were applied on 108 strains of *E. coli*, 88 strains of *Ps. aeruginosa* and 8 strains of *Serratia* isolated from different clinical lesions in Mansoura University Hospitals, Egypt.

**Results:** The distribution of antibiotic resistance among the isolated strains showed high incidence of resistance and imipenem was the most active antibiotic. Using the active pyocin typing, 72 strains of *Ps. aeruginosa* could be typed into 35 pyotypes. SDS-PAGE of total cell protein extracts showed that the presence of fifteen patterns among *E. coli* strains and eleven patterns among *Ps. aeruginosa* strains. Using PCR technique it was found that 84% of the 50 tested strains were found to have at least one of the tested ESBLs. Also *TolC* and *AcrA* genes were present in all tested *E. coli* except 4 strains and did not present in *Ps. aeruginosa* except 4 strains. Plasmid profiles of 23 tested *E. coli* appear to be diverse. Also the prevalence of plasmids in 22 tested *Ps. aeruginosa* strains was lower than in tested *E. coli* therefore 59.1% of tested *Ps. aeruginosa* strains harbored plasmids. Using Pyrosequencing technique, the sequenced region of *gyrA*, *gyrB* and *ParC* were able to differentiate between the tested strains and neighbor-joining tree was constructed to determine relatedness between the isolated strains. Moreover, Molecular cloning of the whole sequence of *bla-TEM*, *bla-SHV* and *bla-CTX-M* was carried out experimentally to study the expression of these genes and determine which genes of them responsible for the resistance

**Conclusion:** Molecular-based methods of typing are more advantageous compared with phenotypic methods of typing in terms of better discrimination and reproducibility. Significant genetic variation was observed among different strains represented by the diversity of their plasmid profiles. All molecular genetic methods for distinguishing organism subtypes are based on differences in the DNA sequence.

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Extended spectrum beta-lactamases in *Escherichia coli* and *Klebsiella* spp. from Eastern Romania

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**Background:** The emergence and dissemination of ESBL are problems of major importance for the population health; ESBLs represent a first example of factor that contribute to the global crisis concerning the treatment of *Klebsiella pneumoniae* and *Escherichia coli* against which the third generation cephalosporins are not effective anymore.

**Methods:** Clinically isolates of *E. coli* (n= 642) and *Klebsiella* (n=92 ) were collected from patients with different types of infections (sepsis, urinary tract infections, etc), hospitalized between september 08 and september 09 in a Hospital of Infectious Diseases from Eastern Romania. Double disc synergy test using cefotaxime and amoxicillin/clavulanic acid discs was used to screen ESBL producers and these strains were subsequently subjected to confirmatory Etest.

**Results:** *E. coli* resistance to cefotaxime, ceftazidime, cefoxitin, ciprofloxacin and imipenem was found to be 40, 29, 6, 30 and 1% respectively. *Klebsiella* resistance to cefotaxime, ceftazidime, cefoxitin, ciprofloxacin and imipenem was 70, 57, 22, 41 and 4% respectively. % of ESBL producers *E. coli* was 15% (97 strains) and *Klebsiella* was 38% (35). All the ESBL producing strains were susceptible in 100% to imipenem and meropenem.

**Conclusion:** Carbapenems remain the most active agents against Gram-negative isolates, including ESBL producers strain of *E. coli* and *Klebsiella* spp. isolated from community-acquired and nosocomial infections from Eastern Romania.

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Can we rely on automated VITEK2 system the detection of KPC and other class A carbapenemase producers enterobacteriaceae?

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**Background:** Class A carbapenemases have become more prevalent within Enterobacteriaceae. Proficient methods are needed for their early detection in clinical microbiology laboratories in any attempt aimed for targeting optimal antimicrobial therapy and controlling their spread. Automated systems, as VITEK2, are increasingly used for routine susceptibility testing to decrease the in-laboratory turnaround time. However, the performance of VITEK2 for the whole class A carbapenemase family detection has never been assessed before. Objective: to determine the performance of VITEK2 for carbapenemase detection compared with both, CLSI agar dilution MIC and the genotype obtained by molecular methods.

**Methods:** Methods: we designed a panel composed by diverse bacterial genera with distinct carbapenem susceptibility patterns composed by 37 carbapenemase producers and 34 nonproducers (n): KPCs (17) Sme (10), NMC-A/IMI (2), GES (4), VIM/IMP (4) and CTX-M (12), AmpCs (12), combined mechanisms and others (10), respectively. The resistance mechanisms of the strains were assessed by PCR/DNA sequencing. Each isolate was tested with the VITEK2 using the AST-N082 cards specifically designed for South American countries (which included only imipenem -IPM- and meropenem -MEM-), according to the manufacturer's instructions and by CLSI agar dilution MICs for both carbapenems. Discrepant results were resolved by retesting the isolates.

**Results:** Overall categorical interpretations with VITEK2 showed a 72% and 79% of agreement with reference MICs for IPM and MEM, respectively. Very major (VM), major (MA) and minor (MI) errors were: IPM, VM 3%, MA 11% and MI 13%; MEM, VM 4%, MA 6% and MI 11%. Most of the errors (>80%) occurred among carbapenemase producers. The expert system showed sensitivity (SN) of 76% and specificity (SP) of 87% for carbapenemase detection, when confronted to the genotype, with the greatest SN for KPCs (82%) and the lower for MBLs (25%). The recognition of suspected carbapenemase producers could be increased with the combined use of IPM and MEM with modified cut-off points of  $\geq 2.0$  mg/L and  $\geq 1.0$  mg/L, respectively (SN 97%, SP 90%).

**Conclusion:** VITEK2 may be suitable in clinical laboratories for Class A carbapenemase detection, but should be accompanied with modifications in the cut-off used for screening of suspected carbapenemase producers to ensure their proper detection.

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**Type: Poster Presentation**

Emergence of multidrugresistant gram negative bacilli and enterococci from rectal swabs of newborn and their mothers from Central India

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**Background:** Newborn babies acquire gut flora mainly from mother and surrounding. We had observed in the faecal samples of newborns prevalence, colonization of multidrug resistant, ESBL pandrug resistant gram negative bacteria, vancomycin resistant enterococci as a new threat in the newborn admitted in hospital. The influx of these bacteria into hospitals has major implications for infection-control and empirical treatment strategies

**Methods:** A total of 140 samples of faeces from neonates and mothers admitted in general maternity ward and ICU of two hospital in central India were examined within 24-48 hours for presence of ESBL, pandrug resistant gram negative bacilli, and vancomycin resistant enterococci. Antibiotic susceptibility test were performed using Kirby-Bauer disc diffusion method and results were interpreted according to CLSI. Van A and ESBL gene was confirmed using E test, PCR and RT PCR.

**Results:** 1. A total of 48 *E.coli*, 49 *Klebsiella*, 21 *Pseudomonas* and 52 *Enterococci* isolates were obtained. The percentage of multidrug resistant *E.coli*, *Klebsiella*, *Pseudomonas* and *enterococci* was 78.79, 66.67, 58.8 and 91.18% respectively. For *gram negative bacilli* % resistance for chloramphenicol (47%), cabapenem 58.9% and ampicillin (74.3%) aminoglycosides (70.9%), quinolones (65.8%) and cefoparazone+ sulbactam (58.1%). piperacillin+tazobactam (69.2%) cotrimoxazole (47%) cephalosporin (71.1%). The prevalence of ESBL gene (TEM and SHV) among *E.coli* and *Klebsiella* was 100% and 75% respectively. The pandrug resistant was 18.15% among *E.coli* and 20.4% among *Klebsiella* and 1.5% among *Pseudomonas*. Of 52 enterococci, 47.06% of them were vancomycin resistant strain and harboured van A gene. Enterococci were showing a high level resistance to aminoglycosides (82.35%), ampicillin (82.35%), chloramphenicol (38.24%), teicoplanin (44.12%) and linezolid (8.82%).

**Conclusion:** We report high rates of colonization with ESBL and pandrug resistant gram negative organism and vancomycin resistant enterococci among faecal samples from newborn admitted in ICU, maternity ward with in 24-48 hours from two hospitals in central India. The colonization is mainly due to antibiotic usage by the mother as well as newborn in ICU. Antibiotics used were cefotaxime, gentamicin amikacin and ciprofloxacin. It poses a great threat to infection control measures and complicates the selection of empirical treatment.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Patients with hematological malignancies who are prescribed quinolone prophylaxis post-chemotherapy in a hospital with high gram-negative quinolone resistance are at a higher risk for febrile neutropenia

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**Background:** Febrile neutropenia (FN) remains a major cause of morbidity and mortality in patients receiving chemotherapy. Major prophylactic strategies include granulocyte colony stimulating factor and antibiotics, the most widely used of which are quinolones. While quinolone prophylaxis has been shown to be effective in areas where fluoroquinolone resistance is low, this same efficacy has not been proven in areas where resistance is high. We reviewed the efficacy of quinolone prophylaxis in preventing FN in patients with leukemia and lymphoma undergoing chemotherapy at our institute - a tertiary academic medical center where quinolone resistance among aerobic gram-negative bacilli (GNB) exceed 40%.

**Methods:** A retrospective chart review was performed on all patients with acute leukemia and lymphoma who underwent chemotherapy and were at high risk (>5%) of FN at our institute between 1 October 2008 and 30 September 2009. Demographic and clinical characteristics for each episode of chemotherapy were collected, with the main outcome being presence/absence of FN. Generalized estimating equation (GEE) models were used to analyze the data to adjust for serial autocorrelation due to repeat measurements of both outcome and covariates for each patient.

**Results:** There were a total of 510 chemotherapy episodes from 119 patients within the study period, with median number of chemotherapy episodes being 3. Demographic and clinical characteristics - including disease stage - were similar between patients on and off quinolone prophylaxis. Among all patients, those on quinolone prophylaxis were at significantly higher risk for both FN (OR:5.4; 95%CI:3.1-9.4;  $p<0.01$ ) and FN with bacteremia (OR:5.3; 95%CI:2.7-10.6;  $p<0.01$ ). Subgroup analysis for patients with acute myeloid leukemia and lymphoma showed similar higher risk of FN for patients with quinolone prophylaxis (OR:7.1; 95%CI:2.2-23.0;  $p<0.01$  and OR:3.8; 95%CI:1.7-8.7;  $p<0.01$  respectively). All GNB isolated in these patients were resistant to quinolones.

**Conclusion:** In a hospital with high quinolone resistance rates, quinolone prophylaxis failed to protect patients with hematological malignancies from developing FN as well as FN associated with bacteremia. A prospective case-control study should be conducted to validate these findings.

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Multidrug resistant and sensitive strains of *Pseudomonas aeruginosa*: Establishing clonal relationship by Pulsed Field Gel Electrophoresis and in vitro antibiotic synergy testing by E test

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**Background:** The emergence of multidrug resistant *Pseudomonas aeruginosa* (MDRPA) as one of the leading nosocomial pathogens imposes a serious health threat due to limited antibiotic options. In this study, a sample of clonally distinct MDRPA and sensitive strains of *Pseudomonas aeruginosa* (SSPA) were tested against various antibiotics combinations to establish synergistic activity against these strains.

**Methods:** A total of 39 retrospectively collected MDRPA strains isolated over three years with a particular antibiogram (sensitive to colistin but resistant to ceftazidime, cefepime, cefoperazone, cefoperazone/sulbactam, ciprofloxacin, imipenem, meropenem, piperacillin, piperacillin-tazobactam, gentamicin, netilmicin, with varying susceptibility to amikacin) and 19 SSPA collected prospectively were genotyped using *Spe1* DNA macrorestriction analysis and pulsed-field gel electrophoresis (PFGE). The strains were from different patients from various sites (blood, tissue, fluid, catheter tip, bronchiol alveolar lavage (BAL), urine, sputum, swab, tracheal secretion and nasal swab). Clonal relatedness was determined at  $\geq 85\%$  level of similarity and grouped into different clusters. A representative strain from each cluster was tested against 10 different paired combinations of various antimicrobials ( $\beta$ -lactams, quinolone, aminoglycoside, tigecycline, rifampicin and colistin) to establish synergistic activity against these strains using the investigational E test fixed ratio method.

**Results:** There were 46 distinct clones out of a total of 58 MDRPA and SSPA resolved by PFGE from which 10 clonally distinct strains (8 MDRPA and 2 SSPA strains) were selected for synergy testing. The antibiotic combination which most frequently demonstrated synergy was cefepime with amikacin (4/10 strains). Other synergistic combinations were aztreonam with either ceftazidime or cefepime, and meropenem with ciprofloxacin (3/10 strains respectively), ceftazidime with either ciprofloxacin or amikacin (2/10 strains respectively) and imipenem with ciprofloxacin (1/10 strains). The combination of ceftazidime with amikacin was antagonistic in 1/10 strains. The other antibiotics combinations were either additive or indifferent.

**Conclusion:** PFGE was a useful and discriminative molecular subtyping tool to establish clonal relatedness among strains. Results of antimicrobial synergy testing indicate that although certain combinations may act synergistically, it was still strain dependent. These preliminary findings require further confirmation with testing of a larger number of isolates.

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**Session: Antibiotic Resistance Gram-Negative**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

KPC Antibiogram in a large teaching Brazilian Hospital

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**Background:** Describe susceptibility profile of KPC *Klebsiella pneumoniae* producers recovered in a teaching hospital from São Paulo.

**Methods:** We analyzed 27 isolates of KPC, one per patient, from July 2008 to April 2009.

Identification was performed by GNI650 Cards Vitek™ (Biomérieux, Marcy l'Etoile, France); susceptibility test was performed by disk diffusion and the Hodge Test was done according to CLSI-M100S19 recommendations. blaKPC detection was done by PCR with previously described primers and Pulsed Field Gel Electrophoresis (PFGE) was also performed. Minimal Inhibitory Concentration (MIC) for polymyxin B, tigecycline and carbapenems was performed by Etest™ (Biomérieux, Marcy l'Etoile, France).

**Results:** KPC antibiogram showed multiple resistance as expected: 100% to ertapenem, 81,4% to meropenem, 77,7% to imipenem, 93,6% to amikacin; 82,5% to gentamicin, 89% to cefepime and ceftazidime and 100% to ciprofloxacin, sulphametoxazol/trimetropim, piperacillin/tazobactam and to aztreonam. MIC 50/90 were 2mg/l and 3mg/l to tigecyclin, and 2mg/l and 48mg/l to polymyxin B. PFGE showed that 89% belonged to the same molecular profile.

**Conclusion:** KPC isolates showed very few therapeutically options being tygecyclin the most active drug among those isolates. In our institution treatment with polymyxin B alone has to be carefully monitored until MIC is available. The same molecular profile emphasizes the horizontal spreading of these multiresistant bacteria.

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**Session: Antibiotic Resistance Gram-Negative**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Decreased susceptibility to polymyxins emerging during treatment for carbapenem-resistant *Enterobacter aerogenes* infection

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**Background:** Emergence of carbapenem resistant enterobacteriaceae (CRE) is worrisome and polymyxins are possible therapeutic options. The objective of this article is to describe emergency of polymyxins resistance during therapy.

**Methods:** A 30 years old male patient with bilioma after liver transplantation had eighteen successive cultures isolates of *E. aerogenes* (*Ea*) from blood and peritoneum fluid recovered during thirty days hospitalization. Identification was done by Vitek™ (Biomérieux) and antimicrobial susceptibility test were done by disk-diffusion (DD) and Etest™ (AB Biodisk) according to CLSI. Polymyxins agar dilution was also performed. All isolates were submitted to pulsed field gel electrophoresis analysis.

**Results:** In the third day of hospitalization the first blood culture was positive for *Ea* susceptible to carbapenems. MIC for polymyxin B was 1 mg/L and for colistin 0,5 mg/L. DD showed 14mm of zone inhibition for colistin. After six days of meropenem therapy *Ea* isolates became resistant to carbapenems with MIC higher than 32mg/L. Colistin therapy was initiated until patient's death.

The eight initials isolates recovered before colistin treatment had MIC less than 2 µg/ml for polymyxins and eight *Ea* isolates recovered after this period had MIC above 12µg/ml for polymyxins. The fourteenth isolate, one day before the patient's death, had MIC of 64 mg/L for polymyxin B and colistin, DD inhibition zone for colistin was 7mm. Correlation between DD and MIC above 8µg/ml for polymyxins was seen with disk inhibition zones inferior than 13mm. All *Ea* belonged to the same clone.

**Conclusion:** CRE is a therapeutically and epidemiological challenge in every hospital. Colistin is one of the therapeutically options but resistance may emerge during treatment and its *in vitro* activity is not routinely recommended by CLSI.

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**Session: Antibiotic Resistance Gram-Negative**  
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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Outbreak of (OXA-66 carbapenemase) multidrug-resistant *Acinetobacter baumannii* in a Spanish tertiary-care hospital: Epidemiology and study of patient movements  
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Hospital Clinico San Carlos, Madrid, Spain

**Background:** *Acinetobacter baumannii* is an increasingly common nosocomial pathogen. Carbapenems have been the agents of choice for severe *Acinetobacter* infections. We describe an outbreak of multidrug-resistant (MDR) *A. baumannii* that produced OXA-66 carbapenemase and was resistant to imipenem. We also analyze the relationship between the spread of this strain and patients' movements within the hospital.

**Methods:** Thirty-one isolates of *A. baumannii* with very similar susceptibility patterns from 15 patients, recovered in a 2 months period, were studied. We analyzed 8 more isolates recovered during the following year. ERIC-PCR and RAPD genotyping methods were used to define clusters of clonally related isolates. PFGE was used to confirm the results and to check the maintenance of the epidemic strain over the following year. Patterns of possible transmission were analyzed by recording patient movements within the hospitals. Antibiotic susceptibility testing to 28 agents was performed by microdilution and by E-test. The isolates were screened by PCR analysis with primers specific for 6 carbapenemase genes. Amplification products were sequenced to determine the gene present.

**Results:** Twelve of the 15 patients studied were hospitalized at the ICU. The most frequent sites of isolation were the respiratory tract (16) and the blood (11). With the exception of colistin (0% resistance) there were no antibiotics with good activity against these isolates. Of the other antibiotics tested, tigecycline showed the best activity with an MIC<sub>90</sub> value of 2 mg/L. The genotypic study revealed that the same strain was responsible for all the infections. All the isolates harboured the *bla*-OXA-51-like gene and the 6 of them chosen to sequence the gene were identical and 100% homologous with the *bla*-OXA-66 gene. PCR showed that the insertion sequence IS<sub>Aba1</sub> was present upstream of the oxacillinase gene in all the isolates.

**Conclusion:** **a)** Molecular typing revealed that the outbreak detected in our hospital was due to a single *A. baumannii* clonal group. **b)** The epidemic strain of *A. baumannii* produces an OXA-66-IS<sub>Aba1</sub> carbapenem-hydrolyzing oxacillinase. **c)** Even though the outbreak was controlled, and the number of isolates decreased significantly, the clone responsible of the outbreak persisted at the hospital during the following year.

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**Session: Antibiotic Resistance Gram-Negative**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Online games teaching children hygiene and antibiotic resistance: Evaluation of the e-Bug games  
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**Background:** e-Bug is a EC-funded antibiotic, hygiene teaching resource aiming to reinforces an awareness of microbes, respiratory hygiene and prudent antibiotics use among junior and senior school children across Europe. e-Bug junior web games were developed for children age 9-12 years.

The e-Bug junior game has a number of “levels” teaching the given set of learning outcomes (LOs). Player, shrunken inside human body, interacts with good and bad cartoon microbes (Fig 1) as well as antibiotics and viruses. Teaching the LOs is implemented through interaction with microbes (making yogurt (Fig 3), finishing course of antibiotics (Fig 2). Knowledge is tested seamlessly before and after each level in a Game Show style similar to the game “Do you want to be a Millionaire?”

**Methods:** Evaluation was primarily conducted in the UK and online demonstrating statistically significant knowledge gain of the learning outcomes. This was complemented by focus groups and observational studies with 29 pupils taking part (and fully completing the pre and post questionnaire) from three schools. Before playing the game, only 4 pupils “agreed” that fungi were microbes while after playing 18. Smaller improvements were seen in other questions including: “We use microbes to make things like bread and yogurt” (11 correct before, 23 correct after) and “Soap can be used to wash away bad bugs” (20 before vs 24 after).

**Results:** The main evaluation took place in May – August 2009. Each of the completed levels was evaluated for statistical significance of knowledge change of the LOs. As many questions were correct before and after the game, the statistically significant improved responses were measured (using McNemar’s test), for the following questions: “if you cannot see a microbe it is not there”, “most coughs and colds get better without medicine” and in particular “we use good microbes to make things like bread and yogurt”. There was a trend towards improved knowledge however in other questions did not reach statistic significance.

**Conclusion:** The study demonstrated that computer games can teach children about hygiene and antibiotics in an enjoyable way. Further study is needed to evaluate an impact on behaviour change.

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**Session: Antibiotic Resistance Gram-Negative**

**Date: Wednesday, March 10, 2010**

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**Type: Poster Presentation**

Antimicrobial utilization and susceptibility patterns of a sentinel group of bacterial isolates prior and subsequent to the introduction of Ertapenem to the hospital formulary

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**Background:** It has been suggested that after the introduction of ertapenem into hospital use, the amount of other carbapenems and the rate of resistance to other antimicrobials decreases. We conducted a retrospective study to evaluate these outcomes.

**Methods:** We studied 48 months (two 24 month-periods) before (PRE) and after (POST) the introduction of ertapenem in August 2004. Antibiotic use was determined using the standard defined daily dose (DDD) per 1000 patient/days. Antimicrobial susceptibility testing to *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* was performed with the Vitek automated system. We used chi-square for trend to compare rates of resistance and changes in antimicrobial use.

**Results:** In the PRE-period, we analyzed 4072 *E. coli*, 677 *P. aeruginosa*, 571 *K. pneumoniae*, and 67 *A. baumannii* isolates; in the POST 4648, 884, 559, and 109, respectively; antibiotic consumption was as follows: PRE-period: meropenem 89.9 DDD/1000/patients/days, antipseudomonal cephalosporins 90.9, and ceftriaxone 195.65; POST period: 75.3, 103.58, and 184.77, respectively. The rate of antimicrobial susceptibility was: *E. coli*, to meropenem in PRE-period 99.6% and in POST-period 98.05%, to ceftazidime 88.67% and 84.75% ( $p < 0.000$ ), respectively; to *P. aeruginosa*, meropenem 67.41% and 61.74% ( $p = 0.004$ ), respectively, ceftazidime 63.74% and 62.12% ( $p = 0.3388$ ), respectively, and piperacillin/tazobactam 69.17% and 67.21% ( $p = 0.15$ ), respectively; *K. pneumoniae*, meropenem 100% and 98.7% ( $p < 0.05$ ), respectively, and ceftazidime, 86.60% and 90.29% ( $p = 0.018$ ); *A. baumannii*, meropenem 95.2% and 79.09% ( $p = 0.08$ ), respectively, and ceftazidime 76.14% and 58.88% ( $p = 0.12$ ), respectively.

**Conclusion:** Our data show that after the inclusion of ertapenem, the use of other carbapenems and ceftriaxone significantly decreased, however the use of antipseudomonal cephalosporins significantly increased; of note, there was a slight decrease in the susceptibility to carbapenems and broad-spectrum cephalosporins during the POST-period, which could be attributed to the intensive use of both, ceftriaxone and antipseudomonal cephalosporins.

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**Type: Poster Presentation**

Clinical characterization of patients with carbapenem-resistant versus carbapenem-susceptible *Acinetobacter baumannii* infections

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**Background:**

Steady rise in carbapenem resistance in *Acinetobacter baumannii* has become a major challenge for clinicians. The objective of this study is to characterize clinical features of infections secondary to carbapenem-resistant organisms compared to carbapenem-susceptible organisms.

**Methods:** We conducted a retrospective analysis of data for 39 patients from two tertiary care medical centers who had positive cultures for *A. baumannii*. Collected data included clinical presentations, underlying illnesses, treatment course, clinical outcome, microbiological data and other laboratory data. Statistical comparisons were done between patients infected with carbapenem-resistant (CRAB) and carbapenem-susceptible (CSAB) isolates using Fisher's exact test and Student's T-test. Multidrug resistance (MDR) was defined as resistance to more than two classes of antibiotics generally active against *A. baumannii*.

**Results:**

Total 17 (44%) patients had carbapenem-resistant (CRAB) and 24 (62%) had multidrug resistant (MDR) *A. baumannii*. Mean age ( $53 \pm 5.7$  years) and male predominance (70%) were comparable in both groups. Predisposing factors included diabetes mellitus (15), pressure ulcers (12), malignancy (8), paraplegia (7), burn (5), peripheral vascular disease (5), and chronic renal failure (5). Majority of the infections involved wound (13) and bone (10) followed by sputum (8), urine (5) and bloodstream (3). Mean duration for first positive *A. baumannii* cultures after admission to the hospital was 6.6 ( $\pm 3.4$ ) days. Patients infected with CRAB had significantly higher rates of respiratory or other organ failures (47% vs. 14%,  $p < 0.05$ ), and were more frequently admitted to the intensive care unit (53% vs. 18%,  $p < 0.05$ ), compared to the patients with CSAB. Patients with CRAB were also more likely to be admitted for prolonged durations in the hospital (mean = 31.5 vs. 8.5 days,  $p < 0.01$ ) and in the intensive care unit (mean = 25.9 vs. 1.2 days,  $p < 0.05$ ). Mortality was significantly higher among patients with CRAB compared to those with CSAB (29% vs. 4.5%,  $p < 0.01$ ). *A. baumannii* infections were treated for an average duration of 24 ( $\pm 7$ ) days. Antibiotics used for treatment included carbapenems, ampicillin-sulbactam, tigecycline, aminoglycosides, colistin and polymyxin B.

**Conclusion:**

Carbapenem-resistant *A. baumannii* infections were associated with severe morbidity, requiring prolonged hospitalization and ICU admissions, and also resulted in increased mortality.

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**Session: Arboviruses**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Doxycycline modify the cytokine storm in patients with dengue and dengue hemorrhagic fever

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**Background:** Dengue virus infection is an acute febrile disease caused by a virus of the genus *Flavivirus*, family *Flaviridae*. Usually is a mild self-limiting acute illness, but may evolve to a hemorrhagic form characterized by plasma leakage and hemorrhagic manifestations. The treatment of both forms has been limited to the management of the signs and symptoms, and sometimes a retroviral treatment has been used. The cytokine cascade has a crucial role in the pathogenesis of dengue (DF) and dengue hemorrhagic fever (DHF), and the presence of the Th1 and Th2 cytokines are responsible to cross-regulate the disease from a mild illness (DF; Th1-type response), to a severe illness (DHF; Th2-type response). Recently, doxycycline was show to inhibit the cytokine storm caused by Staphylococcal exotoxins, specifically the cytokines involved in the Th2-type response. In this work, we use the doxycycline and tetracycline treatment in patients with dengue and dengue hemorrhagic fever, in order to regulate the Th1 and Th2 cytokines.

**Methods:** Thirty patients were included in the work. The patients were divided in groups of 5 each: DF, DF+ doxycycline, DF+ tetracycline, DHF, DHF+ doxycycline, DHF+ tetracycline. The doxycycline groups were treated with oral doxycycline 200 mg/day/7 days, the tetracycline groups were treated with oral tetracycline 1.5 g/day/7 days. The patients were bleeding in days 0, 3 and 7. Serum concentrations of IL-6, IL-1 $\beta$ , IL-1ra, TNF- $\alpha$ , and sTNF-R1 were determined by ELISA. Clinical laboratory were performed with each bleeding, and a clinical control of signs and symptoms were carry out every day.

**Results:** Doxycycline groups shows the higher immune-regulation of cytokines IL-6, IL-1 $\beta$ , and TNF- $\alpha$ , with an increase in the levels of IL-1ra and sTNF-R1, followed by the tetracycline groups which presents an slightly higher regulation than the control group without treatment. The improvement of the patients was better in the doxycycline groups, with a faster remission of the symptoms than the other groups.

**Conclusion:** The use of doxycycline in the treatment of dengue and dengue hemorrhagic fever patients could regulate the cytokine cascade and improve the recovery of the patients with dengue and dengue hemorrhagic fever.

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**Session: Arboviruses**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Field hospital for fluid intake: The solution for the decrease mortality in dengue fever

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**Background:** December 2007, some cases of dengue hemorrhagic fever were diagnosed in children in Rio de Janeiro, Brazil. Along the following months, the has ravaged the state, infecting more than 150,000 people with 232 suspected deaths. 42% of fatal cases were in children. The deaths showed us that the plasma leakage and shock are more common than hemorrhagic phenomena. In many cases, the presence of pleural, pericardial and peritoneal effusions were associated with a severe disease. The emergency rooms in the state were not capable to absorb the extra demand and causing the collapse of the healthcare system. The last outbreak in Rio de Janeiro was happen in 2002 with the serotype 3 and now the serotype 2 e 3 has been reported.

**Methods:** On February 2008, a hundred of new dengue cases were being reported/ hour, so the health department of Rio the Janeiro State, the Myilitary Fire Corps and the Armed Forces assembled 7 field hospitals to support the emergency rooms, working with more 1000 health care providers. The average field hospital was equipped with an electronic blood cell counting machine, 30 beds for hydratation and 1 advanced ambulance. This intervention was based in the cocept of disaster medicine. The patients were triaged in the hospitals, had their blood taken for diagnosis, kept in observation and hydratated. The caes with deterioration were admitted to the hospital and if the patient got better, he was sent home.

**Results:** The intravenous fluid administration during 12 hour observation period was associated with a decreased risk for the death and complications. On April, 29,000 cases were treated in the field hospitals and less than 2% of the patients were admitted to the emergency hospitals.

**Conclusion:** The Field hospitals were a practical solution to reduce the mortality and morbidity in this outbreak.

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**Type: Poster Presentation**

Utility of dengue antigen-capture ELISA in the diagnosis of dengue Fever in the real world

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**Background:** Dengue fever is an endemic and potentially fatal viral infection threatening more than 2.5 million people in over 100 regions around the world - hence the need for timely and accurate diagnosis using an easy and affordable assay.

**Methods:** In our study, the use of a commercial dengue antigen-capture ELISA (PLATELIA DENGUE NS1 AG by Bio-Rad) was evaluated to demonstrate its usefulness in diagnosing acute dengue viral infection in an acute tertiary centre in Singapore. Our country is endemic for dengue fever with more than 7000 cases reported in 2008. Retrospective analysis was performed on 197 patients in whom serum dengue antigen-capture ELISA was carried out. The diagnosis of dengue was made based on dengue IgM positivity and the presence of classic clinical features of dengue fever.

**Results:** A total of 75 patients were identified to have dengue fever. The overall sensitivity of dengue antigen-capture ELISA was 64% (48/75). Sensitivity was 81.5% when testing was carried out on serum samples taken during Day 1 to 4 of fever, 59.4% during Day 5-6 and 42.9% during Day 7-8. In patients who did not have dengue fever, the specificity was 100%.

**Conclusion:** The results suggest that the dengue antigen-capture ELISA is most useful in the diagnosis of dengue fever within the first 4 days of fever.

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**Type: Poster Presentation**

Specific point mutations in the envelope protein of Tick-borne encephalitis virus enhance non-viraemic transmission efficiency in a tick vector.

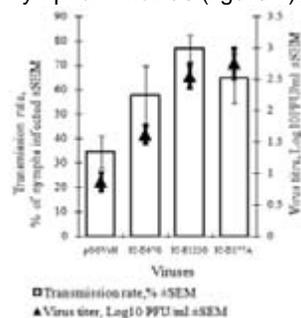
**M. Khasnatinov**<sup>1</sup>, K. Ustanikova<sup>2</sup>, T. V. Frolova<sup>3</sup>, V. V. Pogodina<sup>3</sup>, N. G. Bochkova<sup>3</sup>, L. S. Levina<sup>3</sup>, M. Slovak<sup>4</sup>, M. Kazimirova<sup>4</sup>, M. Labuda<sup>4</sup>, B. Klempa<sup>2</sup>, E. Eleckova<sup>2</sup>, E. A. Gould<sup>5</sup>, T. S. Gritsun<sup>6</sup>

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**Background:** Tick-borne encephalitis virus (TBEV) is transmitted to humans by Ixodid ticks causing >10,000 cases of disease annually. The risk of human infection relates to the efficiency of virus transmission between infected and uninfected ticks. Here we identify specific mutations in the viral envelope protein that affect transmission efficiency of TBEV between ticks.

**Methods:** The genomes of 4 field isolates of TBEV deficient in haemagglutination, were sequenced and recreated by site-directed mutagenesis, in a TBEV infectious clone. They were then compared with the wild-type infectious clone in mice, porcine kidney PS cells and adult and nymphal *I. ricinus* ticks.

**Results:** Sequence analysis revealed unique amino acid substitutions D67G, E122G or D277A in the envelope glycoprotein. Each mutation resulted in an increase of net charge and hydrophobicity on the virion surface. When introduced individually into the TBEV infectious clone (IC), each substitution inhibited haemagglutination and reduced mouse neuroinvasiveness from 65% to 15-30%. Antibody production in infected mice was 1.5-3 times lower for IC-E122G and IC-D277A suggesting lower levels of viraemia and/or deficient immune stimulation induced by these viruses. All mutants demonstrated delayed growth in PS cells during the first 24hpi; however, mutant IC-D67G exhibited significantly better growth characteristics than IC-E122G and IC-D277A. The reproduction of IC-E122G and IC-D277A in fasting ticks was similar to that of control HA positive virus whereas the titres of IC-D67G were significantly lower (2.5-3 vs. 1-2 log<sub>10</sub>PFU/ml, respectively). In feeding ticks, the titre of IC-E122G increased approximately 1000-fold and IC-D277A and IC-D67G - approximately 300-fold, whereas for control virus the increase was about 10-fold. Non-viraemic transmission efficiency from infected to uninfected ticks was increased by each individual substitution in nymphal *I. ricinus* (figure 1).



**Figure 1. Tick-to-tick transmission rate (clear bars) is expressed as the proportion of infected *I. ricinus* nymphs. Black triangles show the average virus titres in individually infected recipient nymphs as determined by plaque assay.**

**Conclusion:** We hypothesize that the mechanism of adaptation of TBEV to its host utilizes the shift of charge/hydrophobicity at several critical amino acid residues exposed on the virion surface. This shift results in different biological consequences depending on the localisation of certain amino acid residue. The results provide valuable information concerning the maintenance in nature and the emergence of pathogenic variants of TBEV.

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**Type: Poster Presentation**

A pre-exposure prophylactic for arenaviral hemorrhagic fever in the pirital virus-Syrian golden hamster model

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**Background:** Arenaviral infections in humans have the capacity to lead to hemorrhagic fever and may be fatal. Supportive care and ribavirin remain the only options for arenaviral infections in humans; however, treatment is often ineffective because of the time frame before the disease is recognized. Pirital virus (PIRV) is a New World arenavirus that was isolated from the cotton rat (*Sigmodon alstoni*) in the Municipality of Guanarito, Portuguesa State, Venezuela in 1994. This virus is not associated with any form of disease in humans and can be studied in a biosafety level 3 (BSL-3) laboratory environment; therefore, the development of a small animal model system is ideal for testing and/or screening of vaccines and therapeutics against arenavirus hemorrhagic fever.

**Methods:** Treated and mock-treated female Syrian golden implanted with telemetry units measuring temperature and activity, were utilized to test the antiviral effects of the compound named BAT-V1. The animals were followed for 14 days post-challenge. Clinical signs of disease were monitored. Temperatures and activity levels were recorded by telemetry. Clinical chemistry, hematology, and coagulation parameters were measured and viral titers in the tissues and blood were analyzed.

**Results:** We demonstrate that PIRV infection in Syrian golden hamsters leads to morbidity, fever, lethargy, hemorrhagic fever manifestations, viremia, and replication in select tissues and results in 100% mortality within 8 days after challenge. Treating hamsters with BAT-V1 prior to challenge significantly protected the animals from death, which is important because survival of hamsters infected with PIRV has not previously been reported. Abnormal temperatures, hematology, clinical chemistry, and coagulation parameters associated with PIRV infection in hamsters all rebounded to normal levels in the treated animals. Lower viremia and inhibition of viral replication in select tissues were also observed in treated animals when compared to mock-treated animals.

**Conclusion:** Results from this study demonstrate a BSL-3 arenaviral hemorrhagic fever animal model that can be utilized to test and screen multiple antivirals in a time efficient and cost effective manner. Additionally, the data demonstrate pre-exposure protective efficacy of an antiviral against PIRV-induced hemorrhagic fever in the Syrian golden hamster model.

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**Type: Poster Presentation**

Characterization of a novel neutralizing monoclonal antibody that recognizes the fusion loop of Flavivirus envelope protein

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**Background:** Dengue, West Nile and yellow fever viruses are major human pathogens that belong to the *Flavivirus* genus, and cause large epidemics and deaths worldwide. Given the lack of approved antiviral treatment, recombinant monoclonal antibodies (MAbs) have been verified as candidate for the treatment of flavivirus infections.

**Methods:** A panel of MAbs against dengue 2 virus was produced according to the standard procedure. Indirect immunofluorescence assay and ELISA were performed to identify the cross-reactivity against flaviviruses. *In vitro* and *in vivo* experiments were performed to analyze the neutralizing and protection profiles of a selected MAb against dengue and other flavivirus. Epitope mapping and *in vitro* binding inhibition assays were further carried out to characterize this specific MAb.

**Results:** Plaque reduction neutralization test demonstrated that MAb 2A10G6 was active to neutralize dengue 1-4, yellow fever and West Nile viruses. *In vivo* protection experiments showed that mAb 2A10G6 protected sucking mice from lethal dengue 1-4 viruses challenge in a dose-dependent manner. Thus, we have established a novel flavivirus cross-reactive neutralizing mAb 2A10G6. Phage-displayed random peptide library mapped the epitope of mAb 2A10G6 to a common antigenic site within the highly conserved N-terminal fusion loop peptide of flavivirus envelope protein. Functional assays confirmed that mAb 2A10G6 bind with the fusion peptide and blocks infection primarily at a step after viral attachment.

**Conclusion:** Together, these experiments define the characteristics of a novel flavivirus cross-reactive neutralizing MAb 2A10G6 and make it a suitable candidate for humanization into a therapeutic antibody to treat severe flavivirus infections in human.

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**Session: Arboviruses**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Gene optimization for expression of Crimean-Congo haemorrhagic fever viral nucleoprotein

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<sup>1</sup>University of the Free State, Bloemfontein, Free State, South Africa, <sup>2</sup>UNIVERSITY OF THE FREE STATE, 9324, ZA, South Africa

**Background:** Crimean-Congo haemorrhagic fever (CCHF) virus causes a severe and often fatal infection. The virus has the propensity to cause nosocomial infections and hence a rapid and sensitive diagnosis is important for isolation of the patient for protection of health care workers and implementation of supportive therapy. Current serological diagnostic assays are based on enzyme linked immunosorbent assays (ELISA) or immunofluorescence (IF) tests using inactivated virus which necessitates biosafety level (BSL) 4 facilities for preparation of reagents. The aim of the study was to prepare a safe recombinant nucleoprotein (NP) and determine its suitability for detection of antibody responses in survivors of CCHF infection. To facilitate protein expression the gene encoding the nucleoprotein was optimized for *E.coli* usage.

**Methods:** The CCHF nucleoprotein gene coding sequence was submitted to GenScript (USA). OptimumGene software was used to optimize codon usage, GC content, eliminate polyadenylation sites and modify cis-acting sites. The optimized gene was synthesized and the nucleoprotein expressed in an *E.coli* bacterial expression system with a His tag for purification. CCHF antibody positive human sera were tested in an ELISA using the recombinant NP antigen.

**Results:** A direct ELISA was developed in which plates were coated with the recombinant NP antigen and reacted with convalescent human sera from confirmed CCHF patients. The antigen expressed from the optimized NP gene was found to detect IgG antibodies against CCHF virus.

**Conclusion:** Recombinant proteins have been shown to be safe, cost effective reagents that can be prepared for biohazardous pathogens without the requirements of BSL 4 facilities. Optimization of the CCHF N gene was essential for high expression of soluble NP. The protein expressed was shown to react against antibodies in convalescent CCHF patients. A panel of serum samples from confirmed patients will be used to determine the specificity and sensitivity of the ELISA for diagnostic purposes.

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**Session: Arboviruses**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Preparation of antigenically active recombinant yellow fever viral envelope domain III protein

**S. Smouse**<sup>1</sup>, F. J. BURT<sup>2</sup>

<sup>1</sup>University of the Free State, Bloemfontein, Free State, South Africa, <sup>2</sup>UNIVERSITY OF THE FREE STATE, 9324, ZA, South Africa

**Background:** Yellow fever virus belongs to the genus *Flavivirus*, of the family *Flaviviridae*. It is a mosquito-borne virus endemic in tropical regions of Africa and South America. Although an effective vaccine is available, the virus remains a major public health threat, particularly in Africa where vaccination is limited by poverty, civil wars and the inaccessibility of rural areas prone to outbreaks. It is a re-emerging pathogen with case-fatality rates that can exceed 50%. The diagnosis of infection and testing of the immune status of vaccinees require reagents that are prepared in biosafety level 3 and 4 facilities. The viral envelope protein plays an important role in eliciting antibodies and hence serves as an ideal diagnostic and research tool for the detection of antibodies. The aim of the study was to compare various bacterial expression systems for the preparation of a recombinant yellow fever viral envelope protein for the detection of antibodies against yellow fever in vaccinated individuals.

**Methods:** The domain III region of the envelope (EDIII) gene was amplified with primers identified using sequence data retrieved from GenBank. The EDIII gene was cloned into three different *E.coli* bacterial expression systems for comparison of protein yield, solubility and suitability in an ELISA. Proteins were all expressed with an N+ terminal His tag for purification. A direct ELISA was developed in which the plates were coated with antigen and reacted with serum samples from vaccinees.

**Results:** Each antigen was evaluated using serum samples collected from vaccinees and serological cross reactivity of the antigen against heterologous flaviviral antibody was determined using convalescent serum samples from patients with known flaviviral infections, such as West Nile. Protein yields varied significantly between the different expression systems with higher yields and increased solubility obtained from expression at lower temperatures. Higher reactivity was associated with homologous antibody.

**Conclusion:** Preliminary results suggest that bacterially expressed recombinant EDIII protein is antigenically active and potentially useful for detecting antibodies against yellow fever virus.

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**Session: Arboviruses**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Development of a recombinant antigen and multiplex PCR for differentiation of tick-borne and mosquito-borne flaviviruses.

**L. MATHENGTHENG, F. J. BURT**

UNIVERSITY OF THE FREE STATE, 9324, ZA, South Africa

**Background:** Crimean Congo haemorrhagic fever (CCHF) virus is widely distributed in Africa. Though cases of suspected CCHF infections are routinely investigated, less than 10 % are confirmed. A proportion are usually *Rickettsia* spp. infections but a large number remain undiagnosed. This warrants investigation of other tick-borne pathogens such as flaviviruses. Although the presence of mosquito-borne flaviviruses in southern Africa is known, that of tick-borne flaviviruses was suggested by serological evidence in cattle but not confirmed. *Ixodes* ticks are the known principal vectors of tick-borne flaviviruses and are endemic in southern Africa. The domain III of the *flavivirus* envelope protein was reported to differentiate between the highly cross-reactive tick- and mosquito-borne flaviviruses. Our aim was to prepare a non-cross reactive representative tick-borne recombinant antigen that will differentiate between antibodies against the tick- and mosquito-borne members, and to develop a nested multiplex PCR that can be used for differentiation. Langat virus, a tick-borne flavivirus, was selected as representative for preparation of a recombinant antigen because it can be handled in a BSL 2 laboratory.

**Methods:** The domain III region of the envelope protein (EDIII) of Langat virus was amplified by PCR and cloned into a pQE-80L expression vector. The recombinant protein was expressed in a bacterial expression system, purified in Nickel-charged columns and characterized using Western blot. The protein was evaluated in an ELISA for cross-reactivity with mosquito-borne flaviviruses. For the nested multiplex PCR, primers that amplify conserved regions of flaviviruses and CCHF were identified.

**Results:** The assay was nested to increase sensitivity and detected the viral nucleic acid of 10 known flaviviruses and CCHF virus. A 13kDa EDIII recombinant protein was expressed and purified. The antigen was able to differentiate between antibodies directed against tick- and mosquito-borne flaviviruses and also showed clear cut-offs when tested against known positive antibodies directed against louping ill, tick-borne encephalitis and West Nile viruses.

**Conclusion:** The recombinant antigen is an important tool for the differentiation of flaviviral groups and will be used to screen human and cattle sera for antibody against tick-borne flavivirus. Similarly the multiplex PCR will be a useful tool for screening acute serum samples and ticks.

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**Session: Arboviruses**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

A novel cell encapsulation mode for delivery of therapeutic antibodies against West Nile Virus infections that maintains steady plasma antibody levels throughout therapy

**A. J. S. Chua**<sup>1</sup>, E. M. Brandtner<sup>2</sup>, J. A. Dangerfield<sup>2</sup>, B. Salmons<sup>2</sup>, W. H. Gunzburg<sup>2</sup>, M. L. Ng<sup>1</sup>  
<sup>1</sup>National University of Singapore, Singapore, Singapore, <sup>2</sup>SGaustria, Singapore, Singapore

**Background:** West Nile virus (WNV) is currently endemic in various parts of all five continents in the world. Being a member of the Japanese encephalitis virus subgenus, WNV can cause potentially fatal neuro-invasive diseases such as encephalitis and meningitis. Unfortunately, to date, no vaccine or antiviral therapy has yet been approved. One antiviral strategy in development involves the passive administration of neutralizing antibodies. As with most immunotherapies, plasma antibody levels diminish between treatments. This is especially detrimental in flavivirus immuno-therapies as sub-neutralizing concentrations of antibodies can instead enhance infection. Here, we report a proof-of-concept for a novel mode of delivery using encapsulation of hybridoma cells producing therapeutic antibodies which enables the maintenance of a steady level of antibody in the plasma, thus preventing any possible antibody-dependent enhancement (ADE).

**Methods:** Recombinant domain III of the envelope glycoprotein (rE-DIII) of WNV was cloned, expressed in bacteria, and purified. It was then inoculated into balb/c female mice and splenocytes harvested to generate hybridomas. Limiting dilution was subsequently performed and producers of antibody specific to WNV were selected. Following which, the selected hybridomas were encapsulated in polymers of sodium cellulose sulfate. Viability of the encapsulated hybridoma clones, their ability to continuously secrete antibodies, and most importantly, whether antibodies can be released from the capsules were then characterized.

**Results:** Pilot batches of hybridomas remained viable and divided beyond 100 days post-encapsulation. Moreover, the encapsulated hybridoma cells and their progeny were able to continuously secrete WNV-specific antibodies, and the secreted antibodies could be released from the capsules.

**Conclusion:** Our preliminary results showed that encapsulation of cells producing therapeutic antibodies can indeed be a potential solution to improving treatment outcomes for immunotherapies. This is especially essential for immunotherapies against flavivirus infections so as to minimize any potential ADE side-effect. However, the application of this technology for the treatment of infectious diseases is still at its infancy and we are currently in the midst of further optimizing and characterizing this technology.

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**Type: Poster Presentation**

Association of tumor necrosis factor-alpha gene promoter regions polymorphism in Japanese encephalitis patients

S. K. Pujhari<sup>1</sup>, **R. K. Ratho**<sup>2</sup>, S. Prabhakar<sup>1</sup>, B. Mishra<sup>1</sup>, M. sharma<sup>1</sup>, M. Modi<sup>1</sup>

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**Background:** More than three billion populations are living under the threat of Japanese encephalitis in the south East Asian countries including India. The pathogenesis of this disease is not clearly understood, and is possibly attributed to the genomic variation in virus as well the host genetic make up. The present study focused to determine the role of -238G/A, -857C/T and -863C/A polymorphism of tumor necrosis factor-alpha (TNF-a), gene promoter polymorphism in Japanese encephalitis patients as a part of host role in disease severity and pathogenesis.

**Methods:** Twenty encephalitis cases (PCR/IgM positive) 16 JE IgM positive fever cases with out encephalitis were considered as subject group I and II respectively, and from A 46 healthy individuals were taken as controls. The genomic DNA was extracted from whole blood/clotted blood. TNF-a promoter polymorphism was performed by PCR restriction fragment length polymorphism. The statistical analysis was performed by X2 using GraphPad software.

**Results:** The genotype frequencies of C/C at -863 are 90 % and 93.75% in subject group I and II respectively and 47.83% in controls. The distribution AA in subject group I and II are 10% and 6.25% respectively and 32.61% in control, in fever and in encephalitis patients. Where as distribution of C/A was found only in controls (19.56%). Significantly higher frequency of distribution was observed in JE encephalitis (Subject group I) and fever cases (Subject group II) as compared to that of healthy controls (X2 10.522, p=0.005, X2 10.77, p= < 0.005).

The frequency of C/C at-857 are in the range of 65.22 to 75%, of C/T are 15% to 32.6%, where as the frequency distributions of G/G at -238 position are in the range of 87.5% to 100% and of G/A 6.25% to 12.5% There was no difference observed in the subjects and control groups.

**Conclusion:** With the available TNF-a promoter variations data analysis reveals that position -863promoter likely to play a substantial role in severity of Japanese encephalitis viral infection.

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**Type: Poster Presentation**

A novel antagonistic relationship between human Sec3 exocyst and flavivirus capsid protein

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National University of Singapore, Singapore, Singapore

**Background:** The *Flaviviridae* family comprises several medically important pathogens such as West Nile virus (WNV) and Dengue virus (DENV). Flavivirus capsid (C) protein is a key structural component of virus particles. However, the role of C protein in the pathogenesis of arthropod-borne flaviviruses is poorly understood. Examination of whether flavivirus C protein can associate with cellular proteins and contribute to viral pathogenesis would define the platform towards the development of new anti-virals against flavivirus infection.

**Methods:** Yeast-two-hybrid screening of brain library was employed to identify the host interacting partners of WNV/DENV C protein. Co-immunoprecipitation and mutagenesis studies were used to delineate the interacting domains of host and C proteins. We used a combination of lentivirus-mediated gene knock-down/over-expression analyses, real-time RT-PCR, co-immunoprecipitation, Western blotting, densitometry, pull-down, proteasome activity and competition assays to unveil the biological significance of identified host-C protein association.

**Results:** This study identified human Sec3 exocyst protein (hSec3p) as a novel interacting partner of WNV/ DENV C protein. Mutagenesis studies showed that SH2 domain-binding motif of hSec3p binds to the first 15 amino acids of C protein. We reported that hSec3p can modulate virus production by affecting viral RNA transcription and translation through the sequestration of elongation factor 1 alpha (EF1 $\alpha$ ). We also demonstrated that flavivirus C protein degrades hSec3p through proteasome-mediated pathway.

**Conclusion:** This study highlighted for the first time that hSec3p functions as a new transcriptional and translational repressor of flavivirus replication. Our study also demonstrated that flavivirus C protein plays an important role in nullifying the antiviral checkpoints imposed by hSec3p and allowing the virus to establish a microenvironment that facilitates successful replication and infection. Understanding the molecular mechanism of flavivirus C protein and hSec3p interaction, besides revealing the new cellular functions of hSec3p, could possibly pave the way towards the development of potential hSec3p-derived antiviral agent against flaviviruses.

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**Session: Arboviruses**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Effect of dengue virus infection on the host signal transduction pathways

M. K. Chong, **A. J. S. Chua**, M. L. M. Ng

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**Background:** Dengue virus (DENV) is a single, positive-stranded RNA virus from the family *Flaviviridae*. Dengue infection is a medically important infectious disease affecting many tropical and subtropical countries. To date, vaccine and anti-viral drugs for DENV infection is still not available. This is mainly due to the incomplete understanding of the pathogenesis and the underlying molecular mechanism involved during infection. This project aims to study the virus-host interplay by investigating the signal transduction pathways that are activated during DENV infection.

**Methods:** RT2 Profiler PCR array from SABiosciences was employed to screen for the pathways that were affected during DENV infection on Human Embryonic Kidney (HEK)-293 cells. A panel of 84 genes from 18 biological pathways were analyzed and quantified simultaneously. The HEK293 cells were infected with DENV at multiplicity of infection of 1 and the total RNA were extracted after 12hr, 24hr, and 48hr post infection (p.i.). The RNA of infected cells and mock infected cells were reverse-transcribed into cDNA which were then served as templates for real-time polymerase chain reaction in a 96-well plate containing pre-dispensed gene specific primer sets. The gene expression profiling of DENV-infected cells were compared to that of mock-infected cells.

**Results:** The PCR array data showed that there was no significant change in the gene expression profile of infected cells at 12hr p.i. as compared to mock infected cells. Nevertheless, the number of activated genes increased from 24hr p.i. to 48hr p.i. Among 18 signal transduction pathways, the most affected pathways are NF $\kappa$ B, CREB, Jak-Stat, phospholipase C, insulin and low density lipoprotein pathways. Besides, there are also novel genes identified to be activated in this study. All the activated genes were analyzed and an interactome map was generated. It provides a better understanding of the virus-host interplay during different stages of DENV infection.

**Conclusion:** PCR array allows fast and convenient analysis of many gene expression profiles simultaneously for different samples. Therefore, the comparison of the gene expression profiles of all four different DENV serotypes can be carried out to study the similarities and differences in virus-host interaction.

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**Type: Poster Presentation**

Jumping over the sharp edge of dengue shock syndrome

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**Background:** Dengue is a mosquito-borne viral infection which sometimes can lead to a potentially lethal complication called Dengue Hemorrhagic Fever (DHF) and dengue shock syndrome (DSS). In severe cases, the patient may rapidly go into a critical state of shock and die within 12 to 24 hours. Major pathophysiological hallmark of DHF is an increasing vascular permeability leading to leakage of plasma and hypovolaemic shock. Also the haematological abnormalities, leucopenia, thrombocytopenia, immune complex formation, vasculopathy, thrombopathy myocarditis and Disseminated Intravascular Coagulopathy (DIC) with massive bleeding contribute to shock and fatal outcome. They also can complicate with pericardial and pleural effusions, ascitis and Liver failure.

**Methods:** With the available data of case studies with 11 mortalities and 3 successfully managed cases two phases of fluid derangement were identified in severe DHF. Namely leaking phase and auto transfusion phase which can lead to more complex situations like pulmonary oedema. Both phases were very difficult to diagnose as well as to manage since the situations can complicate with internal bleeding and myocarditis.

Several protocols had been forwarded and the aggressive management according to the protocols is proved to be effective. Though these protocols were not addressing the different phases, a clear identification of each phase was mandatory since each phase has complete opposite management strategies.

**Results:** Evidences were forwarded from retrospective analysis and management of 9 fatalities of DHF, and successfully managed patient with DHF grade IV who was complicated with Hypotension ascitis, bilateral pleural effusion mild pericardial effusion and acute liver failure .

**Conclusion:** These comparison of dilemmas of identifying the phases and complications and maintenance of critical fluid balance will be discussed in this paper . .

This will also prove the importance of early involvement of the intensive care for the management of patients with DHF with severe leakage.

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**Type: Poster Presentation**

Japanese encephalitis virus and neuronal cell interaction : a study on cellular receptor and gene expression profile

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**Background:** Japanese encephalitis virus (JEV) is a mosquito borne flavivirus responsible for acute encephalitis in humans. Very little information is available on the cellular receptor for JEV as well as changes in host gene expression following JEV infection in the CNS.

Consequently, the present study was undertaken to (i) identify the cellular proteins involved in JEV entry and (ii) to study JEV mediated alteration in cellular gene expression.

**Methods:** A 'Virus Overlay Protein Blot Assay (VOPBA) was used to identify cell membrane protein on mouse neuroblastoma cells (Neuro2a) interacting with JEV. The identity of the interacting protein was established using MALDI TOF. A series of experiments including 'infection inhibition assay', and flowcytometric analysis further confirmed the identity of the protein.

Additionally, using the bioinformatic tool - FTDOCK, protein-protein interaction was studied.

Total RNA extracted from JEV infected Neuro2a cells was subjected to microarray analysis to study alteration in gene expression profiles. Standard assays for apoptosis (TUNEL and Real-time PCR) were used to validate the microarray results.

**Results:** Heat shock protein 70 (Hsp70) was identified as the receptor for JEV on Neuro2a cells based on the following observations (i) surface expression of Hsp70 on Neuro2a cells (ii) reduction in virus infectivity using anti-Hsp70 antibodies, co-immunoprecipitation results demonstrating JEV -Hsp70 interaction and delineating the residues in the interacting pockets using bioinformatic tools.

Microarray analysis revealed upregulation of 660 genes and downregulation of 949 genes in JEV infected Neuro2a cells. A large number of differentially expressed genes were found to be involved in apoptosis, oncogenesis, cellular metabolism, neurodegeneration and immunological functions. Upregulation of pro-apoptotic genes (p53, VEGF, Gadd45) and downregulation of anti-apoptotic gene (bcl-2) were observed. TUNEL assay and DNA fragmentation followed by conventional and real time PCR further confirmed apoptosis in JEV infected Neuro2a cells.

**Conclusion:** In conclusion, this study for the first time identified Hsp70 as the receptor for JEV on Neuro2a cells. Further, this study illustrated that apoptosis is one of the mechanisms of JEV induced damage of infected neuronal cells.

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**Type: Poster Presentation**

Chikungunya virus (CHIKV) infection: Analytical performance of real-time NASBA assay

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**Background:** Chikungunya virus (CHIKV), an alphavirus belonging to the Togaviridae family, is transmitted to human by several species of mosquitoes, with *Aedes Aegypti* and *A. Albopictus* being the two main vectors. The virus is endemic in Africa, India, South-East Asia and recently in southern-Europe and is responsible for an acute infection of abrupt onset characterized by high fever, asthenia, headache, rash, myalgia and a painful polyarthralgia. Occurrence of CHIKV asymptomatic infections, whose epidemiological consistency is still to be assessed, leaves hypothesize spread of infection by blood transfusion or by tissue or organ transplantation and highlights the need for highly sensitive CHIKV-specific tests. Objective of this study is to analyze the analytical performance of real-time nucleic acid sequence-based amplification (RT-NASBA).

**Methods:** The analytical sensitivity of the assay was validated with a panel of blood donor plasma samples spiked with 10-fold serial dilutions of CHIKV, previously quantified by TCID50 assay, with viral titers ranging from 10<sup>5</sup> to 1 TCID50/mL. 10 replicates for each viral concentration were analyzed. Following RNA extraction, all samples were amplified by RT-NASBA and for each virus concentration, the detection rate (n.positives/n.total) was evaluated. Finally the analytical sensitivity of the NASBA assay has been compared with real- time PCR based methods (RT-PCR).

**Results:** RT-NASBA assay has an amplification rate of 100% for plasma samples spiked with CHIKV titers >1 TCID50/mL. RT-NASBA has higher analytical sensibility than RT-PCR, which in turn has an amplification rate of 100% for viral concentrations > 20 TCID50/mL.

**Conclusion:** The results of this study document a good analytical sensitivity of the RT-NASBA assay, even higher if compared with RT-PCR methods. Thus, this assay can be used as routine laboratory test for diagnosis of CHIKV infection in plasma samples. Furthermore, the high sensibility of the test and shortness of turnaround time to obtain the results, make this assay suitable for routine screening of blood donations and organ transplantations.

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Wild yellow fever cases in Sao Paulo state, Brazil, 2009

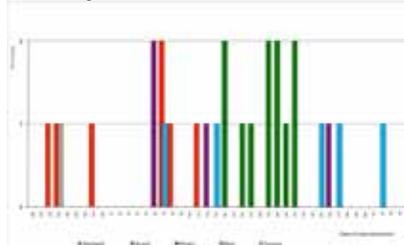
**M. Mascheretti**<sup>1</sup>, A. Ribeiro<sup>1</sup>, C. Tengan<sup>1</sup>, H. K. Sato<sup>1</sup>, P. Opromolla<sup>1</sup>, A. Suzuki<sup>2</sup>, R. Brasil<sup>2</sup>, C. Fortaleza<sup>3</sup>, F. Chudk<sup>4</sup>, M. S. Carl<sup>4</sup>, R. Albernaz<sup>1</sup>, R. Souza<sup>2</sup>

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**Background:** Yellow fever (YF) is an arboviral disease caused by a virus from *Flaviviridae* family and genus *Flavivirus* endemic in tropical regions of America and Africa. Transmission occurs after mosquito bite, *Aedes* and *Haemogogus*. Urban YF was eradicated in Brazil in 1942, sporadic wild transmission has been maintained in Amazon area. Two laboratory-confirmed cases were reported in 2000 and two cases in 2008 in Sao Paulo state suggesting a re-emergence after 50 years.

**Methods:** Descriptive study of YF cases in Sao Paulo state, Brazil in 2009.

**Results:** From February to April 28 confirmed cases of YF were reported including 11 deaths, case fatality rate 39,2%. 18 were male (64,3%), the mean age was 29 years old (range 8 days to 52 years old). Four cases occurred in children under 16 years old (8 and 12 days; 14 and 15 years old). Newborns mother's YF onset symptoms started two to five days before delivery suggesting perinatal transmission. Symptoms varied from mild to severe disease, 75% (21/28) of the cases were hospitalized. Most common symptoms were fever (25/26), headache (15/19), jaundice (5/20), abdominal pain (16/25), vomit (9/18) and hemorrhage (12/27). Aspartate aminotransferase mean was 4,772UL (range 32–28,900UL; reference value 40,00UL), missing data in 6 cases. Direct bilirubine mean was 2,82mg/dL (range 0,20–21,50mg/dL; reference value 1,5mg/dL), missing data in 7 cases. Four cases had renal failure. All cases were laboratory-confirmed: YF IgM antigen-capture ELISA (24/26), blood or tissue virus isolation (5/15), blood or tissue RT-PCR (14/16), immunohistochemistry (5/13). Human transmission was associated with leisure and work activities in rural areas of Sarutaia, Piraju, Buri, Avare and Tejupa municipalities. All cases occurred among unvaccinated person. Mass vaccination campaigns were implemented in 50 cities with more than 1 million doses (vaccination coverage was 87%). Five fatal cases of YF vaccine-associated viscerotropic disease were reported during February to October 2009. Other epidemiological control measures were adopted including entomologic assessments and monkey deaths surveillance investigation.



**Conclusion:** The early identification of YF transmission re-emergence in Sao Paulo state was important to establish effective public health intervention to prevent and control future outbreaks.

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Clinical significance of polymicrobial bacteremia as determined by the pattern of gram stain  
**M. NORIZUKI**, S. YAMAMOTO, N. HOSOKAWA  
Kameda Medical Center, Kamogawa, Japan

**Background:** Polymicrobial bacteremia has been associated in higher mortality in past studies. The objective of this study was to investigate etiology, source of infection and mortality.

**Methods:** We retrospectively reviewed microbiological result of blood cultures in 865-bed tertiary hospital in Japan during the past 22-months ends 31 September 2009. In-hospital mortality, 28-day mortality, follow up period, site of infection, past medical history, gram stain result of blood culture (gram positive cluster, gram positive chain, enterobacteriaceae like gram negative rod, non-fermenting gram negative rod (e.g. *P.aeruginosa*), gram positive rod and fungi) and organisms were collected.

**Results:** Ten-thousand eight hundred thirty-eight blood cultures were taken and 16.3 % (n=1,771) were positive. Seventeen cases of polymicrobial bacteremia were excluded because of contamination. There were fifty-one (2.9 % of positive blood culture) episodes of polymicrobial bacteremia. Four patients were excluded because of admission at the end of investigation. Sixty percent of patients were male and the average age was 72.7 years. Although over all hospital mortality was 42.3%(n=20), 28-day mortality was 21%(n=10). The three leading sources of infection were biliary tract infection (49%), urinary tract infection (15%) and neutropenic fever (11%). There were 24 cases of hospital acquired infection and 23 cases of community acquired infection. Hospital acquired polymicrobial bacteremia had higher mortality than community acquired (63% vs.22%). *Enterococcus* species (18%), *E.Coli* (17%) and *Klebsiella* (15%) were common pathogens cultured. The hospital mortality was different in combination of organisms. Three or four organisms were higher mortality than two organisms (57% vs. 36%). The combination of gram positive coccus and non-fermenting gram negative rod were higher mortality than gram positive coccus and enterobacteriaceae like gram negative rod (57% vs.27%).

**Conclusion:** We conclude that polymicrobial bacteremia is an important entity especially in hospital acquired setting. We were able to predict mortality, prognosis or determination of contamination from the gram stain result of blood culture.

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**Type: Poster Presentation**

Infective endocarditis after dental surgery

**V. Krcmery**<sup>1</sup>, A. Demitrovicova<sup>1</sup>, E. Kalavsky<sup>1</sup>, P. Kisac<sup>1</sup>, M. Karvaj<sup>2</sup>, F. Bauer<sup>2</sup>, R. Kovac<sup>2</sup>, P. Milkvy<sup>2</sup>

<sup>1</sup>St. Elizabeth University College of Health and Social Sciences, Bratislava, Slovakia, <sup>2</sup>Slovak Medical University, Bratislava, Slovakia

**Background:** Dental surgery is one of the major risk factor for occurrence of infective endocarditis, mainly in patients with pre-existing cardiac disorders (prior cardiac surgery, rheumatic heart diseases, prosthetic valve, congenital vitium cordis, previous endocarditis etc.).

**Methods:** Within 606 cases in our national database, 52 (8.6%) patients with infective endocarditis occurred within 7 days after dental surgery (teeth extraction 40 – 76.9%, periodontal abscess 3 – 5.8%, caries debridement 3 - 5.8% and other dental procedures 6 – 11.5%). Ten (19.2%) of them were breakthrough – occurred despite of antibiotic prophylaxis with amoxicillin (3 – 5.8%) roxithromycin (6 – 11.5%) or clindamycin (1 – 1.9%).

**Results:** Comparing risk factors of 52 patients with infective endocarditis after dental surgery and entire group of 606 patients, neoplasia (9.2% vs. 0%;  $p<0.04$ ), prosthetic valve (19.9% vs. 3.8%;  $p<0.05$ ), hearth failure (15.5% vs. 1.9%;  $p<0.02$ ) and patients cured only with antibiotics (57.4% vs. 42.3%;  $p<0.04$ ) were less frequently among patients with endocarditis after dental surgery. Vice – versa vitium cordis congenital heart (3.3% vs. 15.4%;  $p<0.01$ ), right side (2.5% vs. 9.6%;  $p<0.01$ ) and patients cured with antibiotics and surgery (42.6% vs. 57.7%;  $p<0.04$ ) were more frequently observed among infective endocarditis after dental surgery. Mortality was insignificantly lower (15% vs 9.6%; NS) in patients with infective endocarditis after dental surgery.

**Conclusion:** Surprisingly, viridans *streptococci* (5.8 %), and previous cardiac surgery (5.8 %) were not significantly associated with infective endocarditis after dental surgery as expected. Viridans *streptococci*, *Staphylococcus aureus* and HACEK group were replaced by gram-negative bacteria ( $P<0.004$ ) (*Escherichia coli*, *Enterobacter* spp., *Pseudomonas aeuginosa*) in ethiology of infective endocarditis after dental surgery.

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**Type: Poster Presentation**

Efficacy of serum semi-quantitative procalcitonin measurement kit PCT-Q for bacteremia  
**T. Kodama**, H. Wakatake, M. Yanai, S. Fujitani  
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**Background:** Serum procalcitonin (PCT) concentration has been used as a specific biomarker for diagnosis and severity of the bacterial infections. Although data of quantitative PCT concentration for bacterial infections have been accumulated, clinical implications for semi-quantitative PCT concentration have not been well defined. Thus, we report the clinical utility of PCT-Q especially for cases with bacteremia.

**Methods:** PCT-Q concentration was measured in those who were suspected bacterial infections among all patients who were evaluated in our Emergency Department from September 2007 to March 2008. PCT-Q concentration was divided into four classes (<0.5, >=0.5, >=2.0, >=10.0ng/mL) and above 0.5ng/mL that was the cut-off value were defined as bacterial infections. We compared the results of PCT-Q with quantitative PCT concentration, white blood cells (WBC) and C-reactive protein (CRP). The results of blood culture of all recruited patients were collected and the results of PCT-Q positive for bacteremia were also analyzed. Furthermore we compared the rates of detection of Methicillin-resistant Staphylococcus aureus (MRSA) in this period with that of previous one year.

**Results:** A total of 291 patients among all 16,700 patients were evaluated. The concentration between PCT-Q and quantitative PCT were almost concordant. Discordance between PCT-Q concentration and WBC was observed, while significant correlation between PCT-Q concentration and CRP concentration was obtained. The sensitivity and specificity of PCT-Q among patients with bacteremia was 72.1% and 64.4%, respectively. The rate of detection of MRSA fell from 6.5% (50/766) to 3.8% (29/773).

**Conclusion:** PCT-Q is useful for diagnosing severe bacterial infections including bacteremia. PCT-Q could be useful to restrain from the emergence of bacterial resistant strains by decreasing unnecessary antimicrobial usage.

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Salivary IgA responses in newborn against pathogen of oral cavity. Influence of prematurity in this response

**R. D. Nogueira**, M. L. T. Sesso, M. C. L. Borges, L. R. Roberti, V. P. L. Ferriani  
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**Background:** Analysis of the mucosal immune system represents an interesting way to understand the microbial colonization in early life, particularly the response of SIgA (Secretory IgA) present in saliva because it represents the first line of defense. Previous study showed in children with 6 months of age, high complexity of SIgA response to antigens of *Streptococcus mutans* (MS), main pathogen of the dental caries, but little is known about the ontogeny of the mucosal immune system in the first day of life especially in preterm newborn (below 37 weeks of gestation). Thus, we compared the levels and specificity of SIgA to MS and others species enrolled with initial infection in fullterm (FT) and preterm (PT) early in life.

**Methods:** Stimulate saliva from 160 children, with 0 day of life, were enrolled in this study. Salivary IgA and IgM levels were determined by ELISA. Subsets of 24 fullterm (FT) and 24 Preterm (PT) children showing similar salivary IgA levels were paired and matched for gender, racial background, breastfeeding. SIgA antibody reactivity to MS, *Streptococcus sanguinis* (SSA), *Streptococcus mitis* (SMI) and *Streptococcus gordonii* (SGO) Ags was determined in Western blot assays.

**Results:** Levels of SIgA were statistically different (Whitney test,  $p < 0.05$ ) between groups and in FT were 2.5 times higher than PT children. Fifty and 37.5% of PT and FT respectively not show any response to antigens of the microorganisms tested. Significant diversity was observed in IgA antibody response patterns to Ags. The number and intensity of reactive bands was higher in FT than PT children for all antigens tested. Some antigens were more frequently detected in salivas, such as: 165KDa of SGO, 172 KDa of SSA, 202 KDa of SMI, 185 and 160 KDa of MS. Responses to 165KDa of SGO were unique among those antigens that presented different in their pattern of recognition between FT and PT (Chi-square,  $p < 0.02$ ).

**Conclusion:** The data indicate that salivary IgA responses to Ags can occur in the first day of life and children PT show a diminished response of SIgA to SSA, SMI, SM and SGO Ags which may be due to lower concentrations of IgA (FAPESP:07/57346-5; 07/50807-7)

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Infectious complications of venomous snakebite: 2 cases from Eastern Nepal  
**S. K. Sharma**, S. Shrestha, B. Badhu, C. S. Agrawal, B. Khanal  
BP Koirala Institute of Health Sciences, Dharan, Nepal

**Background:** Venomous snakebite is a common and deadly disease throughout the tropics, but there are few reports of infections associated with snake envenomation in Asia. We describe two patients with distinct infectious syndromes following venomous snakebites in Eastern Nepal.  
**Methods:** Two case reports.

**Results: Case 1: Endophthalmitis and necrotizing fasciitis.** A 21 year old female victim of snake bite (common cobra) to the foot presented within 30 minutes and received 24 ampoules of antivenom (Bharat Serum, India) over 2 days. After 18 hrs she developed dusky, ascending wound discoloration associated with (pain, blisters, fevers, and leukocytosis). Necrotizing fasciitis was diagnosed and treated with antibiotics and surgical debridement. On hospital day 2nd, the patient developed right eye pain and blindness. Analysis of vitreous humor aspirate revealed gram negative bacilli, and intravenous and ocular antibiotics were administered; aspirate cultures revealed growth of *Serratia marcescens*. The patient was discharged after 21 days, with moderate foot dysfunction and total eye blindness.

**Case 2: Local soft tissue infection with Enterococcus.** A 62 years old alcoholic male presented within 1 hour after snakebite to the right hand from an unknown species. He had applied two tourniquets (to right forearm and arm) that were loosened in the emergency ward. He developed mild local swelling and neurological symptoms (ptosis, ophthalmoplegia) within two hours of the bite, and received 200 ml of antivenom (Bharat serum, India) over 2 hours. (Although the neurologic symptoms resolved) the patient subsequently developed fevers and the hand swelling increased over 2 days. The wound developed gangrene and purulence over requiring surgical debridement. Wound cultures grew *Enterococcus* species, and he received gentamicin, metronidazole, and vanomycin. He was discharged on day 18th with moderate loss of hand functions.

**Conclusion:** Snakebite-associated infection in the tropics can manifest variously, with local, regional and remote effects on injured tissues. More studies are warranted for this important complication of envenomation.

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Clinicomicrobiological profile of infective endocarditis in a tertiary care centre of Nepal

**M. Ghimire**

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**Background:** Infective endocarditis is a common problem and data regarding its clinical and microbiological pattern from developing countries are sparse. We studied clinical features and the microbiological pathogens in patients with Infective Endocarditis.

**Methods:** A hospital based, cross sectional descriptive study was carried out over a period of 1 year.

**Results:** A total of 54 patients with history of fever and underlying heart disease were evaluated for IE. Out of them 11 patients (20.4%) had Dukes definite IE. Fever was present in 100% cases (n=11) as it was the inclusion criteria of the study, followed by SOB 81.8% (n=9), anorexia 81.8% (n=9), malaise 63.6% (n=7), cough 54.4% (n=6), palpitation 45.5% (n=5), swelling of body 45.5% (n=5), myalgia 36.4% (n=4). Past history of RHD was present in 18.2% (n=2) and only 18.2% (n=2) of patients were in penicillin prophylaxis. No patients had undergone any dental procedure in last 2 weeks prior to the presentation. One patient (9.1%) was intravenous drug abuser and 36.7 % (n=4) of patients were smoker. History of antibiotic therapy prior to the presentation was present in 36.7 % (n=4) patients. None of the patients had a history of prior IE. Pallor was the most common sign 63.6% (n=7), followed by edema 54.5% (n=6), icterus 36.4% (n=4). Embolic events seen in 18.2% (n=2). Dental caries and focal neurological deficit were seen in 9.1 % (n=1) each. Peripheral signs in IE were not seen in any of the cases. Petechial hemorrhage in 18.2% (n=2) patients. Anemia (Hb<10gm %) in 36.4% (n=4) cases, leucocytosis and microscopic haematuria in 72.3% (n=8) and 27.3% (n=3), respectively. Blood culture positivity was seen in 36.4 % .The most common pathogens were *Staphylococcus aureus* in 27.3% (n=3), and *Acinetobacter species* in 9.1% (n=1).

**Conclusion:** Clinical spectrum of IE in our study was different from the west. Majority of patients being young, RHD still being the common underlying heart disease. However microbiological pattern was similar to western studies, i.e. *staphylococcus aureus* being the commonest isolate. So, we need a large study to know the real epidemiological, microbiological pattern of IE in our country.

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The relation of the contraction period and the bacteria of the otorrhea of chronic otitis media  
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**Background:** Chronic otitis media [ COM ] is an infectious disease to need treatment of the antibiotics many times for a long time. It is often that COM suffers from treatment because the appearance frequency of resistant bacteria or fungus increases. Aim of our study is to clarify cause bacteria of the otorrhea of COM and what kind of patient resistant bacteria increase with.

**Methods:** 103 patients aged 2 years to 79 years [median age 53.2 years ] who complained otorrhea with COM were enrolled in a study at the Tokyo Metropolitan Police Hospital during September from 2007 to 2009. Diagnosis was made by otolaryngologists, the otorrhea was collected. The perforation of ear drum to last for more than three months was defined as COM. Antibiotic treatment within one month was excluded. The relation of the contraction period and numbers , kind, sensitivity of bacteria was examined.

**Results:** The patients who had illness under 1 year were 47 people, for 1 -20 years were 29, for more than 20 years were 27. In total , 85 of 103 patients had positive culture. Staphylococcus aureus was found in 37[ 43 %]. Pseudomonas aeruginosa was found in 23 [ 27 %]. Candida or Aspergillus was found in 11 [13%]. Streptococcus pneumonia was found in 2. In the 27 patients who had illness for more than 20 years, Pseudomonas aeruginosa was found in 10, Candida or Aspergillus was found in 9, MRSA was found in 2, and Serratia marcescens was found in 2.

**Conclusion:** In this study, the cause bacteria of otorrhea of the COM found that obviously it was different from that of the acute otitis media. Specially, it found that the bacteria in such case as the Pseudomonas aeruginosa and MRSA that it had a difficulty in the treatment increased as much as the year which COM took increased, and fungus which treatment by the antibiotic was invalid to had identification given, too.

We have to do treatment by the antibiotic carefully until cause bacteria have identification given by a bacteriological examination about the otorrhea of the COM.

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Bone and joint infections in elderly and young adults patients: Comparison of clinical features and outcomes (1991-2007)

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**Background:** The effect of age on the presentation and outcome of BJI is unclear. Available data is mostly based on the analyses of mixed populations.

The objective was to evaluate the clinical and microbiological features and outcomes of BJI in young adults vs. older patients.

**Methods:** We carried out a retrospective cohort study comparing 341 episodes (ep) of BJI in elderly pts (>65 yo) vs. 336 ep in a younger (>16 yo and <50 yo).

**Results:** From 1/91 to 11/07 we registered 908 ep: 341 (37.5%) were >65 yo (47.2% male; mean age 73.5, r:65-103) and 336 (37%) <50 yo (72.1% male; mean age 32.4, r: 16-50). Mean follow-up was 36.7 months (r: 1-168).

Comparison between elder vs. younger patients ep.: a) Type of infection: osteomyelitis (OM) 25.5% vs. 36.6% (p<0,001), septic arthritis (SA) 2.9% vs. 11.9% (p=0.0018), implant-associated infections (IAI) 64.8% vs. 43.2% (p<0,001); b) Location: upper extremities 10.1% vs. 14.6% (p=0.037), lower extremities 75.6% vs. 69.6% (p=0.079), spine 4.7% vs. 9.5% (p=0.014), sternum OM 7.3% vs. 0.6% (p<0.001); c) Source of infection: hematogenous, contiguity and post-trauma (p=NS), post-surgical 70.2% vs. 59% (p = 0.002); d) Comorbidities 75% vs. 19% (p<0.001); c) clinical picture: pain and phlogosis (p=NS), fever 16.4% vs. 36% (p<0,001), fistula 29% vs. 37.2% (p=0.023), purulent discharge 34% vs. 41.4% (p=0.048), sed. rate > 50 mm/h 41.6% vs. 28.6% (p <0.001); e) Microbiology: grampositive cocci (p=NS), gramnegative bacilli 16.4% vs. 8.6% (p=0.0022), polymicrobial 19.1% vs. 13.1% (p<0,001), negative cultures 5% vs. 1.9% (p = 0.0012); f) Surgical treatment: 75.4% vs. 63.7% (p=0.0057); f) Outcome: cure/improvement 79.2% vs. 83% (p=NS), failure 5.3% vs. 2.4% (p = 0.049), relapse 12.6% vs. 10.4% (p=NS).

**Conclusion:** In our experience, BJI affect most often upper extremities (OM and SA) and spine (OM) in male young adults. Post surgical IAI in lower extremities and sternum show higher compromise in older female and the clinical picture is often more insidious than in young adults. Normal sed. rate does not exclude the presence of BJI in both groups. Gramnegative bacilli and polymicrobial infections are more common in elderly. Surgical treatment is implemented most frequently in the elderly; however due to a more conservative approach is usually implemented in IAI, this could partially explain the higher failure rates found in the elderly as compared with young adults.

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Prosthetic joint infections: A multidisciplinary approach (1992-2008)

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**Background:** The management of prosthetic joint infections (PJI) requires combined medical treatment and surgery. The one or two-stage exchange arthroplasty (EA) or resection arthroplasty (RA) are the conventional surgical approach suggested.

The objective was to analyze the clinical and microbiological features and the outcome of PJI treated with the conventional surgery approach.

**Methods:** We performed a retrospective cohort study of PJI episodes (ep.) treated with EA or RA. The medical and surgical approach were not previously standardized, but individualized according to the patient and the treating team. Statistical analysis: we used the Stata 10, applying the Chi 2 test. Difference was considered statistically significant at  $p \leq 0.05$ .

**Results:** From 01/92 to 05/08, 309/1026 ep. (30.1%) in our database were PJI; 129/309 ep. (41.7%) were treated with EA or RA. Mean age 68,2 ± 11,4; 58.1% female.

Late PJI 75.6%, 21.3% early, 3.1% IO.

Location: hip 61.7%, knee 32.8%, shoulder 3.9%, elbow 1.6%.

Source of infection: postoperative 93.6%.

Clinical features: pain 72.1%, flogosis 33.3%, sinus tract 22.5%, purulent discharge 21.7%, fever 14.0%. Sed rate >50 mm/h: 66.3%.

Microbiology: 57.6% grampositive cocci (*S. aureus* 35.2%, CoNS 13.6%, other 9.2%), 10.4% gramnegative bacilli (*P. aeruginosa* 8% *Enterobacteriaceae* 4%); polymicrobial 24%, fungi 1.6%, anaerobic bacteria 0.8%, culture negative 5.6%.

Antibiotic therapy: TI: 20 weeks (median; IR 12-26); oral 50%, switch to oral therapy 37.5%, parenteral only 12.5%. TS in 28,9% ep; median 24 weeks (IR 11-37).

Surgical treatment: two-stages EA 50.4%, one-stage EA 13.9%, AR 35.7%.

Follow up: 27 months (median; IR 16-50).

Outcome: cure/ improvement: 109 (87.2%), relapse 9 (7.2%), failure 3 (2.4%), not evaluable (3.2%).

In the univariate analysis, only PJI treated with two-stages EA had a better outcome ( $p = 0.04$ ).

**Conclusion:** The management of PJI should be individualized according to the patient characteristics and evaluated with a team (orthopedic surgeons and infectologist). This is the key in determining the functional status of the prosthesis, in order to define the surgery strategy. Two-stages EA is the method that achieves better control of infection. There were no significant differences in the outcome with the others variables studied.

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Cerebrospinal meningitis outbreak in Kano state, Nigeria

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**Background:** *Neisseria meningitidis* (Nm) is the leading cause of epidemic Cerebrospinal meningitis (CSM). Kano State in northern Nigeria is located within the 'African meningitic belt' and has reported frequent CSM outbreaks in the past. In March 2009, a suspected outbreak of CSM was reported from Kano. We conducted an investigation to describe the magnitude of the outbreak and institute public health control measures.

**Methods:** We reviewed patient hospital records, interviewed healthcare workers and hospitalized cases. A total of 60 hospitalized cases chosen systematically in one of the reporting Local Government Areas (LGAs) were interviewed with a structured questionnaire. We collected Cerebrospinal fluid (CSF) specimens from suspected cases from the first 15-20 suspected cases from each LGA. A suspected case of CSM was defined as any person aged two years and above with sudden onset of fever (>38°C axillary) and any one of these signs: neck stiffness, altered consciousness, and other meningeal signs. A confirmed case was defined as a suspected case with laboratory confirmation. We analyzed data with Epi info version 3.3.2 and Microsoft excel office 2003.

**Results:** A total of 1036 suspected cases were reported with 30 deaths, a case fatality rate of 2.9%. The overall attack rate was 10.0 per 100,000 persons but varied over the weeks and by LGA. Majority of the cases were aged 2-15 years {812 (78.4%)}. The age-specific attack rate for age group 2-15 years was 19.6 per 100,000 persons. Of 60 cases interviewed, only 3 (5.0%) had received CSM vaccination within previous 3 years. Out of 124 CSF samples analyzed, 69 (55.6%) tested positive for Nm serogroup 'A' (Nm A). The recommended drugs for case management in meningitis outbreaks – oily chloramphenicol and ceftriaxone were not available.

**Conclusion:** Nm A was the cause of the CSM outbreak that affected mostly age group 2-15 years in Kano. The authorities should conduct a reactive vaccination campaign targeting this age group and procure adequate drugs for effective case management.

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A comparison of infective endocarditis related Sequelae incidence among population- based, multicenter, and hospital-based cohorts: Data from a systematic review for the global burden of disease project

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**Background:** Referral bias has been shown to influence infective endocarditis (IE) epidemiology in a previous study comparing IE patients from Olmsted county, Minnesota and those referred to Mayo clinic from other hospitals. Due to scarce data from population based studies in many countries, less representative IE patient populations may be needed to estimate the burden of this disease. We aimed to compare the incidence of IE related sequelae among published population- based, multicenter, and hospital-based cohorts.

**Methods:** We conducted a systematic review of published studies, surveys, and other data sources in order to assess the global epidemiology of IE and related disabling sequelae. A simplified model was used and it included cure, valve surgery, stroke, and death. Electronic databases searched included MIDLINE, EMBASE, LILACS, koreaMED, AMED, EXTRAMED, scopus and web of science. To estimate IE sequela and mortality, data from population based studies were used preferentially whenever available for a given country followed by multicenter cohorts and then hospital series. We compare the incidence of IE related sequelae among published population- based, multicenter, and hospital based cohorts.

**Results:** Data were from 40 countries and 2 international collaborations. we identified 121 cohorts, 21 were population-based, 21 were multicenter, and 79 were hospital based. The incidence on IE related sequelae did not differ significantly among the different cohorts ( table).

Population based Studies	Valve replacement (%)	Stroke (%)	Mortality (%)
Mean(Standard Deviation)	25(13)	7(13)	25(10)
Median (Range)	21(44)	15(32)	21(35)
Multicenter Cohorts			
Mean(Standard Deviation)	38(16)	14(19)	20(8.8)
Median(Range)	43(47)	15(29)	18(37)
Hospital Cohorts			
Mean(Standard Deviation)	28(16)	16(21)	23(25)
Median(Range)	26(63)	12(42)	23(57)

**Conclusion:** In this systematic review of IE studies, the incidence of IE related sequelae did not differ significantly among different types of sampled populations. Due to the paucity of population-based data from many countries, hospital based series could be used as an alternative source to estimate the global burden of IE.

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The impact of a microbial sealant to reduce surgical site infection reduction in cardiac surgery

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**Background:** Surgical site infection (SSI) do have a serious impact on patients undergoing cardiac surgery. This study was performed to prove if additional preoperative care by using a microbial sealant can reduce surgical site infection.

**Methods:** To improve preoperative surgical care two groups of patients were identified during the same period of time. Between January and August 2007, a control group (n=718), receiving standard institutional preoperative preparation and between January and August 2008, the InteguSeal group (n=780) who received additionally a microbial sealant prior to sternotomy. Both groups were evaluated by patients characteristics and a pre-operative risk scores. End-point of this study was freedom from SSI.

**Results:** Follow up was 100% completed. The values of the pre-operative risk score of the control group and the InteguSeal group was significantly different in both groups, respectively  $9.7 \pm 1.5$  and  $10.1 \pm 1.8$  ( $p=0.001$ ).

The clinical end-point, however showed a highly significant decrease of SSI in the InteguSeal group 1.4% (n=11) compared with the control group 4.3% (n=31), ( $p<0.003$ ) although they were at higher risk for SSI.

**Conclusion:** Thus, the pre-operative risk score for patients of the InteguSeal group was significantly higher compared to the control group, there was a highly significant reduction of surgical site infections seen.

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The global burden of infective endocarditis: Methodology for a systematic review to assess disease burden and trends in 21 world regions for 1990-2005

**I. M. TLEYJEH**<sup>1</sup>, M. George<sup>2</sup>, A. Bin Abdulhak<sup>3</sup>, E. Patricia<sup>4</sup>, V. Chu<sup>5</sup>, M. Ezzati<sup>6</sup>, B. Hoen<sup>7</sup>, L. M. BADDOUR<sup>8</sup>

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**Background:** Infective endocarditis(IE) is an important contributor to mortality and morbidity worldwide. However, previous work in the global burden of diseases, injuries, and risk factors(GBD) has not included specific assessment of the burden of IE and its sequelae. We aimed to describe the methodology for a comprehensive assessment of IE burden trends in 21 world regions for 1990-2005 as part of the current GBD 2005 effort.

**Methods:** A systematic review of published studies, surveys, and other data sources is being conducted in order to assess the global epidemiology of IE and related disabling sequelae. Cases of IE were defined according to the Duke, modified Duke, Steckelberg, Von Reyn and modified Von Reyn. A simplified model was used for the systematic review and it included cure, valve surgery, stroke, and death. Electronic databases included MEDLINE, EMBASE, LILACK, KoreaMED, AMED, EXTRAMED, scopus and web of science. Only population based studies were used to estimate the incidence.

**Results:** We identified 121 studies: 21 population-based, 21 multicenter studies, and 79 hospital cohorts. Data originated from 40 countries and 2 international collaborations. Only 18 population based studies reported on the incidence of IE/100,000 in 9 countries. Australia(3), France (2.2,3), Denmark(2.4,2.7), Italy (3.6), Netherlands(1.5,9.6), Sweden(0.39,6.2), Tunisia(5.5), UK(1.6,2.3), USA(1.7,3.8,4.2,4.95,11.6). Valve replacement was performed on 30%(mean) and 29%(median) of IE cases. Stroke occurred in 15%(mean) and 14%(median). Mortality occurred in 23%(mean) and 21%(median) of cases.

Bias secondary to incomplete data, non representative populations, and missing data for national or regional populations remain important challenge. Specific strategies to address this limitation are ongoing and include (1) translating non-English studies (2) searching the gray literature; and (3) contacting IE experts in world regions with limited or no data.

**Conclusion:** A comprehensive and systematic assessment of the global burden and trends in IE mortality and disability using a rigorous methodology is being conducted. IE is a relatively uncommon disease but is associated with significant morbidity and mortality. Completion of this effort will add substantively to the summary estimates of cardiovascular mortality and disability.

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Psoas abscess in Korea: Etiology, clinical features, treatment and outcome  
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**Background:** Abscess of the psoas muscle is a rare disease. Because of nonspecific presentation, it is difficult to diagnosis. There is a wide spectrum in etiology, time to diagnosis, and therapeutic options. This study is to describe the etiology, clinical features, treatment, and outcome of psoas abscess from multicenter in Korea.

**Methods:** We retrospectively reviewed the medical records of patients with psoas muscle abscess who were admitted to three university hospitals in Korea over 10 years.

**Results:** A total of 87 patients (46 male, 41 female) were included in this study. The mean age was  $56.3 \pm 18.5$  years old. The mean duration of hospitalization was  $31.9 \pm 24.37$  days, time from admission to diagnosis was  $13.6 \pm 82.9$  days. Fourteen cases as "secondary" abscess, and pyelonephritis (7/14, 50%) were most frequent etiology. Positive blood culture were obtained in 25% (27/81) and the causative pathogens were *S. aureus* (17/27, 62.8%), *E.coli* (4/27, 14.8%), *Coagulase negative staphylococci* (3/27, 11.1%), *K. pneumoniae* (2/27, 7.4%) and *Candida* species (1/27, 3.7%). Among 49 patients who were cultivated from abscess material, 41 microorganisms were grown in 34 patients (35/49, 71.4 %) and the most frequent organisms were *S. aureus* (17/35, 41.5 %) *E. coli* (6/35, 14.6%), *K. pneumoniae* (5/35, 12.2%). Forty patients (45.9%) underwent surgical debridement, 11 percutaneous drainage, 4 aspiration, and 31 patients were received only antibiotic treatment. The overall mortality was 10% (n=9). Comparison of treatment options showed mortality was not significant different between patients for only antibiotics and for antibiotics plus invasive procedure (12.9% (4/31) vs 8.9% (5/56), p=0.55).

**Conclusion:** The etiology of psoas abscess can vary with each countries. In Korea, "primary" psoas abscess was more prevalent than "secondary" psoas abscess, and *S.aureus* was the most prevalent organism. The causative organisms can be identified by abscess culture in most cases. High index of suspicion is important to early diagnosis.

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Mortality and ICU-admission in community-acquired pneumonia: CURB-65 score validation in Uruguay

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**Background:** Community-acquired Pneumonia (CAP) is still an important health problem with high mortality. Early identification of patients with severe CAP should improve the results. CURB-65 score of the British Thoracic Society is one of the simplest predictor's score of mortality and intensive care unit admission (ICU).

**Objective:** to validate the CURB-65 score in CAP patients admitted to three community hospitals in Rivera, Uruguay.

**Methods:** A prospective cohort of CAP patients admitted between 1st May 2005 and 30th April 2007 was included and followed up until one year of hospital discharge. Area under the ROC and specificity and sensibility were estimated for CURB-65 score for 28 days-mortality and ICU-admission.

**Results:** 495 patients (63 ± 19 years, male 53.9%) were included. 28-days mortality and ICU-admission were 20.8% (102/491) and 19.9% (98/492), respectively. 28-days mortality in patients with CURB-65 0, 1, 2, 3 and 4-5 was 4 (6/150), 14.2 (30/211), 43.6 (41/94), 67.7 (21/31) and 80% (4/5), respectively (p<0.001). ICU admission in patients with CURB-65 0, 1, 2, 3 and 4-5 was 8 (12/150), 15 (32/213), 29.5 (28/95), 74.2 (23/31) and 100% (3/3), respectively (p<0.001). Area under the ROC was 0.79 (CI 95% 0.74 – 0.84, p<0.001) and 0.73 (CI 95% 0.67- 0.79, p<0.001) for 28-days mortality and ICU-admission, respectively.

**Conclusion:** CURB-65 score showed a good discrimination capacity for ICU-admission and 28 days-mortality in CAP in Uruguay and could be used for early identification of patients with high mortality and requiring ICU-admission in Uruguay.

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**Time: 12:30-13:30**  
**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Encrusted Pyelitis: A kidney stone disease of infectious origin

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**Background:** Encrusted Pyelitis is a rare stone disease related to group D2 Corynebacterium. It was first described in 1993 by Aguado- Morales et-al in transplanted patients and later in patients with native kidney having predisposing factors which were underlying urologic disease and/or urologic manipulation, debilitating disease, hospitalization, and prolonged antibiotic therapies.

**Methods:** A 31- year-old female with a history of repeated urinary tract infection who required a left nephrectomy was studied. She was admitted in our service because of persistent symptoms of pyelonephritis with no growth of bacteria on urine culture, and the presence of obstructive acute renal failure.

Ultrasound of the kidney detected hyperechogenic material in the pelvis. Mild dilation of right ureter with a 4 mm diameter stone in distal urether.

Abdominal CT: right kidney with abnormal morphology and loss of cortico-medular differentiation. Hyperdense lesions with coralliform lithiasic aspect.

A percutaneous nephrostomy was performed.

A coralliform stone was seen and the presence of Corynebacterium Urealyticum in the urine culture and culture of the stone was detected. Treatment with vancomicine was completed for 21 days.

**Results:** Encrusted Pyelitis is characterized by accumulation of struvite crystals in ulceronecrotic lesions of an inflamed and infected chorion of the urinary tract.

It's related to the colonization with C. Urealyticum, aerobic gram positive bacilli, non sporulated, with frequently groups with a V shape, positive catalasa, negative nitrate, positive ureasa.

C. Urealyticum is a common hast of skin and mucosa, particularly in genital area which, in presence of predisposing conditions, colonizes the urinary tract causing infection.

The clinical manifestations are a triad of pyuria-hematuria, alkaline urine and presence of struvite crystals.

The growth of C. Urealyticum in urine samples increases when a selective culture is used and the period of incubation is of 48 hours.

C. Urealyticum is generally resistant to B-lactamic and aminoglucozides and sensible to vancomicine and teicoplamine; with variable sensibility to quinolones, erithromicin, rifampicin and tetracycline.

**Conclusion:** It is the purpose of this work to study this case because of its low incidence in not transplanted patients.

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**Type: Poster Presentation**

Distant infection and the risk of Prosthetic Joint Infection (PJI): A case control study  
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**Background:** The risk of prosthetic joint infection (PJI) associated with prior distant organ infection has not been assessed.

**Methods:** We performed a single-center, case-control study between December 2001 and May 2006 in a 2000 bed tertiary care hospital in Rochester, Minnesota. Cases were patients hospitalized with total hip or knee arthroplasty infection. Controls were patients with a total hip arthroplasty (THA) or total knee arthroplasty (TKA) hospitalized within the same time period without a prosthetic joint infection. Controls were frequency matched to cases by prosthesis location. Data regarding demographic features and potential risk factors related to the development of a PJI were collected. Logistic regression was used to assess the association of variables with the odds of infection.

**Results:** There were 339 cases and 339 controls. 164/339(48.4%) cases had a THA and 175/339(51.6%) had a TKA. The two most common organisms responsible for PJI were *Staphylococcus coagulase negative* and *S. aureus*, encountered in 101/339(30%) and 95/339(28%) of cases respectively. 111/339(32.7%) of cases and 62/339(18.3%) of controls had a distant organ infection in the preceding two years prior to the development of PJI (adjusted odds ratio, 2.18 [95 % CI 1.46 - 3.26] ; p-value < 0.0001). The two most common distant infections were Urinary Tract Infection (33/339 cases and 22/339 controls) and Respiratory Infection(31/339 cases and 23/339 controls). Blood stream infection within two years preceding the development of PJI occurred in 14/339 cases and none in controls.

**Conclusion:** Prior distant organ infection is independently associated with an increased risk of PJI. Patients with an arthroplasty and a distant organ infection should be evaluated and treated promptly.

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**Type: Poster Presentation**

Infective endocarditis in children: Analysis of cases admitted to a cardiac surgery referral hospital from January 2006 to July 2009

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**Background:** Paediatric infective endocarditis(PIE) is overall not a common disease, and there are few recent series describing this condition in developing countries. We describe our local experience and identify risk factors for acquisition of PIE.

**Methods:** This is a prospective study, using the ICE case report form (CRF). Cases were defined by the modified Duke criteria. Data extracted from these CRFs were analysed on Microsoft ExcelR. Means were expressed  $\pm$ standard deviation. Chi-square values were calculated in Statcalc (EpiInfo)

**Results:** Twenty six children were included; they were divided into 2 groups: A (age < 1 year) and B (age  $\geq$ 1 year). Group A (gpA) had 5 children (4 male, M, 1 female, F), mean age  $41 \pm 46$  days. Group B (gpB) had 21 children (14M, 7F), with mean age  $12,1 \pm 4,4$  years. Congenital heart disease was present in 15/26 (58 %), 5 in gpA, 10 in gpB. Rheumatic heart disease was present in 7/21(33%) of gp B. Diagnosis of PIE was definite in 21/26 ( 81%; 2 gpA,19 gpB). Mitral, tricuspid, aortic and mitroaortic valves were affected in similar proportions. Acute clinical presentation was present in 20/26 cases (81 %; 5 gpA,15 gpB); and subacute in 6 (6 gpB). Hospital acquisition of infection was present in 11/26 (42%; 5/5 gpA e 6/21 do gpB). Causative microorganisms are shown in figure 1 and clinical features in figure 2. Intravenous (IV) access was a predisposing procedure in 12/26 (46%) patients (5 gpA e 7 gpB). Recent (same admission) cardiac surgery was present in 11/26 (42%; 5 gpA e 6 gpB). Transthoracic echocardiograms showed major criteria in 25/26(96%). Complications were emboli (2 gpA, 9 gpB), persistently positive blood cultures (1 gpA,4 gpB), central nervous system events (8 gpB) and cardiac failure (1 gpA ,8 gpB). Mortality was 4/5 (80%) in gpA and 9/21(43 %) in gpB.

**Conclusion:** Children less than 1 year old had acute presentation, hospital acquired infection and high mortality more often, when compared to older children. Previous IV access and cardiac surgery were frequently seen in this group as predisposition to infection  
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**Session: Clinical Bacterial Infections**

**Date: Wednesday, March 10, 2010**

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**Type: Poster Presentation**

Blood culture negative infective endocarditis (IE). Clinical features, long-term outcome and comparison with positive blood culture IE

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<sup>1</sup>ICBA, Buenos Aires, Argentina, <sup>2</sup>Blanco Encalada 1543, Ciudad Autonoma de Buenos Aires, Argentina, <sup>3</sup>FUNCEI, Ciudad Autonoma de Buenos Aires, Argentina, <sup>4</sup>FUNCEI; Clinical Director, Ciudad Autonoma de Buenos Aires, Argentina

**Background:** Blood-culture negative infective endocarditis (BCNIE) is a challenge for clinicians, due in part to the difficult diagnosis and the appropriate choice of antibiotic treatment.

**Methods:** To evaluate clinical findings and long-term outcome of patients (pts) with BCNIE vs pts with positive blood cultures IE (BCPIE) we analyze a prospective observational registry of consecutive definitive episodes (ep) of IE according to modified Duke criteria admitted at the institution. Patients follow up was made by patient visits and/or phone calls. The outcome (relapse and reinfection) was evaluated by Kaplan Mayer method.

**Results:** From August 1998 through December 2006, 177 ep. of IE in 169 patients were included. One hundred and thirty four (75,7%) were definitive IE; 32 (23,8%) were BCNIE. Median age 67 y/o (range=16-87); male 25 (78,2%). Previous antibiotic therapy was used in 14/32 ep (43,75%). NVIE 12/32, PVIE 11/32 (5 early) and 9 intracardiac devices. Aortic valve 15, Mitral valve 6, Tricuspid valve 1 and both aortic and mitral valve 1. Clinical picture: fever 25 (78%), sepsis 5 (15,6%), new regurgitation heart murmur 9 (28%). Complications were found in 18/32 patients (56,2%): heart failure 11 (61%), embolic events 6 (75%; 4 were in CNS). Surgery: 24/32 (75%); 13/24 had a positive valve culture or valve PCR. In-hospital mortality: 4/32 (12,5%; 2/24 with surgery). Long-term follow up lasted 1034 days (range 67-3492) and there were 4 relapses (16,5%). The overall mortality was 46,4% (13/28); 8/13 died patients had previous surgery (61,5%). Compared with pts with BCPIE, it was noted that pts with BCNIE more frequently have intracardiac devices (prosthetic valves and pacemakers), surgical treatment and a double rate of mortality in the long-term follow up group. The other variables did not show a statistically significant difference.

**Conclusion:** BCNIE represents a challenge in the clinical practice. It is noteworthy that half of cases had previous antimicrobial therapy; this observation must conscious us about antibiotic use, especially in those patients with intracardiac devices. The mortality and complications makes necessary a lasting and rigorous follow up of pts with BCNIE.

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**Session: Clinical Bacterial Infections**

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**Type: Poster Presentation**

Treatment of Chronic Osteomyelitis (COM): Rwanda experience of in situ sterile Plaster Of Paris (POP) pellets containing antibiotics

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**Background:** COM constitutes a serious public health burden in developing countries. Diagnosis of acute OM is often missed in primary care and patients are referred at a late stage of COM in referral hospitals, thus requiring heavy, long and costly treatment.

Treatment in many developing countries is not standardized and patients' outcomes are often poor.

**Methods:** From June 2000 to March 2001, 67 patients diagnosed with COM at the University Teaching Hospital of Kigali, Rwanda were placed into two treatment groups:

Group 1 (34 patients) received "classic treatment," sequestrectomy, surgical toilet, and closing of the wound over sucking drains. Patients also received I.V. antibiotics until the wound healed and then received wide spectrum oral antibiotics until normalization of the ESR.

Group 2 (33 patients) were also treated by sequestrectomy, surgical toilet, and closing of the wound over sucking drains. The latest was given 24 hours I.V. antibiotics as prophylaxis, received in situ sterile POP pellets containing fusidic acid plus oxacillin or amoxicillin.

All patients were discharged when the wound was closed and fistula dried. Patients were followed post-operatively after 20 months.

**Results:** Of all patients, 37% were referred by a health professional, 21% had self-medicated, and 42% were first treated by a traditional practitioner.

The majority of cases were misdiagnosed as acute osteomyelitis (Group 1, n=22; Group 2, n=27). Aureus Staphylococcus was the most frequent germ (Group 1, n=22; Group 2, n=22): 39% were oxacillin resistant; 5% were fusidic acid resistant; none were vancomycin resistant. Hospital stay was significantly ( $p=.0008$ ) longer for Group 1 (32 days) compared to Group 2 (18 days) patients. Wound healing was significantly ( $p=.0017$ ) longer for Group 1 (48 days) compared to Group 2 (36 days) patients.

At 20-month follow-up, treatment failures and infection recurrence was significantly ( $p=.0017$ ) higher for Group 1 (n=15) compared to Group 2 (n=6).

Excellent results (wound healing and fistula dried) were obtained in 58% of Group 1 patients compared to 87% of Group 2 patients.

**Conclusion:** In situ sterile POP pellets containing antibiotics is an effective and cost-efficient alternative treatment for COM and is suitable for resource poor healthcare settings.

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**Session: Foodborne Diseases and Outbreaks**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Shiga-toxin producing *Escherichia coli* in fresh milk from small-holder dairy farms in Kaduna, Nigeria

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**Background:** Untreated milk is a good source of transmission of human and animal disease causing agents. In Nigeria, milking of cows is generally done using unhygienic procedures and pasteurization of milk is rarely done. Shiga toxin-producing *Escheriachia coli* are food-borne pathogens associated with hemorrhagic colitis and hemolytic-uremic syndrome. We conducted a study to determine hygienic practices during milking, assess the microbial quality and presence of *E. coli* in fresh milk from samllholder dairy farms in Kaduna.

**Methods:** We used a multistage cluster sampling to select forty farms from three senetorial districts in Kaduna State. Fifteen milk samples collected from each farm were analyzed for total plate (TPC), and coliform counts (CC), and presence of *E. coli*. The *E. coli* isolates were analyzed for the presence of virulent genes by polymerase chain reaction. Data on variables that describe the milking facilities and hygienic practices during milking were obtained. The mean TPC and CC were normalized by a logarithm base ten transformation and used as dependent variables. Independent sample t-test and one way analysis of variance were used to check associations.



**Fig.1 PCR amplifications from the *E. coli* isolates.**

**Results:** Thirty eight (98%) farms milked cows by hand and had TPC and CC of  $5.5 \pm 0.17$  and  $3.46 \pm 11$ . In the 23 (57.5%) farms that washed the cows' udders before milking, TPC and CC were  $5.39 \pm 0.09$  and  $3.38 \pm 0.15$ , while in farms that did not wash, TPC and CC were  $5.65 \pm 0.15$  and  $3.51 \pm 0.08$  ( $p < 0.05$ ). TPC and CC of farms that never examined the udders before milking,  $5.61 \pm 0.18$  and  $3.47 \pm 0.10$ , and those that always examined the udders,  $5.30 \pm 0.12$  and  $3.46 \pm 0.11$ , were statistically significant ( $P < 0.05$ ). Four (40%) of the *E. coli* isolates were positive for *Stx2d* genes.



**Fig.2 PCR amplifications from the *E. coli* isolates**

**Conclusion:** All farms had microorganisms in excess of tolerable levels due to unhygienic practices. Presence of virulent *Stx2d* producing *E. coli* has a serious public health implication as milk is sometimes consumed without pasteurization. Farmers were educated on hygienic practices during milking and importance of milk pasteurization. Measures aimed at reducing milk contamination especially during milking should be enforced in all dairy farms.

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**Type: Poster Presentation**

Investigation and control of a meningitis outbreak in Birbhum-Murshidabad border of West Bengal, India, March 2009

**G. Roy**  
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**Background:** Sudden onset of high fever, severe headache, nausea and convulsion, followed by unconsciousness, struck in nine villages of both banks of river Brahmani in Birbhum-Murshidabad border of West Bengal, India, from 19.03.2009 to 25.03.2009 causing panic after eight deaths. We along with Rapid Response Team investigated the outbreak to determine the cause and worked for containment.

**Methods:** We defined the case, confirmed the outbreak and line listed by house to house visit; arranged hospitalization, laboratory investigation and treatment of case- patients; examined, treated and monitored contacts; undertook hygienic, disinfective and environmental measures involving community; strengthened immunization, health- education and advocacy.

**Results:** Attack Rate (AR) of two villages of Nalhati II block of district Birbhum and western part of Brahmani was 2.21 with higher incidence among male and 16-49 years age group with hospitalization of 27 cases. Case fatality Rate (CFR) was 6.45. AR of seven villages of Nabagram and Khargram blocks of Murshidabad district and eastern part of Brahmani was 3.60 with higher incidence among male and 16-49 years age group with hospitalization of 116 cases. CFR was 1.90. These areas are riverine low lands with marshy paddy fields full of culex and other mosquitoes; several water bodies where migratory birds used to come; scattered bushes and trees with bat population; congested unhygienic hutments with backyard poultry and pig rearing by a section. Poverty, lack of awareness and poor nutritional status were common. Frequent migration for better livelihood through masonry and other labouring works is rampant. Blood slides, culture, IgM, virology; urine and CSF examinations were performed. CSF suggested Pneumococcal meningitidis.

**Conclusion:** It was a fatal bacterial meningitis outbreak which was controlled by prompt treatment and public health intervention. Improvement of personal hygiene, nutritional status, housing and environment and persistent advocacy and surveillance are recommended to prevent future outbreak.

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**Session: Foodborne Diseases and Outbreaks**

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**Type: Poster Presentation**

Burden of illness for food-borne *Salmonella*: a cohort study in an agricultural community in Yucatan, Mexico

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**Background:** There is a great need for conducting community-based integrated food-chain surveillance in highly endemic settings to assess the impact of *Salmonella* infections on human health.

**Methods:** A 15-month cohort study was conducted in Buctzotz, a small, well nourished, agricultural community in Yucatan, Mexico. Twice-weekly household visits for surveillance of diarrheal disease, collection of stool samples and health education were performed in 126 infants less than 3 years of age and 120 adults over 74 years. Ten samples each of food-animal intestines, raw retail meat, and ready-to-eat food and beverages were tested weekly. *Salmonella* isolates were serotyped, and tested for antimicrobial susceptibility and PFGE according to standard methods.

**Results:** *Salmonella* contamination rates in chicken, swine and cattle intestine and raw retail meat were 20% and 27%; 82% and 69%; and 74% and 75%, respectively. *Salmonella* was also recovered from 5% of cooked pork, 4% of cooked chicken and 4% of fresh fruit beverages. The annual incidence of diarrhea of any etiology was 2.1 episodes/infant and 0.7 episodes/elder. Incidence of *Salmonella*-associated diarrhea was 21 episodes/100 infants and 13 episodes/100 elders. Asymptomatic carriage was detected in 18% of household members. None of the cohort subjects suffered dehydration or required hospitalization. The distribution of *Salmonella* serotypes in humans closely correlated with those isolated from animals and foods. PFGE analysis of the two top serotypes, Agona and Meleagridis, and two virulent serotypes, Enteritidis and Typhimurium, showed that many human isolates were indistinguishable or closely related to animal isolates. In the few household clusters detected, the infant was symptomatic and older family members were usually diarrhea-free. Overall, 3.5% of isolates were resistant to ceftriaxone, 17.7% to nalidixic acid, and 0% to ciprofloxacin; 5.6% were resistant to ampicillin, chloramphenicol and trimethoprim-sulphamethoxazole.

**Conclusion:** In highly endemic settings, multiple clones of *Salmonella* circulate in the food chain and are continuously transmitted to humans of all ages. Constant exposure results in sporadic symptomatic infections at the extremes of age and asymptomatic infections at other ages. Future preventive interventions should focus on health education aimed at reducing household transmission, and on the rational use of antimicrobials in food animal production.

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**Type: Poster Presentation**

Laboratory investigation for clonality of a foodborne outbreak due to *Vibrio parahaemolyticus* in Singapore, 2009

**M.-V. La**<sup>1</sup>, S. Zulaina<sup>1</sup>, R. Jureen<sup>2</sup>, R. Lin<sup>2</sup>

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**Background:** We report a laboratory investigation of a gastroenteritis outbreak caused by *Vibrio parahaemolyticus* following the consumption of the local salad dish "Indian rojak" from a popular hawker stall in Singapore in April 2009. The total number of involved cases was 154, with 48 cases hospitalized and 2 dead. The National Public Health Laboratory collaborated with the investigation of collected isolates of *V. parahaemolyticus* to determine genetic relatedness of these isolates.

**Methods:** Repetitive extragenic palindromic PCR (REP-PCR), PCR for the thermostable direct hemolysin gene (*tdh*) and the *tdh*-related hemolysin gene (*trh*) as well as serotyping were performed on all isolates from suspected outbreak cases and some unrelated control strains. The REP-PCR fingerprint was generated with the Agilent® Bioanalyzer using DNA 1000 LabChip® kit, and then analyzed with Bionumerics software.

**Results:** REP-PCR profiles obtained from 15 of 16 investigated isolates were identical. REP-PCR typing appeared to be as discriminatory as pulse-field gel electrophoresis in this outbreak investigation. All above outbreak isolates were positive for *tdh*, negative for *trh* and had serotype O4:K55.

**Conclusion:** REP-PCR in this setting was a rapid and useful molecular typing method for the laboratory evaluation of genetic and epidemiological relationships among *V. parahaemolyticus* strains.

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**Type: Poster Presentation**

Detection waterborne diseases associated viruses in the river water Metro Manila and Bulacan, the Philippines

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**Background:** Untreated groundwater is responsible for about half of the waterborne disease. Inadequate water distribution system in that may not be able to provide clean water, and use river water for the living are two main cause of water borne disease in developing countries. In addition, viruses causing diarrhea are stable in environmental water and serve as threat to humans In the Philippines, about 10,000 children died of severe diarrhea annually and data for viruses in the environment is not enough. So, detection of those viruses from river water is important as it indicates what viruses are circulating among human living in the area. We conducted environmental sampling in Metro Manila, the capital of the Philippines, and Bulacan, the area with a fifth of population of Metro Manila, to detect enteric viruses.

**Methods:** From March, April and August 2009 water samples were collected from 14 sites of river running in Metro Manila and Bulacan region. Water was concentrated by Poly ethylene glycol precipitation method. We performed real time PCR and conventional PCR to detect virus that can cause water borne disease. All positive samples by conventional PCR underwent sequence analysis and phylogenic tree were constructed.

**Results:** By both real time PCR and conventional PCR, almost all water samples were positive for viruses, including enteroviruses(100%), adenovirus(64.3%), rotavirus(85.7%), hepatitis A virus (HAV) (100%), astrovirus(42.9%) and noroviruses(87.5%) in these areas. Detected rotavirus belonging to G serotype 1 and this is identical to the virus detected in China Detected hepatitis A virus is belonging to genotype IA. The viral titers of samples in April, enterovirus, hepatitis A virus, norovirus G1, and rotavirus were higher than that of August.

**Conclusion:** The result of our study substantially showed that seasonality of the rotavirus in the environment agreed with that of human rotavirus infection in the Philippines. In addition, our study suggested that other enteric viruses would spread in dry season in the Philippines. We believe focusing on the aspect may improve water quality as well as prevention of enteric viral diseases in the Philippines.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Detection of human enteric viruses in shellfish, vegetables, waters and environmental samples: a preliminary study

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**Background:** Human enteric viruses contaminations of foods destined for human use, as shellfish, vegetables and waters, are considered a Public Health problem. Many epidemiological studies show that *Adenoviruses*, *HAV (Hepatitis A Virus)* and *Norovirus* gastrointestinal infections are increasing in industrialized countries. These viruses are largely excreted in feces and show a high resistance in the environment. Environment pollution can occur in many manners. However one of the major source is represented by the personal hygiene of food-handlers and consumers. Moreover, filter-feeder organisms such as mussels are bio-accumulators of viruses in waters. Thus, undercooked shellfish consumption involves sanitary risk. Irrigation and fertilization of fields with sewage may externally contaminate vegetables and fruits. In order to warrant a high level of food safety, European Commission, introduced the concept of "HACCP" (Hazard Analysis and Critical Control Points). This rule (EC 178/2002) provides for bacteria quantitative limits and analytical methods only. Viral detection methods and limits are being studied. Recently, the Environmental Protection Agency has included *Adenovirus* into the "Contamination Candidate List" among the microorganisms to monitor to warrant foods and environment health. Aim of this study is to improve knowledge about *Adenovirus*, *Norovirus* and *HAV* diffusion in shellfishes, vegetables, waters and environmental swabs samples.

**Methods:** Specimens were collected from food-production centers, hospitals, military and school canteens from January 2008 to October 2009. They were tested through biomolecular methods (Nested RT-PCR, Nested PCR and sequencing) and virus isolation in cell lines (A549 and FrhK-4).

**Results:** 19 samples of a total of 270 tested, resulted positives by Nested PCR for Adenovirus and 4 of them were positive also for virus isolation in A549 cell lines. Only one mussel sample was positive through Nested RT-PCR for HAV and negative by virus isolation into FrhK4. Sequence analysis confirmed the results and showed the presence of Adenovirus serotype 2 and 41.

**Conclusion:** These results support the hypothesis that Adenovirus serotypes 2 and 41 are the most frequent contaminants and their role as indicators of viral environmental contaminations.

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**Type: Poster Presentation**

Gastroenteritis outbreak investigation in Adamawa State Nigeria, 2009

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**Background:** In developing countries *Vibrio cholerae* is the leading cause of rapidly, progressive, large-scale outbreak of gastroenteritis. The transmission is mainly faeco-oral associated with poor sanitation. Adamawa State is among Nigerian States that have reported frequent gastroenteritis in the past. In August, 2009 a suspected outbreak was reported in the State following floods in the northern parts. Over 2,000 people displaced and many of them with no access to clean drinking water. We conducted an outbreak investigations to confirm, the outbreak, described the magnitude in view to instituted public health control measures.

**Methods:** The nine reporting Local Government Areas (LGAs) were indentified. we reviewed patient's hospital records, interviewed patients and health care workers, we conducted active case search in the communities and collected stools specimens from the suspected cases from reporting LGAs. Suspected case is defined as person of any age with profuse, effortless watery diarrhea with three or more stools in 24 hours residing in the affected areas of Adamawa State. A confirmed case is any suspected case with laboratory confirmation (presence of *Vibrio cholerae* in the stools). Data analysis was performed and cases were mapped.

**Results:** From 29th July to 10th October, 2009 a total of 2,373 cases with 204 deaths were reported, case fatality rate {204[8.6%] 2,373} was recorded in nine LGAs. The overall attack rate was {2,373 [163.8] 1,447,725} per 100,000 persons but varies over week and by LGAs. Majority of the cases were aged 2-30 years {1,681 [71%] 2,373}. The attack rate among 2-30 years was {1,681[187.3] 897,580} per 100,000 populations. Of the 22 stools samples analyzed {15 [68.2%] 22} were positive for *vibrio cholerae* sero-group 01, Ogawa.

**Conclusion:** *Vibrio Cholerae* Serogroup 01, Ogawa was the cause of the outbreak that affected mostly age 2-30 years with high attack rate and Case Fatality Rate. Active case management, health education, environmental sanitation and decontamination of wells were mounted.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Phenotypical profile against antibiotics of *Lactobacillus* sp isolated from artisanal cheeses

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**BEZIRTZOGLU**<sup>4</sup>

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**Background:** “Kopanisti” and “Tyrovolia” are traditional artisanal cheeses produced in Mykonos island, Greece, from raw unpasteurized milk of cows and ewes. Their organoleptic properties are exquisite and they are considered to be of high nutritional value. Our aim was to identify the lactobacilli composing the fermenting microflora of these cheeses and to evaluate their susceptibility against commonly used antibiotics.

**Methods:** Isolation and identification of the strains was performed by selective culture in MRS agar and by utilization of the API 50 test respectively. Phenotypical resistance was assayed by the broth microdilution method, curves were plotted against M.I.C s and deviation from the Gaussian distribution (“wild type” and “not wild type” strains) was assessed. Bimodal curves indicating separate subpopulation to the right end of the distributions, “hills”, “valleys” and “tails” were the criteria of assessment. The antimicrobial agents in test represented all possible modes of pharmacological action and were the following: penicillin G, streptomycin, sulbactam/ampicillin, ampicillin, vancomycin, teicoplanin, erythromycin, clindamycin, oxytetracycline, chloramphenicol, gentamicin, metronidazole, trimethoprim, fusidic acid and quinopristin/dalfopristin.

**Results:** *Lactobacillus* dominating the microflora of these cheeses belongs to the following fifteen species: *L acidophilus* (9.77%), *L brevis* (4.89%), *L curvatus* (3.16%), *L helveticus* (7.47%), *L plantarum* (13, 5%), *L paraplantarum* (10.34%), *L paracasei* (8.33%), *L reuteri* (3.74%), *L johnsonii* (2.01%), *L delbrueckii subsp lactis* (7.18%), *L delbrueckii subsp bulgaricus* (6.32%), *L gasseri* (2.58%), *L fermentum* (6.03%), *L rhamnosus* (4.6%), and *L pentosus* (10.5%). Tentative ECOFF values are also proposed, often different than those proposed by SCAN and NCCLS. All species had resistant strains to at least one antimicrobial. MIC 50 and MIC 90 as well as microbiological breakpoints clearly are species dependent. A multiresistance antibiotic profile was effective for most bacterial strains, and pronounced resistance profiles were observed for the commonly used antibiotics. Our results strongly suggest resistant patterns to the *Lactobacillus* genus which could be either intrinsic (against vancomycin, trimethoprim and metronidazole) or acquired (against penicillin, oxytetracycline and erythromycin). However, some strains with MIC close to the breakpoint values can be attributed to natural variation within the species.

**Conclusion:** As antibiotic resistance is a growing problem, an holistic approach strategy based on animal, plant and food control should be capable to stop its spread.

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**Type: Poster Presentation**

An outbreak of Cerebrospinal meningitis in Jigawa state Nigeria 2009.

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**Background:** Cerebrospinal Meningitis (CSM) is a fatal infection with high morbidity and mortality. Jigawa State with a population of 5 million people lies within the nation's meningitis belt. We conducted a descriptive study of the outbreak.

**Methods:** Health workers as well as cases were interviewed. Hospital records, laboratory and CSM surveillance data in the state were reviewed.

**Results:** There were community mobilization/advocacies and health staff sensitization /motivations as part of pre-epidemic preparation. The index case a 13 years old male presented in the clinic on the 23/12/08. The cases increased from 12 and one death in epidemiologic week one to 1,238 cases and 43 deaths in week 13. The most affected age group was 5 – 15 years (>65%). A reactive vaccination was carried out at 11 of the 26 affected LGAs targeting age group 2-30 years at week 15. Of the 113 cerebrospinal fluid samples taken 84 was positive for Neisseria meningitidis type A, two for Haemophilus influenza and others were negative. Case fatality rate (CFR) was dependent on the area. The epidemic ended in the state with 8,616 cases, 306 deaths, CFR of 3.6 and an attack rate of 181.9. The CSM surveillance was 100% timely and 87% complete.

**Conclusion:** The State ran out of prepositioned drugs/vaccine as the epidemic was propagating faster than the control measures. Supplies were delayed from the Federal level due to delay in resource mobilization. Pockets of vaccination were done at those LGAs that had crossed the alert threshold and schools that were experiencing outbreaks through the assistance of medicine san frontiers. Most cases had lived in overcrowded rooms and had not been vaccinated in the last five years. The laboratories ran out of supplies.

Unequal distribution of qualified personnel led to the varied CFR observed in various part of the state and this was further complicated by erratic drugs supplies to the hospitals. The recommendations from this investigation led to the establishment of an active Federal Emergency Preparedness and Response (EPR) committee and the decision by all States to preposition vaccines and drugs independent of the Federal Government.

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**Session: Foodborne Diseases and Outbreaks**

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**Type: Poster Presentation**

Risk factors for repeated cholera outbreak in Arua municipal council, north-western Uganda

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**Background:** Cholera outbreaks have occurred annually in Arua district especially in the Municipal Council (AMC) since 2005 with an average case-fatality rate (CFR) of 2.1%. The study objective was to establish the risk factors for repeated cholera outbreak in AMC with a view of designing appropriated strategies for preventing future cholera outbreaks.

**Methods:** Unmatched case control study was conducted in July 2009. Cases were defined as individuals who had lived in the Municipality for at least two years and had symptoms of cholera, while controls were persons that lived in the neighbourhood of a case, but did not have symptoms of cholera. Case definitions of the Epidemiology and Surveillance division, Ministry of Health Uganda were used as eligibility criterion for cases. A total of 23 cases and 46 controls were interviewed using a semi structured questionnaire and analysis done using EPI INFO 2008 version 3.5.1. Univariate and bivariate analysis was done. Odds ratios and 95% confidence intervals were used to determine association and statistical significance at  $p < 0.05$ .

**Results:** Factors identified for repeated outbreak of cholera in AMC included: being younger than 25years of age (OR = 4.3, CI = 1.29 – 14.21); having no post primary education (OR = 5.6, CI = 1.46 – 21.50); having source of information on cholera from IEC materials (OR = 0.84, CI = 0.11 – 0.85); not covering latrine after use (OR = 6.48, CI = 2.11 – 19.90); using un protected water source (OR = 5.65, CI = 1.66 – 19.99) and drinking untreated water (OR = 5.34, CI = 1.78 – 16.01).

**Conclusion:** Risk factors for repeated cholera outbreak were: being younger than 25years; having no post primary education; not covering latrine after use; using unprotected water source and drinking untreated water, while having source of information on cholera from IEC materials was protective. The findings emphasised the importance of personal hygiene, communal sanitation and being knowledgeable and educated.

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**Type: Poster Presentation**

Top health concerns in rural Honduras following the introduction of clay water filters

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**Background:** Access to clean water is a major health issue for many living in rural Honduras. In June 2008, clay water filters were distributed to individual homes in La Hicaca, a rural mountain community in the Yoro area of Honduras. In June 2009, a needs assessment survey was administered to residents in this area.

**Methods:** In June 2009, a needs assessment survey was administered in La Hicaca and other communities in the Yoro area that collected data on demographics, water source and principle health concerns. One question asked, "What are the three biggest health care problems that you worry about? (Check all that apply)" There were 15 options in the areas of water sanitation, nutrition, education, maternal/child health, access to doctors and medicine and specific disease concerns. Survey respondents in La Hicaca who used clay water filters and all other respondents were compared regarding their principle health concerns. Respondents were excluded if they filtered their water but did not live in La Hicaca or if they did not respond to the question regarding health concerns.

**Results:** Of 101 completed surveys, 12 survey respondents from La Hicaca used clay water filters and 70 respondents from the Yoro area did not. For the respondents who did not use filtered water, water sanitation (48.6%) and nutrition (42.9%) were the principle health concerns. For the La Hicaca residents who utilized water filters, access to healthcare in terms of distance (41.7%) and overall cost of healthcare (50%) were the principle concerns.

**Conclusion:** When comparing the primary health concerns of rural Hondurans in La Hicaca who utilize clay water filters to those who use a different water purification method, the principle health concerns differ. Water sanitation and nutrition were the principle concerns for those who do not use filtered water, and access to healthcare in terms of distance and cost were the concerns for those respondents who utilize water filters. As far as perceived health concerns are an indicator, the distribution of clay water filters in one rural Honduran community may have had a positive health impact.

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**Type: Poster Presentation**

E.coli infectious complicated with HUS in Georgia

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**Background:** Infectious diarrhea with its morbidity is the main issue of Georgian population and at the same time it is the multifactor problem ( climato-geographic, social-economic, etiological). Noticeable increasing rate of E.coli infectious, (including EHEC- Enterohemorrhagic E.coli, 0124, 0111, and et al.) has been revealed in etiological structure of infectious diarrheas in recent years. During this period some sporadic cases of hemorrhagic colitis have been registered, and among the patients of different ages they were complicated with HUS syndrome that indicates to possible circulation of E.coli. 0157.

**Methods:** Since 2009. 07- up to 2009. 09 18 cases of E.coli complicated with HUS syndrome have been registered. Most patients were rural residents ( 14 - 78%), only 4 ( 22%) were urban. 8(44% ) patients were children, 10 (55%) were adults. Among male and female patients HUS complication rate was equal. All the patients associated the onset of the disease with the ingesting of unwashed and raw fruits or vegetables. Hemorrhagic colitis dynamics in clinical estimation of cases revealed development of hemolytic anemia, thrombocytopenia, and renal failure which were confirmed by clinical-laboratory findings.

**Results:** The onset of disease was acute: low grade fever ( 37,5-37,8 ° C), severe diffuse pain in abdomen accompanied with cramps, frequent bowel movement with loose, watery stool 5-6 times a day. On the 2-3rd days of disease condition was worsened by increasing of intoxication and frequent bowel movement with bloody stool about 10-15-20 times a day. Acute renal failure developed in 11( 61%) patients , in spite of adequate treatment with infusion of crioplasma and polyionic solutions 9(50%) patients required haemodialysis in specialized department. Severe Thrombohemorrhagic syndrome - 2(11%) patients, poliserositis - 6( 33% ), ileus -1( 5%), encephalopathy - 2 (11%), psychosis - 1(5%) were revealed in various rates.

**Conclusion:** Outcome was lethal in 5(28%) cases due to delayed hospitalization and inadequate therapy.

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**Type: Poster Presentation**

Epidemiology of alimentary toxiiinfection in western Romania  
**S.-M. Draghici**, C. Laslau, A. Jarca  
University of Oradea, Oradea, Romania

**Background:** Knowledge of the epidemiology of alimentary toxiiinfections (AT) is one of the main tools for public health care control in Romania.

**Methods:** We made a retrospective study of the cases of alimentary toxiiinfections in Bihor county between the years 2005 and 2009, emphasizing the epidemiological process.

**Results:** In the past 5 years in Bihor county (610,000 inhabitants), there were reported 601 cases (9.85%000 inhabitants) of AT, 442 sporadic cases, and 29 foci with 159 cases. The 17 familial foci were prevalent (74%), versus the 6 collective foci (27%). We presume that 40-50% of food borne illnesses go unreported to health departments due to mild symptomatology and quick recovery. Etiology was found in 220 cases (38.26%), *Salmonella* (29.13%), *Staphylococcus* (20.45%), *E. coli* (15.45%), *Clostridium botulinum* (14%), *Campylobacter*, *Pseudomonas*, *Bacillus cereus*, associated germs. As sources of infection there were identified eggs, poultry, dairy products, cream-filled cakes and pies, home-packed cans, sausages, ham, and sometimes water contaminated by animal feces.

Improper food handling from the farm to the table creates conditions for the growth of bacteria that make people sick. Vegetables that are eaten raw may be contaminated by bacteria in the soil, water, and dust during washing and packing. Home canned and commercially canned food may be improperly processed at too low a temperature or for too short a time to kill the bacteria. Cooked food can also be contaminated after cooking by bacteria carried by food handlers or from bacteria in the environment. The preparation of vegetable cans and keeping of home-produced smoked meat in unhygienic conditions in the presence of vectors (flies, cockroaches) are also involved in producing AT in the western region of Romania, mainly botulism.

**Conclusion:** Contaminated, adulterated, and mishandled food and beverages are the key element or source for contracting alimentary toxiiinfections.

The main etiology was *Salmonella*, followed by *Staphylococcus*, *E.coli* and *C. botulinum*. The public health authorities must take further measures to stop the illegal commerce with alimentary products and to keep the population informed on the food associated risks.

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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

The vertical transmission of the HIV/AIDS. 22 years of experience in Cuba

**I. González Nuñez**

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**Background:** Since 1986 a controlled program was established in the primary health care system in order to reduce vertical transmission in Cuba.

**Methods:** The usual approach followed with each HIV+ pregnant woman who decides to keep her pregnancy, is to administer HAART independently her immunologic status, from week 14 to the time when a caesarean operation is carried out (week 38). Maternal nursing is strongly discouraged. The newborn child receives ZDV (2mg/Kg/dose) every 6 hours for the first 6 weeks. The children are followed-up in IPK outpatient office, where their HIV infection status is determined. Infected children are treated with antiretrovirals according to the presence of opportunistic diseases, CD4 cell count and viral load. Quantification of CD4 is determined by flow cytometry, using a FACScan cytometer. Quantification of viral RNA levels was made with the Nuclisens system from Biomerieux.

**Results:** A total of 2074 seropositive women have been reported between January 1st, 1986 and December 2008 of all the seropositive cases (10655) reported for the country (19.4%). Three-hundred thirty and seven seropositive women (16,2%) have given birth to a total of 364 children (22 women have delivered twice and 5 women have delivered twins); of the 364 children 35 (9,6%) are HIV+ , 33 (33/35=94,2%) have been classified as AIDS patients, 21(21/35=60.0%) are under treatment with HAART ; 3 (3/35=8,5%) are asymptomatic and 11(11/35=31,4 %) have died. No infection has been shown in 255 children (255/364=70,0%) by PCR and Western Blot, and 74 (74/364=20,3%) are still under study.

**Conclusion:** The Program of Prevention and Control of Vertical transmission is effective since the number of infected children is low, similar to the figures reported for developed countries.

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**Type: Poster Presentation**

Community involvement in HIV/AIDS prevention

**C. OKONKWOH**

Youth Repositioning Foundation, 234, Nigeria

**Background:** Due to high prevalence rates of HIV in limited resource locations in Nigeria, communities have been trained to be more involved in designing and implementing HIV/AIDS prevention programmes. This project after months of research identified community members as “significant others” in the prevention of HIV/AIDS. Mother to child transmission of HIV/AIDS have been used as a case study to buttress this fact.

**Methods:** The data was generated through a descriptive cross sectional study among one hundred positive nursing mothers in Ijesha community in Surulere Local Government Area, Lagos State, Nigeria. Household interviews were carried out and these interviews were used to assess community knowledge, beliefs, opinions, perception and attitude on Mother to child transmission of HIV/AIDS and the need to reduce the gap and misinformation on the prevention of mother to child transmission of HIV/AIDS.

**Results:** The data generated revealed that seventy percent of the research population responded to medical treatments because they received support from their families and other community members. Furthermore mother-to-child transmission of HIV/AIDS was greatly reduced. Contrarily, thirty percent of the research population had a high rate of mother to child transmission of HIV/AIDS due to cultural barriers from the community and fear of stigma and discrimination from the community.

**Conclusion:** To be most effective, biomedical breakthroughs on mother to child transmission of HIV/AIDS must be combined with educational efforts that inform communities about HIV/AIDS prevention. Interventions to reduce the risk of mother to child transmission of HIV/AIDS will succeed to a large extent when adequate information, education and services are properly packaged for community members who represent significant others in women’s lives. Involving community members in mother to child transmission of HIV/AIDS is an excellent approach to breaking down barriers that influence HIV transmission from mother to child.

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**Time: 12:30-13:30**  
**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Community awareness including male involvement in prevention of mother to child transmission of HIV infection is crucial

**K. A. Myint**

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**Background:** Myanmar is one of the developing countries in South East Asia and HIV prevalence among pregnant women is 1.4%. UNFPA has established the Prevention of Mother to Child Transmission of HIV infection (PMCT) programme collaborated with National AIDS Programme since 2005. Low education status, social, traditional, cultural, and financial barriers limited many women not access to sexual and reproductive health information and services including PMCT services. Therefore, number of pregnant women access to PMCT services and HIV testing is still low and should promote community awareness including male involvement in HIV/AIDS activities.

**Methods:** Sharing knowledge and information via TV spots, video clips, role plays, stage shows, and distribution of pamphlets, posters, and wall sheets are effective and it reaches to grass root level where 70% of total Myanmar population live and most of them are illiterate. It also improves awareness on HIV/AIDS and male participation. Peer knowledge and information sharing among men and women, health talk to targeted population with two ways discussion improve knowledge and utilization of services. Delivery of information by influential people is also effective in male involvement of PMCT activities. Partner counseling is better than individual counseling. Basic health staff are main actors in PMCT activities. PMCT activities should be integrated with other health activities.

**Results:** Address and stress on community awareness and male involvement by different ways made improvement of male participation in health care of pregnant women and it enhanced the utilization of PMCT services. Partner involvement has been increased as there was no partner has taken HIV testing in the project started time and now gradually increased in number of male HIV testing. Husbands become aware on safe deliveries of their wives and taking regular antenatal care. In some areas, husbands got peer information sharing from their friends and they lead and bring their wives to health sectors.

**Conclusion:** Community awareness and male involvement in sexual and reproductive health care activities including HIV/AIDS is important and crucial for utilization of PMCT services. If male involvement is improved, PMCT programme would be improved and successful. Integration of other activities is also needed to improve the PMCT programme.

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Frequency of isolated antibody to hepatitis B core antigen in HIV- HCV coinfecting individuals

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**Background:** An association between isolated anti-HBc and HCV infection has been noted in human immunodeficiency virus (HIV)-infected persons. Isolated anti-HBc was more frequently seen in HIV-HCV coinfecting patients than subjects with HIV infection alone. Occult hepatitis B may be encountered in HIV-HCV co-infected patients with isolated anti-HBc. This study describes the frequency of isolated anti-HBc and its possible value for detection of HBV-DNA in HIV infected patients with or without HCV co-infection.

**Methods:** 92 HIV infected patients were enrolled in this study. HBsAg, anti-HBs, anti-HBc, anti-HCV, ALT, HIV viral load and CD4 count were tested in all subjects. We compared 63 subjects with HIV-HCV co-infection with 29 subjects with HIV infection alone regarding isolated anti-HBc (HBsAg negative, anti-HBs negative and anti-HBc positive). Presence of HBV-DNA was determined quantitatively by real-time PCR in serum samples of patients with isolated anti-HBc.

**Results:** Of the 63 anti-HCV positive patients, 18 subjects (28.6%, 95% CI, 22.6%-34.6%) and of 29 anti-HCV negative patients 5 subjects (17.2%, 95% CI, 11.5%-22.9%) had isolated anti-HBc. HBV-DNA was detectable in 3 out of 18 anti-HCV positive patients with isolated anti-HBc (16.7%, 95% CI, 9.7%-23.7%) and none of anti-HCV negative patients with isolated anti-HBc. There was no significant difference between the HIV patients with and without HCV coinfection regarding isolated anti-HBc and occult HBV infection.

**Conclusion:** Our study showed that HIV-HCV coinfecting individuals were more likely to have isolated anti-HBc than subjects with HIV alone. It demonstrated that the presence of isolated anti-HBc in HIV-HCV co-infected individuals may reflect occult HBV infection in these patients.

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HIV voluntary counseling and testing (VCT) in three sites in Nigeria

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<sup>1</sup>Ben Gurion University of the Negev Beer Sheva Israel, 84101, Israel, <sup>2</sup>Institute of Human Virology Nigeria, Abuja, FCT, Nigeria

**Background:** VCT is an entry point to many HIV care and treatment programs. This operation is mostly a walk-in clinic, and clients come to be tested by self referral or following guidance from health care personnel. The question of the efficacy of this method is open, and the clients mix does not necessarily reflect the typical clients who need to be tested. In order to learn more about the characteristics of this population, data were collected from three centers of a single project in Nigeria, one in the Muslim north, one in the Christian south, and one near the central region of the capital during the years 2006-2007.

**Methods:** VCT rosters and client forms of consecutive clients were reviewed for demographic data and personal details, compared with HIV test results, from three VCT centers: Kano (1130 clients), Nnewi (4312 clients) and Gwagwalada (1073 clients). Data were logged on EXCEL sheets and analyzed with EPI-INFO and ANOVA software.

**Results:** In Kano 56.6% were HIV+, in Nnewi 30.7% and 53.2% in Gwagwalada. In all three sites female gender and age were risk factors. Muslims had significantly lower rates than others, and marital status was a marker for HIV: Married clients were more frequently positive (66.6%) than single ones (41.7%) OR=2.97), separated or widowed clients had the highest prevalence of HIV (89 and 90%) (p=0.00024). Clients' professions influenced the relative risk for HIV: Among drivers (OR=2.7), musicians (OR=9.3) and restaurant workers (OR=3.9) the prevalence was high, while clergymen (OR=0.5), teachers (OR=0.4) and health care professionals (OR=0.4) were less frequently afflicted. Higher levels of education were found to be protective (71.4% versus 36.7%).

**Conclusion:** The question of who should come to be tested is addressed, but not answered. We identified criteria which identify high and low prevalence groups, these could be useful when populations are targeted for promotion of counseling and testing.

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**Type: Poster Presentation**

Does knowing someone who has died of AIDS affect condom use? An analysis from Ivory Coast  
**A. Ngui Ngamini**  
University of Montreal, Montreal, QC, Canada

**Background:** Prior research indicates that personally, knowing someone who has died of AIDS is associated with greater perceived risk behaviour risk of contracting HIV and changes in sexual risk behaviours.

**Methods:** The current study with a sample of 9,686 persons examined whether personally knowing someone who died of AIDS influence condom use using the 2005 Ivory Coast Demographic and Health Survey (ICDHS).

**Results:** Unadjusted model suggests that those who reported personally knowing someone with AIDS were 1.72 times more likely to have used condom at last sexual intercourse ( $p < .001$ ). After controlling for potentially confounding variables, logistic regression showed no association between condom use and knowing someone who died of AIDS. Condom use at last sex was strongly associated with condom use at first sex, gender, age group, residency, employment status, information, AIDS-related knowledge and lack of homophobia toward Persons Living with HIV/AIDS.

**Conclusion:** Findings suggest that preventive efforts should be expanded given the current level of AIDS mortality in Ivory Coast. Campaigns must reinforce messages that in the absence of vaccine against AIDS, condom is the only best protection against AIDS during sexual intercourses.

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The features of HIV-infected patients at A. Wahab Sjahranie General Hospital Samarinda, Indonesia

**C. gunawan**

Mulawarman University School of Medicine/ A. Wahab Sjahranie General Hospital, Samarinda, Indonesia

**Background:** Indonesia is a country with rapid growth of HIV cases. With population of more than 230 millions, it is estimated there will be one to five million people infected with HIV in 2010. In East Kalimantan Province, until August 2009, more than 1100 cases of HIV are reported, although the exact number of cases must be much higher.

**Objectives.** To know the features of HIV-infected patients who come to *Voluntary Counseling and Testing* (VCT) Clinic and treated at the Infectious Diseases Ward of A. Wahab Sjahranie General Hospital Samarinda, East Kalimantan, Indonesia.

**Methods:**

An observational study was performed at VCT Clinic and Infectious Diseases Ward of A. Wahab Sjahranie General Hospital Samarinda from December 2005 to August 2009. Data collected were included number of visits to VCT Clinic, number of positive HIV test, sex, age, specific groups, route of transmission, stages of HIV, CD4 counts on admission, opportunistic infections, ARV treatment, mortality.

**Results:** During the period of study there were 2591 visits to VCT Clinic, 2176 people were tested for HIV, and positive results were 143 (6.6 %). Most patients with positive HIV were 25-34 years old (55.2 %). Male patients were 87 (60.8 %) and females 36 (39.2 %). Specific groups of infected patients were customers of sex workers (32.2 %), injecting drug users (IDUs) (23.8 %), female sex workers (19.6 %), housewives (18.2 %), children born from HIV positive mothers (4.9 %), male sex workers (1.4 %). Routes of transmission were vaginal sex (69.9 %), shared needles use (22.4 %), perinatal (4.9 %), tattoo (2.1 %), anal sex (0.7 %). Stages of infection while diagnosed were stage IV (60.1 %), stage I-III (39.9 %). Most patients came to hospital with opportunistic infections (72 %), including pulmonary tuberculosis (50.5 %), chronic diarrhea due to parasitic infections (40.8 %), oral candidiasis (16.5 %). Patients on ARV treatment were 67 (46.9 % of total patients). Initial CD4 counts were 1 - 458. Most patients had initial CD4 counts < 50 (62.5 %), while others with CD4 50 – 100 (15.6 %), 101 – 200 (9.4 %), 201 – 350 (9.4 %), > 350 (3.1 %). Total mortality was 44 (10 on ARV treatment, 34 had not got ARV).

**Conclusion:**

The majority of HIV patients are young people 25-34 years old (55.2 %). Vaginal sex is the main route of transmission (69.9 %), while shared needles use is 22.4 %. Most patients come to hospital in late stage (stage IV) (60.1 %) that correlates with high mortality rate. Opportunistic infections are found in 72 % of patients when first diagnosed and the most common opportunistic infection is pulmonary tuberculosis (50.5 % of cases).

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**Type: Poster Presentation**

Bile salt stimulated lipase genotype distribution in Ghanaian couples discordant for HIV-1 infection

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**Background:** Some individuals remain HIV-1 seronegative despite multiple sexual exposures to HIV-1 virus. This study analyzed the possible role of bile salt stimulated lipase (BSSL) genotypes in the lack of HIV-1 transmission in Ghanaian HIV-1 serologically discordant couples (SDCs). BSSL is a Lewis X-carrying glycoprotein secreted by the pancreas and present in human milk, the testes, adrenals and blood plasma of humans. BSSL has been postulated to have variant capacity to bind Dendritic cell-specific ICAM-3 grabbing non-integrin (DC-SIGN) and potentially block viral transmission across a mucosal surface.

**Methods:** A total of 32 couples were enrolled in the study. These comprised of 12 SDCs and 20 serologically concordant couples (SCC). Five milliliters of blood was taken from couples. HIV-1 antibody testing was done using Abbott HIV-1/2 Determine assay and confirmed with Innolia HIV-1/HIV-2 assay. HIV-1 negative serostatus of discordant negative partners was confirmed by polymerase chain reaction (PCR) and BSSL genotypes of all couples were also identified by PCR.

**Results:** HIV antibody testing with PCR confirmation revealed 8 SDC and 24 SCC. BSSL genotypes were grouped into high high (HH), high low (HL) and low low (LL) genotypes based on the number of repeats (ranged from 6 to 19 repeats; 16 repeats or more was denoted as high (H) and less than 16 repeats was low (L)). Each patient had 2 types of the repeats. Fifty five percent of SDCs had HL genotype found to be associated with strong binding of BSSL to DC-SIGN, 20% had HH genotype and 25% had LL genotype both of which are associated with weak binding of BSSL to DC-SIGN. On the other hand, 40% of SCCs had HL genotype, 45% had LL genotype and 15% had HH genotype. SDC positives were also compared to SCCs in terms of BSSL genotype distribution. The HH genotypes in both groups were the same (15%). The HL genotype was higher in SDC positives (60%) than in SCCs (40%) and the LL genotypes was higher in SCCs (55%) than in SDC positive partners (25%).

**Conclusion:** SDCs could be more protected against HIV-1 transmission from DC-SIGN to CD4 cells than SCCs.

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**Type: Poster Presentation**

Safety and immunogenicity of measles vaccine in HIV-infected children: Systematic review and meta-analysis

**P. Scott**<sup>1</sup>, W. J. Moss<sup>2</sup>, Z. Gilani<sup>2</sup>, N. Low<sup>1</sup>

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**Background:** Measles vaccines could be less immunogenic in immunosuppressed people. We conducted a systematic review to identify and synthesize evidence about the immunogenicity and safety of measles vaccination in HIV-infected children.

**Methods:** We searched eight electronic databases for studies published through February 12th 2009. Identified studies were independently screened by two reviewers for eligibility based on predefined criteria. Information was extracted independently by two reviewers. Meta-analysis was conducted where appropriate, and heterogeneity in results between studies was investigated through stratification of results.

**Results:** Seven-hundred and twenty-three articles were identified. Twenty-five studies with comparison groups were included. Thirteen studies without comparison groups and one case report were also examined for adverse event data.

After vaccination at 6 months, measles antibody levels were similar in HIV-infected and HIV-unexposed (combined relative risk (RR) 1.05, 95% confidence interval (CI) 0.83-1.34) or HIV-exposed but uninfected children (RR 0.91, 95%CI 0.80-1.04). Among HIV-uninfected children, slightly more HIV-exposed but uninfected children responded when vaccinated at 6 months than HIV-unexposed children (RR 1.11, 95%CI 0.99-1.25).

By nine months of age, fewer HIV-infected children responded to measles vaccine than HIV-unexposed (RR 0.79, 95% CI 0.61-1.02,) or HIV-exposed but uninfected children (RR 0.70, 95% CI 0.56-0.88). HIV-uninfected children had similar levels of response after vaccination at nine months regardless their mother's HIV-infection status (RR 1.01, 95%CI 0.98-1.04).

Vaccination at twelve months of age resulted in poorer responses in HIV-infected children relative to HIV-unexposed children (RR 0.52, 95% CI 0.21-1.33) and HIV-exposed but uninfected children (RR 0.61, 95% CI 0.50-0.73).

No reference was made to adverse events in half the studies. In studies describing adverse events, most reported no serious adverse events. There were limited data comparing vaccinated to unvaccinated HIV-infected children.

**Conclusion:** Our findings suggest that children of HIV-infected women, regardless of the child's HIV infection status, might benefit from initial vaccination at 6 months of age in regions where children are at risk for measles. Confirmation of the child's HIV-infection status prior to vaccination would not be needed. Measles vaccines appear to be safe in HIV-infected children but there is an absence of studies reporting adverse events.

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**Type: Poster Presentation**

Metabolic changes in HIV-infected children and adolescents from São Paulo city, Brazil

**C. Zanin Palchetti**, R. Vega Patin, A. D. F. Thomé Barbosa Gouvea, F. Bononi do Carmo, A. M. Rufino, V. L. Szejnfeld, R. C. de Menezes Succi, F. L. Ceragioli Oliveira  
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**Background:** HIV infection and the use of antiretroviral therapy can lead to clinical and metabolic changes first described in adults and later in children and adolescents. In this study, we evaluated the presence of lipodystrophy, dyslipidemia, hyperglycemia and insulin resistance determined by biochemical parameters and clinical assessment

**Methods:** Prospective transversal study including 40 prepubertal children and adolescents of both gender, aged 7 to 12 years, attended at the Pediatric Infectious Disease Clinic - Universidade Federal de São Paulo, São Paulo city, Brazil, from august to december, 2008. Epidemiological parameters (age,sex, HIV transmission), clinical and immune status (CDC, 1994) and HAART were recorded. Presence of clinical signs of lipodystrophy was assessed by a trained clinician. Lipid panel, glucose and insulin levels were evaluated after an overnight fast (Kwiterovich, 2008; ADA, 2008). HOMA-IR was calculated to assess insulin resistance. Statistical tests such as t-distribution and chi-squared test were performed. Statistical significance was considered as  $P < 0.05$ , data were analysed using STATA 8.0 software.

**Results:** The mean age was  $9,8 \pm 1,2$  years, 50% were boys, 82,5% children from B e C categories and 97,5% were infected by vertical transmission. Lipodystrophy was present in 11 patients (27,5%) , while hypertriglyceridaemia, hypercholesterolaemia, low HDL cholesterol levels and high LDL cholesterol levels were present in 40%, 32,5%, 32,5% and 17,5% respectively. The prevalence of hyperglycemia (5%) and insulin resistance (2,5%) was lower in these patients. There was a significant association between lipodystrophy and insulin levels ( $P = 0,043$ ). In our study, 52,5% patients received protease inhibitors, which showed significant association with higher levels of triglycerides (  $P= 0,003$ ) and total cholesterol ( $P=0,005$ ).

**Conclusion:** There was a higher prevalence of lipodystrophy and dyslipidemia in comparison with hyperglycemia and insulin resistance. The protease inhibitors were associated with impaired lipid metabolism, increasing the risk of early cardiovascular disease.

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**Type: Poster Presentation**

Body composition measurements and fat redistribution in HIV-infected children and adolescents from São Paulo city, Brazil

**C. Zanin Palchetti**, R. Vega Patin, D. M. Machado, S. Vasconcelos Beltrão, E. R. Paulino, V. L. Szejnfeld, R. C. de Menezes Succi, F. L. Ceragioli Oliveira  
Universidade Federal de São Paulo - UNIFESP/EPM, São Paulo, Brazil

**Background:** Body composition changes are frequently present in HIV –infected individuals. We studied the relation between body composition measurements and lipodystrophy in children and adolescents infected with human immunodeficiency virus.

**Methods:** Prospective transversal study including 40 prepubertal children and adolescents of both gender, aged 7 to 12 years, attended at the Pediatric Infectious Disease Clinic - Universidade Federal de São Paulo, São Paulo city, Brazil, from august to december, 2008. Age, gender, clinical and immune status (CDC, 1994), weight and height were recorded. Body Mass Index (BMI) z-score and height-for-age z-score was calculated according to WHO, 2007. Circumferences and skinfolds measurements were assessed. Body fat mass (%) was determined by DXA. Presence of clinical signs of lipodystrophy was assessed by a trained clinician. Statistical tests such as t-distribution and chi-squared test were performed. Statistical significance was considered as  $P < 0.05$ , data were analysed using STATA 8.0 software.

**Results:** The mean age was  $9,8 \pm 1,2$  years, 50% were girls and 82,5% children from B e C categories. The mean values of BMI z-score and height-for-age z-score were  $-0,10 \pm 1,58$  and  $-0,80 \pm 1,26$  respectively. Lipodystrophy was present in 14 (27,5%) patients ; four had lipoatrophy (10%), three lipohypertrophy (7,5%) and four a mixed pattern (10%). Considering the body composition measurements, the waist circumference ( $61 \pm 7,47$ cm;  $P=0,044$ ) and trunk-arm ratio ( $0,95 \pm 0,28$ ;  $P=0,001$ ) were related to lipodystrophy presence. No significant association was found between lipodystrophy and arm and calf circumferences ( $18,76 \pm 2,58$ cm;  $25,93 \pm 2,93$ cm), body fat mass ( $17,61 \pm 8,26\%$ ), tricipital and bicipital skinfolds ( $8,37 \pm 2,91$ mm;  $5,67 \pm 2,13$ mm).

**Conclusion:** Waist circumference and trunk-arm ratio were sensitive measurements to show body composition changes in patients with lipodystrophy.

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**Type: Poster Presentation**

Antioxidant nutritional status and superoxide dismutase (SOD) levels in school-aged children infected with human immunodeficiency virus

R. Vega Patin, F. L. Ceragioli Oliveira, R. C. de Menezes Succi, O. M. Silvério Amâncio, A. D. F. Thomé Barbosa Gouvea, **C. Zanin Palchetti**, D. M. Machado, F. Ancona Lopez  
Universidade Federal de São Paulo - UNIFESP/EPM, São Paulo, Brazil

**Background:**

Antioxidant micronutrients play an important role in HIV infection. Deficiencies of micronutrients are associated with immune deficiency, rapid disease progression and mortality. The aim of the study was evaluate antioxidant nutritional status in prepubertal school-aged children in two situations: HIV exposure and the control group.

**Methods:** Prospective transversal study including 51 HIV-infected children attended at the Pediatric Infectious Disease Clinic - Universidade Federal de São Paulo, São Paulo city, Brazil and their respective exposed siblings not infected by HIV (n=31) and the ones not exposed to HIV (n=32). Antioxidant substances related to the clinical, dietary and biochemical variables in the groups were evaluated.

**Results:** Vitamin A, C, E, beta-carotene, lycopene, zinc and copper intake and plasma levels of vitamins A, C, E, beta-carotene, serum copper and superoxide dismutase (SOD) and protein C reactive, did not significantly differ in the groups. Major probability of vitamin A inadequacy was identified in the HIV-infected group (43.1%) as well as in the exposed but non-infected by HIV (48.4%), when compared to the non-exposed to HIV (37.5%) ( $P>0.05$ ). Lower rates of dietary vitamin E in HIV-infected children with inadequate trans fatty acid intake ( $P<0.005$ ) were verified. In the three studied groups, vitamin E correlated to the dietary saturated and polyunsaturated fatty acid ( $P<0.007$ ). Lower rates of the erythrocyte and serum zinc ( $P<0.02$ ) were verified in the HIV-infected children, when compared to the other groups. In the same group, correlation between dietary and biochemical values for vitamin C ( $r=0.34$ ;  $P=0.01$ ) and serum zinc ( $r=0.37$ ;  $P=0.008$ ); serum copper with SOD ( $r=0.30$ ) and with serum zinc ( $r=0.43$ ) and an inverse correlation of the erythrocyte zinc with the serum copper ( $r=-0.31$ ) and with SOD ( $r=-0.50$ ) were found. Plasma levels of vitamin E, lycopene and beta-carotene were related to CD4 count ( $P<0.05$ ) but negatively to the viral load ( $P<0.02$ ).

**Conclusion:** HIV-infected school-aged children without severe infectious processes in the last year presented oxidative stress by the chronic viral infection, allied to infectious processes, promoting increase in the demand of antioxidant. Interdisciplinary accompaniment has become essential, emphasizing adequate dietary in antioxidant micronutrient intake by HIV-infected children.

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**Type: Poster Presentation**

Lipodystrophy and antioxidants in school-aged children infected with human immunodeficiency virus

R. Vega Patin, F. L. Ceragioli Oliveira, R. C. de Menezes Succi, D. M. Machado, **C. Zanin**

**Palchetti**, S. Pessoa, O. M. Silvério Amâncio, F. Ancona Lopez

Universidade Federal de São Paulo - UNIFESP/EPM, São Paulo, Brazil

**Background:** To identify association between clinical-metabolic repercussions of the lipodystrophy with micronutrient nutritional status and the enzyme superoxide dismutase in HIV-infected children.

**Methods:** Prospective and transversal study was assessed in 51 HIV-infected children attended at the Pediatric Infectious Disease Clinic - Universidade Federal de São Paulo, São Paulo city, Brazil. The clinical, nutritional and biochemical variables were related to the presence or absence of clinical lipodystrophy.

**Results:** Lipodystrophy was present in 25.5% of the children. This group was identified for subjects of younger age at the moment of diagnosis and antiretroviral therapy (ART) was more damaging concerning current clinical and immune status ( $P<0.03$ ). The current use and the time lapse of stavudine use (D4T) were related to lipodystrophy presence ( $P<0.04$ ). The lipodystrophic group presented higher trunk-arm ratio and lower values of phase angle. The dietary energy, macronutrient and micronutrient intake did not differ in the groups. The prevalence of hipertriglyceridemia and immune damage were verified in the first group ( $P<0.008$ ). Lipodystrophy was related to fast insulin values, glutamic piruvic transaminase (GPT) and HOMA ( $P<0.02$ ). Biochemical levels of micronutrients and superoxide dismutase (SOD) did not vary in the groups ( $P>0.05$ ). Multiple regression analysis showed that 37% of the trunk-arm ratio was explained by triglycerides, HOMA and SOD variables and the time of protease inhibitor use, when controlled by inadequate saturated fatty acid intake (more than 7% of total energy).

**Conclusion:**

Although the variables assessed in this study were associated to trunk-arm ratio, there were other factors that must be investigated in order to prevent or to minimize the damages caused by the oxidative stress, in order to decrease the cardiovascular disease risk factors in HIV-infected children.

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Factors influencing pregnancy among HIV positive women receiving anti-retroviral therapy in Tororo district, Uganda

**M. Busuulwa**

Medical Epidemiologist, Kampala, US, Uganda

**Background:** In Uganda HIV prevalence among women is estimated at 7.5% relative to 5.0% among men. The women are disproportionately affected at the younger age compared with men. This therefore has an implication on their fertility in the era of anti-retroviral therapy (ART). The improvement in health in the advent of ART has resulted in high proportions of unplanned pregnancies in HIV positive women on treatment estimated at 97%. Purpose of study: establish factors for unplanned pregnancies among HIV positive women on ART care to help health district department implement and strengthen family planning services in HIV care in Tororo District and prevent unplanned pregnancies in women with HIV/AIDS on ART to improve their quality of life.

**Methods:** Facility based unmatched case control study. Cases were women who had an unplanned pregnancy or had ever had one whilst on ART; controls were women on ART who had never had an unplanned pregnancy. Sample size was 414 with 207 cases and 207 controls.

**Results:** Age  $\leq 31$  years (OR= 1.70, 95% CI =1.140-2.53), seeking FP information ( $p=0.004$ ), barriers to FP use ( $p=0.00001$ ) and provision of male condoms ( $p=0.00004$ ) by the ART clinics were significantly associated with unplanned pregnancies in HIV positive women on ART. On the other hand, using injectable hormonal FP ( $p=0.00002$ ) and dual contraceptives (OR = 0.22 95% CI=0.12- 0.41) was associated with less likelihood of occurrence of unplanned pregnancies

**Conclusion:** Age  $\leq 31$  years; seeking FP information from health workers and presence barriers to FP use were risk factors to unplanned pregnancies. On the other hand, using male condoms alone; using dual FP methods of contraception was protective against unplanned pregnancies in HIV positive women on ART. Providing comprehensive family planning in HIV/AIDS care settings is necessary to reduce/prevent unplanned pregnancies in HIV+ women.

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Epidemiologic characteristics and transmitted drug resistance mutation patterns among newly diagnosed HIV infected individuals in a large tertiary care hospital in Detroit

**M. Huaman**, J. Aguilar, N. Markowitz, D. Baxa, A. Golembieski, I. Brar  
Henry Ford Hospital, Detroit, MI, USA

**Background:** Although evidence is mounting for earlier initiation of antiretroviral therapy, late presentation and transmitted drug resistance are major obstacles for current treatment and prevention strategies. We describe the epidemiology and transmitted drug resistance mutation patterns of newly diagnosed HIV individuals in a large tertiary care hospital in Detroit.

**Methods:** A retrospective analysis among adults newly diagnosed with HIV infection was performed using a computer-based medical record system. Individuals who received care at Henry Ford Hospital at the time of diagnosis and underwent genotypic resistance testing were included. Demographic, clinical and laboratory data were collected. Drug resistance mutations were detected using the Trugene HIV-1 genotype assay. Mutations evaluated were selected from the IAS-USA mutation list (12/2008).

**Results:** From 01/2006-12/2008, 137 individuals were newly diagnosed with HIV and underwent genotypic resistance testing. Mean age was 37 years; 75% were male; 77% African-Americans. Transmission risk in males: 60% MSM, 35% high-risk heterosexual, 2% injection drug use (IDU). Transmission risk in females: 79% high-risk heterosexual, 9% IDU. Overall, 53% were diagnosed in outpatient clinics, 29% during a hospital admission, and 10% in the Emergency Department. Reasons for HIV testing included: symptoms suspicious for immunosuppression in 45%; routine checkup for 29%; suspected sexually transmitted disease in 14%, partner notification/recent high-risk sexual contact in 12%. At the time of diagnosis, 30% had an opportunistic disease: 14% Pneumocystis pneumonia; 10% esophageal candidiasis. Mean CD4 count was 272 cells/mm<sup>3</sup>; 47% of patients had  $\leq 200$  CD4 cells/mm<sup>3</sup>; 35% had a viral load  $>100,000$  copies/ml. Prevalence of transmitted drug resistance mutations was 18.9%. Prevalence of one, two and three class mutations were 13.1%, 5.1% and 0.7% respectively. NNRTI resistance mutations occurred in 11% (K103N in 8%); 7% had PI resistance mutations (L90M in 5%); and 7% had NRTI resistance mutations. Bivariate and multivariate analyses showed no significant associations between resistance mutations and demographic variables.

**Conclusion:** Late presentation and transmitted drug resistance mutations are common among newly diagnosed HIV infected individuals in Detroit. There is a high burden of disease among young and heterosexual populations. Scaling up of programs to diagnose HIV infection in a timely manner is essential for appropriate treatment intervention and enhanced preventive efforts.

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Occupational risk of HIV infection among Nigerian dentists

**C. Azodo**

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**Background:** Dentists are often victims of occupational injuries by contaminated sharps of projection of contaminated fluids to mucous membranes and are therefore a occupational transmission HIV acquisition.

**Objective:** To analyze the occupational risk of HIV infection among Nigerian dentists.

**Methods:** A multi-staged sampling technique was used to select 300 practicing dentists from all parts of Nigeria. Data collection tool was a self-administered questionnaire. Measures were: frequency of percutaneous injury, and action taken and precautions to mucocutaneous exposure.

**Results:** Percutaneous injury was recorded among 69.3% of respondents and only 1.2% post-exposure prophylaxis. Those with abraded skin that will treat patient with additional barrier was 8.6%. Percutaneous injury was positively related to gender, position, additional qualifications, location of practice and experience ( $p < 0.05$ )

**Conclusion:** Percutaneous injury was significantly high and low preventive measure was utilized at such exposure. This indicated obvious need for training on needle safety and other occupational HIV preventive measures.

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Outbreak investigation of HIV/AIDS in Jalalpur Jattan (JPJ), Pakistan- 2008

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**Background:** In Summer, 2008 an NGO in Jalalpur Jattan (JPJ) arranged two voluntary HIV screening camps after observing many HIV infected persons in their treatment center. 88 (35.8%) of 246 persons screened were positive by rapid test. Intense media coverage made village residents hostile to further inquiries. The Pakistan Field Epidemiology Training and Laboratory Training Program (FELTP) was requested by the Provincial AIDS Control Program to carry out an epidemiological investigation.

**Methods:** HIV-positive persons or family members of patients who died of AIDS and consented for an interview during 15 December 2008 to 2nd January 2009 were investigated. Enhanced contact tracing was done to identify additional cases. A structured questionnaire was used to collect data regarding clinical history, HIV knowledge & practices. The national HIV/STI Referral Laboratory collected blood samples for HIV serology and molecular studies independently following pre and post counseling.

**Results:** Of 53 HIV-infected persons investigated; 47 (88.7%) were alive, 27 (50.9%) female. Median age was 35 years (mean 34.7, range 3-70), including six children of 10 years or less. Clinical symptoms included; unexplained fever (79.2%), diarrhea (64.15%) and skin infections (50.9%). 24.5% had co-infection with tuberculosis and 18.9% with hepatitis (B or C). Unsafe injections (96.2%), dental procedures (40%) and barber shop visits among males (72%) were common risk factors. Extramarital sex was reported by 9.4% including 3.8% who admitted paid sex. Only 19 (35.8%) were aware that HIV can be sexually transmitted or transmitted by blood, 18 (34%). Phylogenetic analysis revealed HIV infection in this group was HIV-1 Subtype A, transmitted over a decade, likely endemic, and not an outbreak.

**Conclusion:** Investigation indicates high rates of HIV infection in JPJ. Unlike other studies from Pakistan, increased rates were observed in females and under 12 children. Socio-cultural norms and stigmatization limited in-depth investigation of sexual practices and history of drug abuse. Shift of HIV from high-risk to general population was evident and requires vigilant surveillance besides targeted health education, VCT and clinical management services to limit disease spread.

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A randomized controlled field trial of HIV-STI risk reduction program among undergraduate students at a University in Northern Nigeria

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<sup>1</sup>Universiti Putra Malaysia, Serdang, Selangor DE, Malaysia, <sup>2</sup>Ahmadu Bello University, Zaria, Kaduna, Nigeria, <sup>3</sup>Infectious Disease Hospital, Kano, Kano, Nigeria, <sup>4</sup>Universiti Sains Malaysia, Penang, Malaysia

**Background:** Nigeria with over 2.9 million people living with HIV is at risk of increased burden and transmission HIV. It has the second largest burden of HIV infection in the world with young people at higher risk. The aim of this study was to evaluate the effectiveness of a peer-led HIV-STI intervention program framed on the Information-Motivation-Behavioral skills model among undergraduate students at Ahmadu Bello University, Zaria- Nigeria.

**Methods:** Randomized controlled field trial design was used to investigate the effectiveness of an intervention program developed for HIV-STI risk reduction. Participants were randomized to either the intervention group or control group. An 8-hour integrated HIV-STI prevention program comprising of four structured modules was developed and delivered to the intervention group while the control group received another 8-hour program on career development. Both programs were delivered by trained peers. Two-way repeated measure ANOVA was applied to assess the effectiveness of the intervention. The outcome measures including HIV-related and STI knowledge, sexual risk behaviors, attitude and stigma were assessed at baseline, immediately post-intervention, at 3 months and 6 months post-intervention.

**Results:** Respondents in the intervention arm showed significant improvements in HIV-related and STI knowledge, sexual risk behaviors and attitudes towards HIV-STI prevention. Conversely, there was no difference in tolerance towards PLHIV, which was assessed using the stigma scale. There were significant main effects for group [F = 155.94, p= < 0.001,  $\eta^2$ = 0.401]; time [F = 248.35, p= < 0.001,  $\eta^2$ = 0.516] and group x time interaction [F = 162.96, p= < 0.001,  $\eta^2$ = 0.412] for HIV-related knowledge. Similarly, the main effects for group, time, and group x time interaction for STI knowledge, sexual risk behaviors, and attitudes were also significant.

**Conclusion:** The peer-led HIV-STI intervention program developed was effective in improving knowledge and attitudes towards HIV prevention and reducing sexual risk behaviors among Nigerian university students.

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Qualitative analysis of a new web-based system for monitoring and evaluation of HIV/AIDS, El Salvador 2009

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**Background:** In February 2009, the Ministry of Health (MoH) of El Salvador launched SUMEVE, a unitary web-based system for monitoring and evaluation of HIV/AIDS. SUMEVE collects data on every person seeking testing or treatment for HIV/AIDS.

In May 2009 we performed an early, qualitative analysis to verify whether the system was operating as designed.

**Methods:** We selected a convenience sample of 5/30 collection centres and 6/30 laboratories at regional and central level and performed a qualitative analysis assessing the system's resources and functioning, following CDC (Atlanta)'s "Updated Guidelines for Evaluating Public Health Surveillance Systems".

Standardized interviews with SUMEVE-professionals were conducted to explore the system's strengths, weaknesses, opportunities and threats (SWOT-format).

**Results:** Patient data are entered on paper forms when HIV rapid testing is requested at primary health-care facilities and forwarded with the HIV test results to the collection-centres for entry into the online database.

97% of primary health care facilities and 100% of HIV laboratories are reporting to SUMEVE, producing exhaustive data and showing wide system acceptability.

Timeliness varies 1-7 days between generating and digitizing notification forms for instant analysis.

SWOT analysis shows as strengths a comprehensive legal framework for SUMEVE which is part of the National HIV/AIDS plan. Registration by name avoids double notification. Results are regularly published on MoH website.

Data confidentiality is guaranteed by password-restricted access.

The system has a flexible modular design allowing to amend and remove indicators.

Identified weaknesses were: lacking reliable internet connection at two of the centres and that notification forms there could not be stored confidentially.

As opportunities, we identified advocating renewed political commitment of the current government to guarantee the stability of SUMEVE and take actions in benefit of vulnerable groups identified by SUMEVE.

No current threats to the system performance could be identified.

**Conclusion:** SUMEVE is operating as planned on all levels, collecting exhaustive data of at least 97% of primary health care facilities. Identified weaknesses are being improved. SUMEVE is the only surveillance system to direct public health interventions for HIV/AIDS. We recommend performing a re-evaluation after one year to assess the value of the SUMEVE for planning and executing intervention measures.

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HIV infection in elderly (patients over 65 years)

**F. Almasi Nokiani**

University Paris 6, Courbevoie, France

**Background:** After 1996 with highly active antiretroviral therapy, not only HIV infected people live longer but also new HIV infection occurs in older people.

**Methods:** This is a Retrospective observational study in Registered HIV infected patients older than 65 years old in order to review special epidemiologic, clinical and biologic aspects of HIV infection in elderly.

**Results:** Among 1680 registered HIV infected patients in this center, there are 61 HIV infected patients older than 65 years old including 13 women (21%) and 48 men (79%). The oldest patient is an 84 years old man and male-female ratio is 4/1. There are 29 homosexual-bisexual transmission (47%), 24 heterosexual transmission (40%), only one patient blood transmission and 7 patients who denied any risk factors (11%).

23 patients (38%) are coming with AIDS defining condition (stage C), 13 patients (21%) are symptomatic (stage B) and 25 patients (41%) are asymptomatic (stage A).

Before antiretroviral treatment, CD4 count was between 9 and 493 as a mean 231cell/ml. There were 24 patients (40%) with CD4 count less than 200 and 7 patients (11%) with CD4 count less than 100 cells/ml. Recent CD4 count, was between 10 and 1080 and as a mean 478. There is an important increase in CD4 count (247 cells/ml) after antiretroviral treatment.

Viral load before treatment was ranged between 6410 to 1740000 copies/ml. Mean viral load was 230184. There are only 5 untreated patients (8%). After antiretroviral treatment 8 patients present viral load more than 500 copies/ml including 2 untreated patients. It means 6 patients (10%) suffered from confirmed virologic failure and 7 patients (11%) present detectable viral load but less than 500 copies.

**Conclusion:** 80% of HIV infected people older than 65 are men. Homosexual contact is the major risk factor in this group. In spite of 40% asymptomatic patients there are near 40% stage C. 40% of patients were coming with low CD4 count (less than 200). Mean CD4 count before treatment was 231 which is less than younger HIV infected patients, may be because of low CD4 count in older patients and/or late diagnosis of HIV infection in elderly. Virologic response is as well as younger patients even better.

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Primary human immunodeficiency virus-1 infection: Clinical, virological and immunological characteristics of a brazilian cohort

**D. Pellegrino**, E. Boccardo, M. Eira, R. Silva, I. Moreira, M. Bocoli Rossi  
Institute of Infectious Diseases Emilio Ribas, Sao Paulo, Brazil

**Background:** Primary HIV-1 infection (PHI) consists in the period of time between viral acquisition and seroconversion, its hallmark is high viremia and consequently increased infectiousness. The occurrence, severity and duration of symptoms are predictive factors of clinical deterioration. We report here the epidemiological, clinical, virological and immunological characteristics of a cohort of patients with PHI.

**Methods:** Prospective observational study of patients with PHI at the Emilio Ribas Institute of Infectious Diseases, a tertiary hospital in Sao Paulo, Brazil. Inclusion criteria included negative or undetermined HIV-1 serology associated with viral detection, or clinical and serological evidence of seroconversion during the last 6 months. Epidemiological history, clinical data, HIV-1 plasma viral load, CD4 cell count, genotypic resistance testing, serology for hepatitis B, C, A, toxoplasmosis, cytomegalovirus, herpes and syphilis were recorded as well as the use of highly active antiretroviral treatment (HAART).

**Results:** Between 2007 and 2009, 10 patients met the inclusion criteria (8 males and 2 females, median age was 34). Two patients were asymptomatic and eight were symptomatic. The main symptoms were fever (80%), myalgia (60%), rash (30%), hepatitis (20%) aseptic meningitis (20%) and renal failure (10%). Only 4 patients had a mononucleosis-like illness. Homosexual transmission route was more frequent (60%). Five patients had plasma viral load above the upper limit of detection and the median CD4 cell count was 395cel/mm<sup>3</sup> (range: 47-835cel/mm<sup>3</sup>). Five patients received HAART and among 5 patients who did not receive HAART, 2 patients had clinical and immunological criteria for initiating HAART after 12 months of follow-up. Genotypic resistance testing was available for 4 patients. Overall patients had triple class susceptible HIV-1 sub-type B strain. One patient had primary resistance to non-nucleoside reverse transcriptase inhibitors and several protease inhibitors mutations and this finding was correlated with clinical severity.

**Conclusion:** Clinical, virological and immunological parameters in PHI may be heterogenous, atypical clinical presentation is frequent. Determinating resistance profile is useful for early therapeutic intervention, which is associated with better outcome.

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Prevalence of metabolic syndrome and estimated Framingham risk score among Brazilian HIV-infected patients

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<sup>1</sup>Instituto de Infectologia Emílio Ribas, Sao Paulo, Brazil, <sup>2</sup>Hospital Universitário, University of Sao Paulo, Sao Paulo, Brazil, <sup>3</sup>Faculdade de Medicina, University of Sao Paulo, Sao Paulo, Brazil

**Background:** Recent studies suggest that HIV infection itself or combination ART (cART) were both associated with increased risk for cardiovascular disease (CVD). The 10-year Framingham risk score (FRS) is used to predict cardiovascular events in the non-HIV-infected patients, and its application in the HIV-infected subjects is under discussion. We evaluated the traditional CVD risk factors and metabolic syndrome (MS) components among HIV-infected patients ART-treated and ART-naïve.

**Methods:** This was a cross-sectional study of HIV-infected subjects ART-treated (n=29), HIV-infected patients ART-naïve (n=28) and controls without previous CVD events (n=32). Subjects were selected for common age range (20 to 69 years) from the Instituto de Infectologia Emílio Ribas, São Paulo. We assessed cardiovascular risk factors, HIV viral load, nadir CD4 count, high-sensitivity C-reactive protein (hs-CRP) and plasma lipid concentrations. MS components included low LDL cholesterol, high triglycerides, high BMI, hypertension and diabetes. The statistical analysis were done using a SPSS 16.0.

**Results:** Groups were matched for age (mean 43.6 years for ART-treated vs 42.0 years for ART-naïve vs 42.8 for controls); 31%, 35.7% and 46.8% are women, respectively. The mean duration of HIV infection was 10 years for ART-treated and 6 years for ART-naïve subjects. The mean nadir CD4 count (cells/ $\mu$ L) was 208 for ART-treated and 449 for ART-naïve subjects ( $p < 0.0001$ ); current HIV-RNA levels were undetectable on ART-treated and 13.683 copies/ml on ART-naïve subjects ( $p = 0.005$ ). There were no significant differences between the groups in levels of hs-CRP, HDL and LDL-cholesterol. Total cholesterol was higher in ART-treated than in ART-naïve (mean 209 vs 182 mg/dl, respectively;  $p = 0.02$ ); triglycerides was higher in ART-treated than in ART-naïve subjects (mean 234 vs 137 mg/dl, respectively;  $p = 0.02$ ). Hypertension was more frequent in ART-treated compared to the others groups ( $p = 0.01$ ). 41.4% of ART-treated patients had MS, compared to 25% of ART-naïve and 28.1% of controls ( $p = 0.0001$ ); 27.6% of ART-treated had a high (> 20%) 10-year FRS compared to 0% in the others groups ( $p < 0.0001$ ).

**Conclusion:** Our results shows a high prevalence of MS and high FRS in HIV-patients under treatment, which can be used to predict cardiovascular risk stratification in this population.

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Factors affecting acceptance of HIV testing among antenatal care attendees in Ethiopia: With emphasis on role of male partners

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EngenderHealth , Hawassa, Ethiopia

**Background:** Counselling and testing is an entry point for PMTCT of HIV infection. To increase uptake of PMTCT interventions and to benefit more generally from HIV testing, the greater involvement of men is important.

This study was designed to assess factors affecting acceptance of HIV testing among antenatal care (ANC) attendees with emphasis on role of male partners, in Wolaita zone, southern Ethiopia.

**Methods:** Cross-sectional study was conducted on 412 pregnant women using structured questionnaire from March to April 2008 in three public health centers of Wolaita zone, southern Ethiopia. The study was complimented and triangulated by focus group discussions (FGDs). In the absence of similar study, the sample size was determined based on the assumption that 50% of women would make joint (with their partner) decisions about HCT. The margin of error was set at 5% and degree of confidence 95%.

**Results:** Acceptance of HIV testing among the interviewed pregnant women was 74.5%. Stigma and discrimination by the community, husband reaction and fear of positive test result were reasons that impede higher acceptance of HIV testing. Acceptance of HIV testing was significantly associated with pregnant women who had attended formal education, reside in urban area, living with their partners and those mentioned ART as PMTCT. Only 27% of pregnant women could decide independently on accepting HIV testing. Male partners, who came to health centers along with their partners for ANC and HIV testing, were only 5.1%. Two third of the study participants (65.5%) have no habit of open discussion on HIV/STI with their male partners. Most women with positive test result do not disclose their test result to their partners. Pregnant women who could get partner support with positive test result were more likely to accept HIV testing than their counterparts.

**Conclusion:** HIV testing acceptance is encouraging but men's involvement was found to be low and they appear to be the secrete ingredient of PMTCT intervention. Generally, pregnant women need their male partners' positive attitude and support to accept HIV testing. Hence PMTCT programs should give emphasis on involvement of male partners.

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Attitudes towards neonatal male circumcision among Hispanic men and women in Miami, Florida

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**Background:** Hispanics in the U.S. with the lowest rates of circumcision (MC) and relatively higher rates of heterosexual HIV transmission may benefit with higher rates of MC. Before interventions to promote MC can be introduced in the Hispanic community, additional information is necessary to determine the factors that are related to its acceptability in this population.

**Methods:** We conducted a qualitative study to assess the attitudes towards neonatal MC in Hispanic pregnant females and males and intensive interviews with 12 providers of the Hispanic community. Gender concordant focus groups were held to address the relative acceptability of MC. Each focus group (2 male, 4 female) was lead by 2 trained facilitators (medical, psychosocial). Key informants of the Hispanic community had an individualized in-depth structured interviews.

**Results:** Qualitative data was analyzed for dominant themes and collapsed into overarching themes. Thirteen themes emerged, including acceptability, appearance, circumcision and children, circumcision and HIV, cost, cultural differences, health benefits, knowledge and personal experiences, pain and injury to the penis, perceived HIV risk, religion, sexual performance and sexual pleasure. Men associated acceptability with the attitudes of health care professionals, knowledge about the procedure and hygiene. Women focused on cost, cultural differences, circumcised family members and decision making for circumcising children. Attitudes regarding MC differed between national/ cultural group and gender; excepting the Mohel, Hispanic male providers related MC acceptability to American Pediatric Association guidelines, personal circumcision status and were skeptical regarding its health benefits for STD/HIV risk reduction. Female providers focused on the financial burden to parents in its provision to neonates, lack of information and the need to increase acceptability among Hispanic men.

**Conclusion:** Both women and men appeared accepting of neonatal circumcision, and the women were assertive regarding their role as decision makers regarding the procedure.

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Patients with newly-diagnosed HIV in 2004 versus 2008: No apparent difference in progression

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**Background:** Trends have recently been observed suggesting more rapid progression in newly-diagnosed HIV-positive patients. This data is limited by an inability to specifically identify the time of infection. Our HIV-positive cohort displays heterogenous acquisition risk and significant immigration from countries of high prevalence, and is ideal to study changing epidemiology.

**Methods:** A retrospective cohort study was undertaken comparing newly diagnosed HIV patients attending in the first half of 2004 with those in 2008. Baseline demographics and virological parameters were gathered. Progression was followed for the first year after diagnosis. Patients with known seroconversion were of particular interest – including those certain of time of infection, who recalled significant seroconversion illness and/or a negative test within the preceding 6 months. Others with previous negative tests outside this window were not designated as known seroconverters. Rapid progressors (RPs) were defined as those with documented seroconversion window <2 years prior to presentation, who progressed to CD4  $\leq$ 350 cells/mm<sup>3</sup> within first year of follow-up. Results were analysed using GraphPadInStat.

**Results:** Of the 200 charts reviewed, 96 met inclusion criteria. Baseline characteristics are summarized in Table 1. CD4  $\leq$ 350 cells/mm<sup>3</sup> at first presentation in 2004 and 2008 respectively was seen in 20(48%) and 31(57%) p=0.42. CD4 count  $\leq$ 350 by end of year 1 was 25(60%) vs 35(65%) p=0.67. Mean change in CD4% from diagnosis to end of year 1 or pre-ART was -2.9%(SD5.1) vs +7.2%(SD 11.0) p<0.0001 (95%CI, -13.7718 to -6.4882). AIDS-defining illness in first year was seen in 6(14%) vs 7(13%) p=1.0. Of those with CD4  $\geq$ 350 cells/mm<sup>3</sup> at first presentation, 18 (82%) vs 18 (78%) maintained CD4  $\geq$ 350 at end of year 1 untreated. Of those with known seroconversion window <2 years, rapid progression was seen in 3/7 in 2004 and 9/20 in 2008 (p=1.0).

**Conclusion:** In a diverse group, there was no difference in progression to CD4  $\leq$ 350 cells/mm<sup>3</sup> in the first year of attendance between cohorts. Knowledge of time of infection eliminates bias of late presentation in assessing true progression. Further large-scale prospective studies should focus on progression from seroconversion and minimising late presentation.

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Effectiveness of "positive prevention" among people living with HIV/AIDS in Yunnan Province, China

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**Background:** HIV/AIDS prevention needs not only a participation of HIV-negative people, but also the HIV-positive. "Positive prevention", which mainly targets on people live with HIV/AIDS (PLWH), aims to improve PLWH's health knowledge, self-esteem and life quality, protect their families and communities, and helps reducing HIV related infections as well as HIV transmission. This project evaluated the effectiveness of "Positive Prevention" among multiethnic people living with HIV/AIDS in Yunnan province, China.

**Methods:** Using a quasi-experiment design and doing surveys before and after intervention.

**Results:** On baseline, total 620 qualified questionnaires were finished, 312 from R county and 308 from L. 227 participators of R county enrolled in training, 6 months later 89.43% were followed, as well as 88.96% of L county. There were no significant differences between initial and tracing population of both counties. Through interventions, R county got higher knowledge rates than L in most questions; its average scores on 4 belief factors - perceived susceptibility and severity of diseases, perceived benefits of engaging in health behaviors, self norms- remarkably increased; in past 3 months, the condom using rate raised slightly (from 89.91% to 92.17%) whenever having sex with his/her married partner, and unprotected intercourses with the out-of-marriage decreased from 23.08% to 5.88%, and for the latest sex, the condom using rate increased from 77.97% to 97.69%. However, there were insufficient evidences on reducing drug using and syringe sharing.

**Conclusion:** "Positive Prevention" effectively improved R county participators' pertinent knowledge and belief, and also reduced high risk sexual behaviors to some extent. Moreover, with participators' praise and welcome, the training was proved to be feasible and proper.

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Demographic and psychosocial factors associated with appointment attendance among HIV-positive outpatients

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**Background:** Appointment attendance is an important strategy for ensuring adherence to antiretroviral treatment (ARV), and also provides valuable opportunities for HIV care professionals to assess the patient's need for medical advice, individualized treatment, and implement behavior interventions when needed. The purpose of the present study was to identify individual and psychosocial characteristics associated with HIV-related medical appointment non-attendance among adult participants attending the Outpatient Adult HIV/AIDS Immunology Clinic at Jackson Memorial Hospital (JMH) in Miami, Florida over a 12-month period.

**Methods:** Patient selection: Treatment naïve or no ARV for the previous 6 months at study entry. Baseline questionnaire included demographic, social and behavioral determinants, depression level, CD4 cell count and viral load. Data on medical appointment attendance was obtained from the Jackson Computer Data System retrospectively for 12 months from baseline. Mean percent of missed appointments was obtained. Pearson and Spearman correlations were used to analyze the relationship between potential predictors. A hierarchical linear multiple regression (HLMR) analysis testing the relation of the factors above to appointment non-attendance was conducted. One-way ANOVA was performed to examine whether missed appointments would vary based on CD4 count at baseline.

**Results:** A total of 178 patients, 110 males (61.7%) and 68 females (38.2%) were included. African Americans represented 70.2% ( $p < 0.001$ ) of participants. The average age was 41.05  $\pm$  10.04 for men and 43.2  $\pm$  12.23 for women. 131 (73.6%) missed at least one appointment, 29 (16.29%) missed all appointments, and 18 (10.1%) attended to all scheduled appointments. Missed appointments averaged 27.9%. HMLR was significant ( $R^2: 0.064$ ,  $F(3,142) = 3.17$ ,  $p = 0.026$ ). Greater non-attendance was associated with younger age ( $b = -0.21$ ,  $t = -2.5$ ,  $p < 0.014$ ), and no family support ( $b = -0.19$ ,  $t = 2.23$ ,  $p = 0.028$ ).

**Conclusion:** Medical appointment non-attendance has been associated with younger age and lack of family support. Non-attendance has been consistently demonstrated in similar studies to be associated with younger age. Interventions targeted to young patients and strengthen family support could address and improve medical appointment attendance as part of the complexity of long term HIV patients adherence.

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The role of HIV Viral Load and CD4+ Cell Count in the prolongation of the QT interval in patients from an HIV outpatient clinic

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**Background:** QTc Interval prolongation is associated with life-threatening arrhythmias and it has been reported to occur more frequently in the HIV-infected population.

**Methods:** The data was collected from the charts of a cohort of 135 consecutive HIV-infected patients from our HIV outpatient clinic. QTc interval was corrected to the heart rate using Bazetts formula .The cohort was divided into two groups(prolonged QTc and normal QTc interval). QTc interval was considered prolonged if it was >440 ms in males and >460 ms in females. Multiple variables and potential risk factors were collected including the CD4+ cell count and Viral Load (VL) measurements which were done at the same day or within few days from the time the ECG was done.

**Results:** 23 patients were found to have prolonged QTc (17%).No significant difference between the groups was observed for any baseline characteristics ;however stastistically significant differences were observed with regard to the CD4+ cell count and VL. The ROC curves for both CD4 count and VL were obtained to establish cut-off points .The cutoff points for CD4 count and VL were 144 and 17.9 x 10<sup>3</sup> respectively. Sixteen of 23 subjects (70%) with prolonged QTc had CD4+ cell counts < 144 cells/mm<sup>3</sup> as opposed to only 27 of 112 (24%) of subjects with normal QTc interval (OR: 7.20; 95% CI: 2.88 to 19.33; p < 0.0001). For VL, 18 of 23 subjects (78%) with prolonged QTc had levels ≥ 17.9 x 10<sup>3</sup> copies/ml, whereas 43 of 105 (41%) of patients with normal QTc had VL greater than the cut-off value (OR: 5.19; 95% CI: 1.79 to 15.05; p = 0.002). The simultaneous presence of both risk factors increased the OR to 14.74 (95% CI: 3.84 to 56.55; p <0.0001).

**Conclusion:** Our study confirmed that the risk of QTc prolongation increases with the progression of the HIV infection. Low CD4 cell count and high Viral load could be considered as independent potential risk factors for QT prolongation in HIV patients in the outpatient-clinic settings.

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Modelling the between-host evolution of set-point viral load in HIV infection

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**Background:** The Human Immunodeficiency Virus (HIV) is capable of evolving rapidly and responding to diverse selection pressures. Previous research has generally focused on the responses of HIV to selection within-host, comparatively little has been done on the response between-host selection. Previous work has proposed that the set-point viral load (SPVL) may evolve due to between-host selection as intermediate values maximise transmission potential. There is also evidence for heritability of SPVL from one infection to the next.

**Methods:** We developed three models to examine the evolution of the SPVL distribution. One modelled change in strain prevalence in discrete generations of infection. Another incorporated continuous time into this framework. The third was extended to include explicit modelling of host dynamics and variable population size. Comparison of the simulated distribution with observed data allowed estimation of parameter values.

**Results:** All three models demonstrated that SPVL distribution would converge on the optimum relatively rapidly regardless of the initial distribution of genotypes. The discrete generation model provided a robust measure of the amount of variation attributable to non-viral effects and mutation from one individual to the next. The dynamic population model showed the response of the SPVL distribution to host dynamics.

**Conclusion:** The models described can be used to simulate the response of SPVL to widespread interventions such as circumcision or treatment, or the response to changing demography.

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Large scale HIV survey in Cameroon by mass HIV testing mobile units: Evidence of HIV epidemic hot spot areas and high HIV vulnerability of women over time

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**Background:** In Africa where the majority of HIV-infected adults do not know their HIV status, the use of HIV testing mobile units offers relevant public health prospects. In order to increase the HIV testing capabilities of voluntary counselling and testing centres, we developed a decentralized, large scale strategy based on bringing the needed services closer to the people, through the use of mobile units.

**Methods:** The National Public Health Laboratory "Hygiene Mobile" acquired a van (in order to propose voluntary HIV screening) comprising separate compartments for the driver, the medical team, and laboratory facilities. The screening of HIV-specific antibodies in serum samples was carried out using SD Bioline HIV 1/2 (Standard Diagnostics) & Determine (Inverness Medical Innovation). Indeterminate or positive samples were immediately retested by the ImmunoComb® II HIV 1 & 2 Bispot /Hexagon HIV. People diagnosed as HIV-infected were referred to health care centres.

**Results:** From April 2005 to November 2008, 114 campaigns were organised in Cameroon (average of 277 volunteers tested per day). Out of 31,610 volunteers who received a pre-test counselling, 31,512 (20,330 males; 11,182 females) tested for HIV (acceptance rate of 99.7%). Their average age was 31 years. Amongst those, 31,210 (99.04%) received post-test counselling. The overall HIV prevalence was 6.06% (HIV infection in women significantly increased from 6.7% in 2005 to 9.73% in 2008). The North West region of Cameroon was the most affected with a prevalence of 15.76% in females and 7.27% in males.

**Conclusion:** HIV testing through mobile units likely constitutes a useful tool to promote large scale up HIV testing in sub-Saharan Africa and should be integrated into national HIV control programmes. Successive campaigns in a given area allow to evidence hot spots of the HIV epidemic, and to follow the variations of HIV prevalence over time.

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Potential impact of a customized peer-facilitators training program on sexual health intervention  
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**Background:** The human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) epidemic remains of global significance. Targeting the youth in which most new infections occur especially in Nigeria where the second greatest burden of the epidemic lies is pivotal in HIV prevention. The use of peer facilitators to deliver an integrated sexual health intervention on HIV/AIDS and sexually transmitted infections (STI) in this age group is an important prevention strategy. This study aimed to determine the feasibility of developing and implementing a customized training program related to the delivery of an integrated HIV/AIDS and STI risk reduction intervention for peer facilitators and to evaluate its potential impact on their knowledge, attitudes, and perceived competence.

**Methods:** We developed and delivered a structured training program and materials about HIV/AIDS and STI prevention in a university setting. The training was offered to facilitators who were planned to be involved in a larger project, known as Integrated HIV-STI Risk Reduction Program. A pretest-posttest survey of single group was conducted. Changes in scores before and after the training program in knowledge, attitude, stigma scores and perceived confidence and competence were measured.

**Results:** Ten potential facilitators participated in the program and completed both the pretest and posttest survey questionnaire. The trainees' median scores in HIV-related and STI knowledge, attitude and stigma scores had significantly increased compared to the baseline (HIV knowledge from 22 to 30.5; STI knowledge from 8 to 22.5; stigma scores from 5 to 9; attitude from 39 to 57,  $p < 0.05$ ). Upon completion of the program, majority of the trainees (80 – 100%) believed that they were competent and confident in performing most of the sexual health intervention activities.

**Conclusion:** This preliminary study suggests that a customized on-site training program on sexual health intervention designed for and delivered to peer-facilitators could significantly improve their knowledge, attitude and confidence to deliver program related to HIV-STI prevention.

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**Type: Poster Presentation**

Molecular epidemiology of HIV-1 in newly-diagnosed treatment-naïve patients in Singapore

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**Background:** The molecular epidemiology of HIV-1 has not been well studied in Singapore. Information on HIV-1 molecular epidemiology obtained from previous studies was either based on a small sample size or on a specific HIV-1 population (seroconverters). In this study we aimed to have a better understanding to HIV-1 molecular epidemiology in newly-diagnosed treatment-naïve patients on the basis of a larger sample size.

**Methods:** Newly-diagnosed treatment-naïve HIV-1 patients were prospectively, consecutively recruited from the Communicable Disease Centre, Tan Tock Seng Hospital, Singapore. PCR products of pol gene that encompass the entire protease gene and two-third of the reverse transcriptase (RT) gene were amplified based on PBMC-associated viruses using an in-house method. Direct sequencing was used to sequence the pol gene PCR products. The pol sequences were used for phylogenetic analysis and for genotypic analysis of drug resistance.

**Results:** One hundred fifty-one patients were analyzed in this study. Of whom 92% (n=139) were men, 49.7% (n=75) were men who have sex with men (MSM), and 49% (n=74) were heterosexuals. Five HIV-1 subtype/CRF were identified, including CRF01\_AE (n=94, 62.3%), subtype B (n=53, 35.1%), CRF33\_01B (n=3, 2%), and one each of CRF34\_01B and CRF07\_BC. MSM accounted for 35.1% (n=33) of the CRF01\_AE infections and 77.4% (n=41) of the subtype B infections. Phylogenetic analysis showed that 55.3% (n=52) of 01\_AE sequences were clustered in each of 8 clusters and 73.6% (n=39) of subtype B sequences were clustered in each of 5 clusters; in addition, MSM was more common in one of the clusters compared to heterosexuals in both 01\_AE clusters (55.7% vs. 37.7%, OR 11.9, 95% CI 3.9-36.7) and subtype B clusters (87.8% vs. 25%, OR 21.6, 95% CI 4.6-101.5). There was no any known drug-resistant mutation was detected by direct sequencing.

**Conclusion:** CRF01\_AE and subtype B remain the primary etiology for HIV-1 infections in Singapore. The high extent of clustering of MSM suggests active transmission of HIV-1 in this specific population, and therefore, more effective prevention intervention measures for HIV-1 transmission are needed for this group of individuals.

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Factors influencing access to HIV care services by clients referred from a district home based HIV counseling and testing program in eastern Uganda, 2009

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**Background:** Between 2006 and 2008 only 64.6% of all HIV positive referrals from the Home Based HIV Counselling and Testing (HBCT) program in Kumi district of Eastern Uganda accessed HIV care services at health facilities. This study to determined the factors influencing access to HIV care services for clients referred in the district

**Methods:** We conducted a descriptive, cross-sectional study in February 2009. The study population comprised of HIV positive adults aged  $\geq 18$  years identified by the district HBCT program and referred to public and private health facilities for HIV care services. We administered face-to-face interviews to 352 respondents using a semi-structured questionnaire. Respondents were selected by systematic random sampling using a sampling interval of 15 out of 5044 HIV positive clients identified in the HBCT program. The main outcome measure was access to HIV care defined as access to Cotrimoxazole prophylaxis in the facility HIV program. Data was entered in Epi-Info version 3.5.1 and analyzed using Stata/SE 10.0. Logistic regression analysis was done and the level of significance used was  $p < 0.05$  at 95% confidence interval.

**Results:** About 80.7% (284/352) of respondents in this study accessed HIV care. Access to HIV care services was positively influenced by; Age (25-34) compared to (18 – 24) year-olds (AOR=5.07,95%CI:1.50-17.12); being male (AOR =2.26, 95% CI:1.17-4.39); urban residence (AOR=2.53,CI: 1.09-5.89); and availability of drugs at health facility (AOR=8.42,CI:3.2122.09). Access to services was negatively influenced by lack of family support to the client (AOR=0.46,CI: 0.23-0.94).

**Conclusion:** Age (25 – 34), male sex, urban residence, availability of drugs at facilities and lack of family support are the main influencing factors of access to HIV care.

Study findings emphasize the importance of the district to place considerations within existing and planned interventions increasing access to HIV care to target; specific age groups, females, rural residents, ensure constant stock of medicines in health facilities and improve client family support.

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Adherence to infant feeding practices among HIV positive women with infants aged less than six months in Rakai district, South-western Uganda

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**Background:** In 2001, the Ministry of Health, Uganda, developed and adopted policy guidelines on feeding of infants and young children in the context of HIV/AIDS, however, little is known about the adherence to infant feeding practices. The study aimed at identifying factors affecting adherence to infant feeding practices among HIV positive mothers with infants aged below six months in Rakai district, South – western Uganda.

**Methods:** We conducted a cross-sectional study in Rakai district, Uganda between February to May 2008. HIV positive mothers, who had infants, aged less than 6 months and had attended ANC were enrolled consecutively on every clinic day, using a semi-structured interviewer administered questionnaire. Purposive sampling of five government and three private health facilities offering PMTCT in the district was done. The outcome measure was adherence to recommended infant feeding practices categorized as a binary variable (yes, no). Logistic regression analysis was performed in S.P.S.S version 12.0 with the level of significance considered at  $p < 0.05$ .

**Results:** Mixed feeding was the most practiced. Factors found to favor adherence to infant feeding practices were; having  $\leq 3$  children (AOR 2.5, CI: 1.1 - 6.4) and having an infant aged  $\leq 3$  months (AOR 3.9, CI: 1.7 - 9.0).

Factors found not to favor adherence to infant feeding were; Low education ( $\leq$ primary seven) (AOR 0.2, CI: 0.1-0.8), received group infant feeding counseling (AOR 0.3, CI: 0.1-0.7), had one counselling session (AOR 0.1, CI: 0.1-0.4), and knowing one route of MTCT of HIV (AOR 0.3, CI: 0.1-0.7).

**Conclusion:** The study found a four-fold higher level of adherence in the first three months of infancy.

Adherence can be improved through the district health team and health workers encouraging HIV positive mothers to practice accelerated weaning (exclusive breast feeding for three months); to attend at least three infant feeding counseling sessions; and to improve individual counseling which provides privacy.

There is a need to use a robust analytical study design to ascertain the influence of numerous factors on adherence to chosen infant feeding options.

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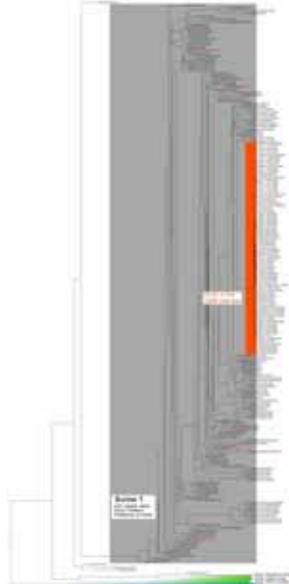
The new North American strain of influenza A virus (H1N1) may not be a novel variant of H1 subtype

**Y.-H. Lu, L.-W. Ju, C.-Y. Jiang, Y.-J. Zheng, Q.-W. Jiang**  
School of Public Health, Fudan University, Shanghai, China

**Background:** The current influenza A virus (H1N1) epidemic raises a serious concern about the nature of the virus. The transmissibility was substantially higher for this virus compared with seasonal flu viruses and close to those from previous influenza pandemics. It's reported that the virus had been already evolving for some time.

**Methods:** The present study was based on HA (hemagglutinin) fragment. A total of 558 H1 full-length sequences collected since 1930 were selected from the GenBank, including 76 H1N1 isolates from 2009 outbreak, 439 H1N1 and 48 H1N2 sequences collected previously. If there were many sequences isolated simultaneously in the same area, three were randomly included. A Bayesian approach (<http://beast.bio.ed.ac.uk/>) was used to construct genetic phylogeny and to explore evolutionary history with incorporation of a time-scale.

**Results:** All the H1 sequences were classified into 5 main clusters. There was a little diversity within Swine 1 (swine H1 in the US, Canada, Japan, Korea, Thailand, Philippines, China Mainland and Taiwan) and Swine 2 (swine H1 in Europe and China Mainland). Swine 2 was more close to clusters Avian 1 (avian H1 in the US and Canada) and Avian 2 (avian H1 in other areas) phylogenetically. Cluster Human consisted of human H1 worldwide with a similar identity. Further research revealed that the time to most recent common ancestor (*tMRCA*) of animal H1 sequences was 93.6 years ago and that of human H1 sequences was 91.6 years ago. The H1 isolates in 2009 outbreak remarkably clustered within Swine 1 with *tMRCA* being 9.1 years ago. It was noted that there were other 7 human H1 sequences clustered within Swine 1. Among them, six were isolated in the US with the earliest isolation time of 1976.



**Phylogenetic tree of HA fragment produced with posterior probability**

**Conclusion:** The present study suggested that H1 virus was originally circulating in human and animals simultaneously and separately. In addition there was marked spatial clustering in the evolution of H1 in animals.

It's likely that there was a long-term cross-species circulation of swine-origin H1 virus in the US and that the current outbreak was attributable to an accumulated genetic mutation induced by zoonosis rather than a novel variation.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

NS1 protein of avian influenza A virus prevents activation of NF- $\kappa$ B through binding to IKK $\alpha$  and IKK $\beta$

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**Background:** Highly pathogenic strains of avian influenza A virus H5N1 have caused the deaths of more than 262 people since 2003, corresponding to a death rate of about 60% for known infections (information from WHO website). If these viruses acquire the ability for efficient transmission between humans, the next pandemic will come. Therefore, it is important to study the factors that limit the transmission of avian viruses within humans.

**Methods:** The non-structure protein NS1 of H5N1 influenza virus contributes to virulence during virus infection by allowing the virus to disarm the IFN-based defence system of the host cell. To identify new potential antiviral genes regulated by NS1, we measured the expression pattern of cellular gene using DNA microarray technology. The down-regulation of several NF- $\kappa$ B-mediated downstream genes, IL-6, IL-8, COX-2, CCL-20, etc, was observed in the presence of NS1. Real-time PCR and ELISA were used to further confirm the microarray results. Luciferase activity assay indicated that the NF- $\kappa$ B binding sites were essential for the regulation of IL-6 and IL-8 by NS1 protein. Further studies demonstrated that NS1 protein can suppress NF- $\kappa$ B activity in a dose-dependent manner. Western blot assay suggested that NS1 did not alter the expression level of NF- $\kappa$ B, but prevented the translocation of NF- $\kappa$ B from cytosol to nucleus. This inhibitory property of the NS1 protein was dependent on its ability to bind IKK $\alpha$  and IKK $\beta$ , which confirmed by the GST pull down, co-immunoprecipitation and confocal assay.

**Results:** We for the first time demonstrated that NS1 can prevent activation of NF- $\kappa$ B through binding to IKK $\alpha$  and IKK $\beta$ .

**Conclusion:** NF- $\kappa$ B, an important transcription factor, plays an essential role in the regulation of immune and inflammatory responses. Therefore, NS1-mediated inhibition of the NF- $\kappa$ B pathway may thus play a key role in regulating the host innate and adaptive immune responses during virus infection.

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**Type: Poster Presentation**

H5N1 NS1 change the cell cytoskeleton and interferes with host cell motility through the GTPase  
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<sup>1</sup>Institute of Microbiology, Chinese Academy of Sciences, Beijing, PR, China, <sup>2</sup>Beijing, China

**Background:** NS1 protein of highly pathogenic avian influenza virus H5N1 contributes significantly to disease *pathogenesis* by modulating virus replication. It can inhibit innate immunity by preventing type I IFN release and inhibit adaptive immunity by attenuating human DC maturation. The ability of the protein NS1 to induce cytoskeleton changes and alter the cell motility in infected host cells is a key event in these processes. And all these may associate with the Rho subfamily of small GTP-binding proteins which mediates many fundamental cellular functions. The commonly studied members (Rho, Rac, and Cdc42) regulate actin reorganization, affecting diverse cellular responses, including adhesion, cytokinesis, and motility.

**Methods:** In our experiment, we use the three-dimensional cell culture system and the scanning electron microscope to detect the cell surface change after transfection of NS1 in A549 cell.

**Results:** We found forced expression the NS1 in A549 cell could curve the stress fibers, decrease lamellipodia and inhibit cell migration. And we found a new interaction about the NS1 and Rap1 $\beta$ , a member of the Ras family of small G proteins, which has been recognized as an important regulator of cell proliferation, differentiation, and adhesion, may impact the Rac1 activity and interfere the cell morphology and motility.

**Conclusion:** Taken together, our results suggest that the avian influenza A virus NS1 protein is a multifunctional virulence factor which can also inhibit the cell motility and change the cell morphology through interfere the GTPase' activity. Demonstrate the importance of the NS1 protein in regulating the host cell response triggered by virus infection.

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**Type: Poster Presentation**

Novel influenza A H1N1 infection among healthcare workers

M. QUERCI, F. HERRERA, E. TEMPORITI, W. ALCALA, L. FIGUERAS, F. BARBERIS, G. CARBALLAL, M. ECHAVARRIA, M. STRYJEWSKI, **P. BONVEHI**  
 CEMIC, BUENOS AIRES, Argentina

**Background:** Knowledge of epidemiological, clinical characteristics and outcomes in healthcare workers (HCW) exposed to novel Flu A H1N1 could be useful in order to improve protection measures.

**Methods:** A longitudinal study was carried out at CEMIC among 1,465 HCW from June-July, 2009. Those with influenza like illness (ILI) were swabbed for detection of novel Flu A H1N1 virus by RT-PCR. Fisher's exact test or Wilcoxon were employed. Logistic regression (LR) model to identify variables associated with H1N1 virus were analyzed.

<b>Results:</b> HCW with ILI n = 85	<b>H1N1 positive</b> <b>n=43 (%)</b>		<b>H1N1 negative</b> <b>n=42 (%)</b>		<b>p</b>
Female	26	(60)	24	(57)	0.83
Age, mean ( $\pm$ SD)	33.4	(9)	34.1	(9)	0.72
Nurse or physician	33	(77)	31	(74)	0.81
Comorbidities	6	(14)	7	(17)	0.77
Contact with suspected/ confirmed case	30	(70)	35	(83)	0.20
Use of adequate protection measures	28	(65)	26	(62)	0.82
Flu vaccination †	32	(76)	32	(78)	1.0
Fever	37	(86)	34	(81)	0.57
Asthenia	31	(72)	02	(48)	0.03
Cough	34	(79)	18	(43)	0.008
Diarrhea	4	(9)	12	(29)	0.03
Complications	5	(12)	4	(9.5)	1.0
Pneumonia	2	(5)	1	(2)	--
Oseltamivir					
Prophylactic	2	(5)	13	(31)	0.002
Therapy	41	(95)	36	(86)	0.16
Days of oseltamivir, median (IQR)	5	(5-5)	5	(3-5)	0.002
Adverse events with oseltamivir	12	(28)	8	(19)	0.44
Days off, median (IQR) †	7	(5-7)	4	(2-6.5)	0.0004
Hospital admission	1	(2.33)	0		1.0
Death	0		0		--

† In 94/96 HCW.

LR with H1N1 as dependent variable showed: cough (OR 6.93; 95%CI 2.24, 21.4); prophylactic oseltamivir (OR 0.08; 95% CI 0.01, 0.43); and diarrhea (OR 0.17; 95% IC 0.04, 0.74).

**Conclusion:** Incidence and complications of ILI was low among HCW. Seasonal flu vaccination seems to give low protection against pandemic strain. Prophylactic oseltamivir was associated with decreased risk of H1N1 infection.

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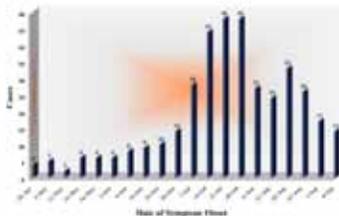
**Type: Poster Presentation**

Demographic and epidemiological characteristics of influenza in HIMA, San Pablo Caguas Hospital, Puerto Rico

**M. Collado**, L. Collado, C. Cortes

HIMA San Pablo Caguas Hospital, 00725, PR, USA

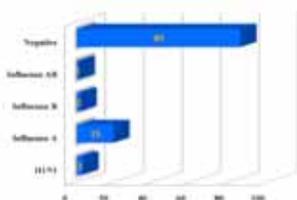
**Background:** On 24 April 2009, the World Health Organization (WHO) informed of an epidemic caused by a novel influenza A/H1N1 originating from Mexico. On 25 May 2009, Puerto Rico confirms its first case. About a month later, 11 July 2009, WHO declares a worldwide pandemic (phase 6), but not before Puerto Rico reports its first death on 15 June 2009. Nevertheless, after results of submitted specimens were reported from CDC, HIMA•San Pablo Caguas Hospital received its first positive confirmation for influenza H1N1 from a specimen collected on May 23, 2009. The purpose of this presentation is to assess the emergence and characteristics of influenza A/H1N1; specifically focused in HIMA•San Pablo Caguas Hospital. The correlation between severity of illness and clinical outcome will be analyzed in cases admitted to ICU. We will also determine the distribution among demographical characteristics, such as age, gender, and locality.



**Epidemic curve of collected specimens (n=379) of influenza A/H1N1 by date of symptom onset, 26 April 2009 to 14 September 2009.**

**Methods:** Clinical specimens, oral pharyngeal and/or blood, were collected and tested using rRT-PCR, rapid antigen testing, or influenza titers from April 26 2009 to September 14, 2009.

**Results:** Influenza A, B, and both A and B viruses were identified in 47, 6 and 3 of the Oseltamivir-treated population (n=375), correspondingly. Less often associated with laboratory-confirmed cases of influenza A/H1N1, were the incidence of mortality in obstetrical cases. Of the 379 confirmed and probable cases of influenza A/H1N1, 2.6% and 8.7% were reported as deceased and pregnant, respectively. Among the 379 clinically suspected cases for influenza H1N1, only 3 were confirmed by RT-PCR sent to the Centers for Disease Control and Prevention in Atlanta. The median age for the 79 confirmed cases for influenza A and/or B was 18 years (range, 6 month to 65 years) with a female prevalence of 63%. Complicated cases of probable and/or confirmed influenza A/H1N1 were admitted to the intensive care unit of which 5 patients (6%) died.



**Reported cases of Influenza-like illness tested with RT-PCR (n=119)**

**Conclusion:** This was an observational study demonstrating demographic and epidemiological information of influenza in HIMA•San Pablo Caguas Hospital.



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**Session: Influenza**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Clinical profile and outcome in 100 patients admitted with pandemic influenza in four intensive care units in Uruguay during the winter of 2009

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**Background:** Pandemic Influenza Virus (AH1N1) has been identified as the cause of respiratory infection worldwide, has been linked to severe respiratory failure requiring frequently ICU admissions

**Methods:** We describe the clinical and epidemiologic characteristics of 100 patients(p) hospitalized at these intensive care units with laboratory confirmed (RT-PCR) or acute febrile respiratory illness epidemiologically linked.

**Results:** 60p were confirmed and 40 were epidemiologically suspected cases. Mean Age: 45 yrs ( $\pm$  16,8); 84%< 60yrs

*Clinical profile:* cough ( 96%), dyspnea (93%), fever(90%), bronchospasm (51%), headache (41%), myalgias(42%), obtundness (35%)

*Risk Factors:* 31p had a body mass index >30; in 13% obesity was the only risk factor. COPD (33%), cardiovascular disease(19%), diabetes(16%), asthma(14%), pregnancy(10%). White count cell in 70p was less than 10.000. 76p had less than 1000 lymphocytes, CPK was elevated in 60p All tested patients had elevated LDH; in 37/80 p>1000 IU, Most p (82) showed bilateral interstitial alveolar images.

Acute Respiratory Distress Syndrome was present in 60 p. Invasive Mechanical Ventilation was instituted in 54 p. Alveolar recruitment maneuvers were necessary in 36 p; in 20 of which oxemia improved. When recruitment failed prone position was instituted: 7/12p also improved

*St pneumoniae* was identified in 22 p (18 in tracheal aspirates and 4 in blood cultures). In 84 p Oseltamivir was indicated; average dose 150 mg per day for 7 days . Pts who did not respond to treatment or in obese the dose was increased to 450 mg. Corticosteroid were indicated most due to bronchospasm(61p)

The most important complications were thrombosis (7p) and Septic shock (35p).The most common cause of death (24/28) was respiratory failure

**Conclusion:** The vast majority of our patients were younger than 60. High frequency of bronchospasm, myalgia and obtundness. Obesity and pregnancy, are special risk factors. Lymphopenia, elevated LDH and CPK represent laboratory findings. Both lungs are usually compromised.

These patients have high mortality linked to respiratory refractory failure.

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**Type: Poster Presentation**

Intravenous peramivir for treatment of influenza A and B infection in high-risk patients

**S. Kohno**<sup>1</sup>, H. Kida<sup>2</sup>, M. Mizuguchi<sup>3</sup>, N. Hirotsu<sup>4</sup>, T. Ishida<sup>5</sup>, J. Kadota<sup>6</sup>, J. Shimada<sup>7</sup>

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**Background:** The ongoing pandemic of 2009/H1N1 influenza has increased the awareness of the impact and unpredictable nature of influenza. This in turn has raised the interest in the development of additional drugs to treat the disease when vaccinations fall short. The patient population of most concern are those classified as "high-risk" where influenza causes substantial morbidity and mortality. This high-risk group comprises individuals with underlying chronic disease. In this study, efficacy and safety of intravenous administration of peramivir were evaluated in influenza patients with one or more high-risk co morbidities.

**Methods:** The study was a double-blind, multi-center study of intravenous peramivir with dosing over multiple days. The study enrolled 42 influenza patients with complications due to one or more qualifying conditions: diagnosis with poorly controlled diabetes mellitus, a chronic respiratory disease requiring pharmacotherapy, or current treatment with any immunosuppressive drug. Peramivir was administered at 300 mg or 600 mg per day, and the duration was adjusted (up to five days) on a case-by-case basis, depending on the patient's temperature and clinical condition as determined by the treating physician.

**Results:** In this study, the median time to alleviation of symptoms in all 37 evaluable patients treated with either 300 mg or 600 mg peramivir daily was 68.6 hrs (90% CI, 41.5 hrs - 113.4 hrs). The median time for each group (300 mg and 600 mg) was 114.4 hrs and 42.3 hrs respectively, while the time for each group categorized as single-dosed (n=10) and multiple-dosed (n=27) was 92.0 hrs and 64.1 hrs, respectively. The incidence of pneumonia, which was the main complication resulting from influenza was 8.1% (3/37). There were no drug-related SAEs throughout the study.

**Conclusion:** The results suggest that intravenous peramivir was effective for treatment of influenza patients with a high-risk for complications co morbidities and increased risk for complications due to influenza infection.

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**Session: Influenza**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Neuraminidase sub typing and drug resistance among influenza A viruses circulating in western India

**V. Potdar**, M. Dakhave, M. Chadha, A. Mishra  
National Institute of Virology, 421001, India

**Background:** Influenza viruses are important respiratory pathogens in India. Clinical, virological and molecular surveillance is being carried out under a Multi- site Influenza Surveillance project (Indian Council of Medical Research-Centers for Diseases Control). National Institute of Virology, Pune, India monitors genetic variations and drug susceptibility in circulating influenza viruses. Most of the influenza A viruses are amantadine resistance worldwide and hence neuraminidase inhibitors (NAI) oseltamivir or zanamivir are recommended for the treatment of infection with influenza viruses.

**Methods:** Neuraminidase sub typing of 42 H1 and 48 H3 viruses from the year 2003 to April 2009 were carried out by one step diagnostic RT PCR using published primers. Universal M13 primers forward and reverse were used for Sequencing and subtypes of isolates were confirmed by blast analysis. 253 bp product of N1 isolates contains established mutation site H274Y leading to oseltamivir resistance. To check the mutation sites E119V, I222V, R292K, N294S and  $\Delta$  244-247 for N2 isolates; further 1103bp N2 gene was amplified using specific primers.

**Results:** NA Subtyping: 90 influenza A isolates from 2003- April 2009 circulating in Pune were amplified. 90 influenza A isolates were confirmed as 42 N1 and 48 N2 respectively by sequencing. No unusual combinations were observed

Detection of drug resistant sites for N1 and N2 isolates:

N1 isolates: 253 bp amplified product of N1 isolates which included the established mutation site H274Y leading to oseltamivir resistance was analyzed by sequencing. All N1 isolates were sensitive to oseltamivir.

N2 isolates: To check the mutation sites for N2 (E119V, I222V, R292K, N294S and  $\Delta$  244-247); further 1103 bp products were amplified. All 44 N2 isolates were sensitive to both the drugs oseltamivir and zanamivir.

**Conclusion:** All seasonal influenza A viruses A/H1N1 and A/H3N2 were sensitive to neuraminidase inhibitors. Antiviral drugs against influenza are rarely used in India as a therapeutic agent. However due to globalization, resistant viruses could be seeded in Pune from elsewhere. Though no resistant viruses were detected, continuous surveillance of influenza viruses is needed to monitor circulating strains, and their drug susceptibility in view of pandemic potential of influenza virus.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Influenza in the tropics - Epidemiology and burden

**L. Da Silva**, M. H. Kyaw

Novartis Vaccines and Diagnostics, Cambridge, MA, USA

**Background:** Although influenza presents a significant burden in the tropics, the belief that this disease primarily affects people in temperate climates and developed countries persists because of peculiar circumstances of disease epidemiology, other local causes of morbidity and mortality, and better surveillance systems in countries in temperate regions. The public health burden of influenza is high in the tropics, particularly in children, yet it has been suggested that vaccination programs would not be cost-effective in this region because little is known about the true impact of the disease.

**Methods:** Systematic literature review of Medline using search terms of influenza, tropical, and epidemiology.

**Results:** In Medline, 155 manuscripts were published between 1969 and 2009. Influenza in tropical regions has gained more attention in the medical literature in the twenty-first century; 118 of 155 papers (76%) appeared after 1999. Twenty-three studies presented data on laboratory-confirmed cases of influenza in tropical regions. Outbreaks and epidemics present a significant public health burden in tropical regions. For example, in Madagascar in 2002, more than 27,000 cases of influenza and 800 deaths occurred within a period of three months despite rapid public health intervention. In tropical regions, viral transmission is usually year-round and commonly has two peaks annually. Co-circulation of what are considered "northern" or "southern" strains occurs year-round in tropical regions. Tropical regions, thus, may be the source of new strains that are then disseminated to other latitudes. However, the clinical characteristics, impact on healthcare services, and seasonal variation of influenza in tropical regions are still not clearly understood, based on the current literature. A third category of strain recommendation for influenza vaccines may be needed for tropical regions.

**Conclusion:** The burden of influenza in tropical countries may be higher than in North America or Europe. More studies in the tropics are necessary to define the impact and seasonality of influenza in these regions. These data could help identify the optimal timing of vaccination programs and other measures for the prevention and control of seasonal and pandemic influenza in the tropics, including protection for travelers and control of emerging strains.

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**Session: Influenza**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Novel influenza A H1N1 (NIA) infection in Argentinean Children – Experience at Hospital de Niños “Ricardo Gutiérrez”

**E. L. Lopez**, A. FALLO, M. M. Contrini, A. Mistchenko, C. Molise, S. Neyro, N. Gonzalez, D. Lazzarini, G. Manonelles, J. Grichener, M. Dastugue, A. Teper, N. Study Group  
Hospital de Niños "Ricardo Gutiérrez", Buenos Aires, Argentina

**Background:** NIA pandemia (P) began in Argentina during respiratory virus season. Hospital Gutiérrez (HG) set up special outfitted examination room trailer; 5000 suspected cases were attended in 2 mo. Health care for P included 2 different stages: May24-June12, 695 outpatients mainly school-age were attended [Containment Phase(CP)]. After 17 ds with a widespread disease: June12-July11, 110 children were admitted to HG due to respiratory failure [Mitigation Phase(MP)]

**Objective** to characterize clinical, lab and treatment of confirmed NIA patients (pts)

**Methods:** IFA was used to rule out other RV; confirmation by RT-PCR

**Results: CP:** 220/695(61.3%) cases were confirmed; 191students, 15 close contacts (CC), 8 international travelers, 3 teachers; 2 health-workers. The 191 confirmed student cases are reported: mean age: 9±3(4-17) yr; 16(8%) had comorbidity(C). Most frequent symptoms: fever(F) 178(93%) and cough(Co)160(64%). Oseltamivir(Os) was prescribed to 150(78.5%); none of them was hospitalized. Duration of F and Co was lower in treated than untreated 2,1 vs 3.6 ds, and 4.2 vs 7 ds respectively( $p<0.001$ ). Secondary cases occurred in 41/297(15%) of CC, 12/31 in CC without Os prophylaxis (OsP) and 29/266 in CC with OsP( $p=0.0002$ ). Mild adverse events(AE) by Os were reported in 12% of treated pts and in 6.3% of CC

**MP:** 110 NIA cases represented 6.9% of admissions during this period (double of usual seasonal flu cases/yr). Mean age 43.4(±54.4) mo; 61(55.5%) were younger than 24 mo. Main symptoms on admission: F 106(97.4%) and bronchiolitis 70(63.6%). C was present in 84/110(76.4%), it was more frequent in children >24 mo: 38/61(62.3%) than younger 46/49(93.9%)( $p<0.0003$ ). Fifteen(13,6%) required ICU. Complications: 69/110(62.7%) pts; pneumonia 52/110(47.3%); pneumonitis 31/110(28.2%) [16/52(30.8%) had both X-Ray patterns]; encephalitis 1/110 (0.9%). Os treatment: 108(98.2%); AE 1/108(0.9%): unspecific exanthema. Mortality 6/110(5.5%); all of them had C; median age:87mo

**Conclusion:** NIA was mild in school-age pts

Admissions by NIA were twice higher than seasonal flu annually

Os was well tolerated by children

Os statistically reduced duration of F and Co

Os was effective to prevent secondary cases in CC

Mortality was low in hospitalized children, associated to Comorbidity

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Assessing the risk of Influenza virus strains isolated from birds and mammals to humans  
**G. Vdovichenko**<sup>1</sup>, A. Sergeev<sup>1</sup>, O. K. Demina<sup>1</sup>, A. Kabanov<sup>1</sup>, A. S. Kudriavcev<sup>2</sup>, A. V. Pal'cev<sup>2</sup>,  
U. E. Narov<sup>2</sup>, L. N. Shishkina<sup>1</sup>, I. G. Drozdov<sup>1</sup>

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**Background:** Influenza A virus is the subject of research of many scientists in the world. So now the attention of scientists focused on the problem of influenza, first because of the increased outbreaks of avian influenza in the world and the incidence of transmission of the disease to people who were in contact with sick birds. Secondly the threat today is the influenza virus of H1N1 subtype, which caused many diseases among people around the world. In this connection assessment of risk to human influenza virus strains of various origins is essential for timely implementation of effective anti-epidemic measures in order to prevent the development of an epidemic or pandemic influenza. We have evaluated parameters of infectivity of influenza virus strains (H5N1, H1N1 and H3N2 subtypes) for primary cell culture obtained from human lung tissues.

**Methods:** Three influenza A/Chicken/Suzdalka/2/05 (H5N1), A/Novosibirsk/1/09 (H1N1) and A/Aichi/2/68 (H3N2) virus strains from the collection of research institute of FRSI SRC VB "Vector" and primary cell cultures obtained from pieces of human lung tissue kindly provided by colleagues from the Regional Oncology Center of the Novosibirsk, Russia have been used.

**Results:** A comparison of the values 50% cell infecting dose (CID50) of strains found that the susceptibility of human lung cells to influenza A/Chicken/Suzdalka/2/05 virus strain in the 10 and 100 times more than values both A/Novosibirsk/1/09 and A/Aichi/2/68 virus strains respectively. However influenza A/Chicken/Suzdalka/2/05 strain had a 10 times lower progeny yield during 20 hours of replication in human lung cells compared with other strains.

**Conclusion:** Thus, our studies revealed influenza A/Chicken/Suzdalka/2/05 virus strain is able to more easily infect cells of the respiratory tract of man, but has a lower replicate activity than both A/Novosibirsk/1/09 and A/Aichi/2/68 virus strains. Influenza A/Chicken/Suzdalka/2/05 virus strain was isolated during an outbreak of avian flu among wild birds and poultry in the Novosibirsk region. During this period of the outbreak have not been established cases of avian influenza transmission from birds to local residents. Perhaps this is due to the low replicate activity of A/Chicken/Suzdalka/2/05 virus strain, which we evaluated in our studies.

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**Session: Influenza**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

In vitro inactivation of avian influenza virus by Caprylic acids and its derivatives

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**Background:** Avian influenza is an important disease as it causes significant economic losses to the poultry industry. Further, transmission of the virus to humans enhanced the major public health significance. Since the virus is highly contagious, strict hygienic measures employed at the poultry farms can greatly help prevent the virus from spreading. Contaminated food, water, and poultry manure are most important vehicles of the virus to infect animals and humans. Effective and safe antimicrobials that kill the virus in the aforementioned vehicles could greatly reduce spread of the virus. Caprylic acid is a natural fatty acid and its other chemical forms, namely sodium caprylate and monocaprylin are highly effective in killing a variety of disease causing bacteria and viruses.

**Methods:** Low pathogenic avian influenza viruses H5N1 and H5N2 were subjected to caprylic acid, sodium caprylate and monocaprylin. Concentrations of viruses were determined using TCID50 in the cell culture. Plaque assays were optimized and 100 PFU viruses were used to infect chicken embryo kidney cells. Serial dilutions of fatty acid compounds were made from 0.005% to 0.5% for each virus to observe their virucidal effects in plaque assays. The effect of the caprylic acid to virus particles was observed using the transmission electron microscope.

**Results:** Caprylic acid and its derivatives were able to inhibit the plaque forming in chicken embryo kidney cells. The minimum inhibitory concentrations were 0.5% for caprylic acid, 0.1% for sodium caprylate and 0.05% for monocaprylin. Using transmission electron microscope, negative staining of avian influenza virus particles treated with 0.5% caprylic acid indicated disruption of cell membrane and envelope of the viral particle.

**Conclusion:** Low concentration of caprylic acid and its derivatives in-vitro can reduce or inhibit avian influenza virus. Therefore, caprylic acid and its derivatives are potential to be effective and safe antimicrobials that can be used in poultry feed in order to reduce transmission to humans.

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**Type: Poster Presentation**

Development of new effective antiinfluenza drugs based on extracts of basidiomycetes

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**Background:** Due to the high incidence of respiratory diseases in humans caused by influenza viruses of different subtypes are now particularly relevant search for new curative and preventive drugs against influenza. Many species and strains of basidiomycetes are producers of natural compounds exhibiting anti-tumor and antiproliferative activity, cytostatic effect and antiviral effect. Specific components of different bio-genetic origin, with antibacterial, anti-fungal, nematocidal, cytotoxic, antiviral, and other pharmacological properties of the basidiomycetes were identified in different investigations. Study of antiviral activity of extracts isolated from basidiomycetes against influenza virus, including highly pathogenic avian influenza and creation on their basis of new non-toxic antiviral drugs is essential for health. In the present investigation was carried out screening and studying of antiviral activity of basidiomycete extracts in experiments on MDCK cells for avian influenza A/H5N1 virus.

**Methods:** In present study more than 70 water extracts of fungi of the genera *Rhizoglyphus*, *Ganoderma*, *Laetiporus*, *Inonotus*, *Lentipus* obtained from the laboratory collection of fungal cultures and the simplest of SRC VB "Vector" were used. The antiviral effect of extracts of fungi was assessed by highly pathogenic avian influenza A/chicken/Kurgan/05/2005 (H5N1) virus strain and MDCK cells.

**Results:** The results showed that many of the studied extracts of basidiomycetes have not toxicity to MDCK cells and thus exhibit a high antiviral efficacy. This fact was noted in the evaluation of A/chicken/Kurgan/05/2005 infectivity in model of MDCK cells, when cultured with influenza virus strain in the presence and absence of tested drugs. Some aqueous extracts of fungi *Ganoderma*, *Laetiporus*, *Inonotus* and *Lentipus* has been shown in vitro to have high up to 1000- 10000 times neutralization activity for influenza A/chicken/Kurgan/05/2005 virus strain.

**Conclusion:** One possible reason for the effectiveness of the audited extracts isolated from the basidiomycetes is the presence of polysaccharides. The spectrum of polysaccharides, flavonoids, melanins, mycelium and fruit of body and other biologically active substances of basidiomycetes is very wide. The results of the studies of fungi extracts suggest about the great prospects associated with the study of substances from basidiomycetes to create new highly effective antiviral therapeutic preparations on their basis.

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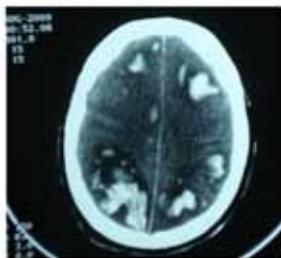
Multifocal cerebral hemorrhages in H1N1 SOIV influenza patients: Report of 2 cases  
E. Carvalho Siqueira, L. M. Carvalho valle, A. D. F. Borges, **P. S. Gonçalves da Costa**  
Hospital Monte Sinai & Suprema Medical School, Juiz de Fora MG, MG, Brazil

**Background:** Neurological complications of influenza have been scarcely reported possible under-recognized. The full description of clinical forms of the current pandemic influenza is currently underway. Several different neurological syndromes have been associated with influenza but no cases of multi-focal cerebral hemorrhages have been reported so far. The aim of this report is to present 2 cases of multiple focal cerebral hemorrhages in patients with influenza H1N1 pandemic.

**Methods:** Clinical, laboratorial and imaging data of 2 cases of influenza and serious neurological symptoms are presented. Cranial CT scan was performed in both cases. Influenza virus identification in upper respiratory secretion (UPS) was performed by real-time-reverse-transcriptase-polymerase-chain-reaction assay. Cerebrospinal fluid (CSF) was analyzed. No necropsy or brain biopsy were performed

**Results:** Case n<sup>o</sup> 1: 32 years old pregnant woman with flu like symptoms, evolved to severe pneumonia and ARDS requiring mechanical ventilation. She developed comma and cranial CT scan has shown multiple hemorrhagic foci scattered throughout the brain and sub-aracnoid hemorrhage (SAH) (Figure 1). CSF was hemorrhagic. No hemorrhagic disorder was found and eclampsia was ruled-out. Despite oseltamivir therapy she eventually died. H1N1 SOIV was isolated from UPS.

Case n<sup>o</sup> 2: 51 years old male with flu like symptoms evolved to ARDS and respiratory insufficiency demanding ICU and mechanical ventilation. He developed status epilepticus. Cranial CT scan showed multifocal hemorrhages. Lumbar CSF was hemorrhagic. H1N1 SOIV was isolated from UPS. Despite therapy with oseltamivir he eventually died.



**Figure 1: Cranial CT scan showing multifocal hemorrhage in frontal, parietal, temporal and occipital lobes, as well as SAH and intraventricular hemorrhage**

**Conclusion:** Influenza has imposed an under-recognized burden of central nervous system disease so far. Well known neurological syndromes associated with influenza virus in humans have been febrile convulsions, acute encephalitis, acute encephalopathy, Reye's syndrome, Guillain-Barré syndrome, Kleine-Levin syndrome and post encephalitic Parkinson's disease. Some encephalitis cases with petechial lesions in the brain have also been reported. The highly pathogenic H5N1 influenza virus has shown neurological damage in both birds and humans. Current medical literature, including some up-to-date publications of H1N1 pandemic haven't shown the occurrence of multifocal hemorrhagic neurological syndromes. The cases here presented should aware practitioners for such possibility especially because many seriously ill influenza patients in ICU have been kept under sedation so hiding several neurological symptoms.

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**Type: Poster Presentation**

Development of a consensus microarray method for identification of different influenza A viruses

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**Background:** Rapid and reliable identification methods are fundamental for the comprehensive characterization of emerging and evolving influenza viruses. A consensus microarray approach for detection and a panel of specific probes covering 16 hemagglutinin (HA) subtypes of influenza type A viruses has been designed.

**Methods:** Nucleotide sequences of all available influenza A virus subtype combinations, isolated from various species on different geographic regions were selected from GenBank and from influenza virus specific databases (FLU Database, Influenza Research Database). Among them 192 sequences of matrix (M) protein gene and 206 sequences of hemagglutinin (HA) gene were aligned and arranged, respectively, using ClustalX and BioEdit programmes.

**Results:** For the influenza A virus detection oligonucleotide probes were selected on the virus M gene. 16 different segments of this gene were used for the construction of 37 probes. The probes for simultaneous identification of different subtypes were selected on 6 different parts of the virus HA gene. For each HA subtype at least 10 probes were designed on the 1770 nts long segment 4.

**Conclusion:** Oligonucleotide microarray method was designed for simultaneous detection and subtyping of influenza A viruses. The evaluation of developed approach including all 16 HA subtypes is in progress.

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**Type: Poster Presentation**

Clinic-epidemiologic identification of the first wave of AH1N1 in adults in Albania  
**N. Como**<sup>1</sup>, **D. Kraja**<sup>2</sup>, **P. Piper**<sup>3</sup>, **S. Bino**<sup>4</sup>, **E. Meta**<sup>3</sup>, **N. Gjermeni**<sup>3</sup>, **E. Muco**<sup>3</sup>, **A. Kica**<sup>3</sup>, **E. Erindi**<sup>4</sup>, **V. Ostreni**<sup>3</sup>, **R. Petrela**<sup>1</sup>, **S. Cibuku**<sup>3</sup>

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**Background:** Our goal is the recognition of demonstration clinical-epidemiologic of AH1N1 first wave. This material includes 32 cases of ages 15 – 66 years old, during June-September 2009. The influenza identification is based on criteria of WHO.

**Methods:** The cases were classified based on chronology demonstration, origin, group-age and gender. There were analyzed symptoms with what influenza was represented; it was conceived its clinical syndrome based on the subject. There were evidenced and complications.

**Results:** Epidemiological aspects:

Chronological: June 9, July 5, August 17, September 1 case.

Imported cases 27 (England 12, Greece 7, Italy 6, Macedonia 1, Malt 1); autochthon 5.

Based on group-age

Age in years	Number of cases
15-24	10
25-44	13
45-59	6
60-66	3

Where 20 were male and 12 female.

Clinical aspects:

General syndrome appeared with subfebrile temperature

Temperature	Number of Cases
37.60C - 37.90C	2
38.20C- 39.40C	30
with hyperpyrexia	0

The fever extended:

Number of days	Number of cases
1	18
2	9
3	3
4	1
5	1

Symptoms	Number of cases
sweating	32
general myalgia	13
lower extremities myalgia	9
headache	29
diarrhea	6
vomiting	7
loss of appetite	25
weakness	27

Local syndrome was presented as

- 1) Rhinitis (nose congestion 14, exudative rhinorrhea 15, sneezing 17)
- 2) Pharyngitis 18 cases (dryness and sore throat 18, pain of pharynx 13)
- 3) Laryngitis 3 cases with hoarseness and 1 with dyspnea;
- 4) Catarrhal angina 19 cases;
- 5) Tracheitis 17 cases (cough 17, sputum 7, chest pain 10)
- 6) Bronchitis 13 cases (unproductive cough 13, productive cough 9; chest pain 7, dyspnea 2, bronchial rale 13)
- 7) Tracheo-bronchitis 9 cases
- 8) Bronchiolitis 5 cases

14 cases were healthy subjects; 7 cases had chronic diseases (diabetes mellitus 4, cirrhosis 1, cholangitis 1, lymphoma 1); 1 was 4 months pregnant.

Complications:

Diagnosis	Number of cases
Otitis	1
Myositis	1
Encephalopathy	2
Pneumonia	8
ARDS	4 cases (2 with multiorgan failure)

**Conclusion:** 1. AH1N1 first wave extended June- September; the maximum reached on August with 53.12% of cases.

2. Cases were imported from 5 countries; majority 37.5% from England.

3. Affected age fluctuated from 15-66 years old; predominated group-age 25-44 years old, 40.62%.

4. The temperature fluctuated from 37.6- 39.4 0C; 6.25% were subfebrile.

5. There were highlighted 7 clinical syndromes and 6 cases of complications.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Analysis of first cluster outbreak of the pandemic influenza (H1N1) in Georgia

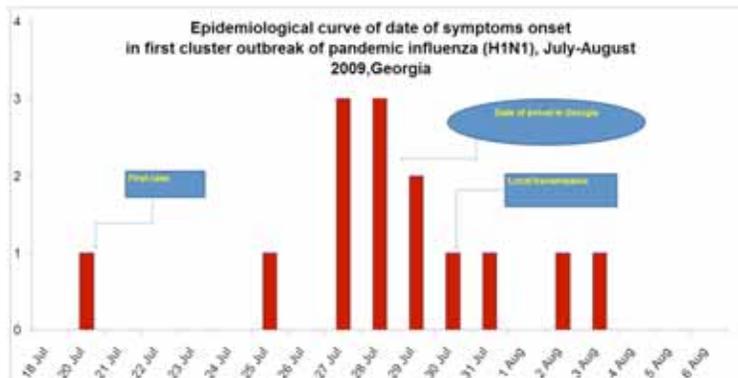
K. Zakhashvili, P. Imnadze, **O. Tarkhan-Mouravi**

National Center for Disease Control and Public Health, Tbilisi, Georgia

**Background:** On 30 July, 2009 a member of a Georgian dance group was confirmed to have influenza A(H1N1). For that time 7 sporadic imported cases of influenza A(H1N1) have been confirmed in Georgia. Group had just returned from touring Western Europe. We investigated transmission of influenza within the group and their close contacts upon return to Georgia.

**Methods:** WHO case definitions of pandemic influenza A(H1N1) virus based on real-time RT-PCR confirmation were used. We collected information regarding age, symptoms, and health practices using a standardized questionnaire. Received data have been analyzed in EpiInfo

**Results:** As of 17 August 2009, we detected 5 confirmed and 9 probable case-patients with influenza A(H1N1) virus (13 of 35 group members, Attack Rate=37%; 1 of 37 in-country contacts, AR=3%). First probable case developed symptoms on 20 July, 2009 during the tour. All the subsequent a case have developed symptoms within the possible period of a transmission of infection from previous cases. Age distribution of cases was 10 to 18 years old; none of 30 adults (4 group members and 26 in-country contacts) was affected. Common symptoms of case-patients were: fever over 38°C (70%), cough (35%), sore throat (35%), runny nose (35%) and, headache (29%). 93% of all cases revealed limited hand-washing during the tour and all of them had been in close contact with each other for 5 days while traveling by ferry and bus. No group members or contacts were ever vaccinated against seasonal influenza. No one received antiviral treatment. No complications or deaths have occurred.



#### **Epidemiological curve of date of symptoms onset in cluster outbreak of pandemic influenza (H1N1), July-August 2009, Georgia**

**Conclusion:** First cluster outbreak in Georgia coincided with summer pick of pandemic influenza A(H1N1) virus in the Western Europe. Investigations showed that more susceptible were under 18 years' old group members and no transmission was identified in adults. Poor hygiene and close contact were the most likely risk factors of transmission among the group. Discontinuation of these conditions after arrival and moderate severity of the disease may explain limited in-country transmission, as evidenced by the differences between the attack rates among group members and in-country contacts. These findings suggest that social distancing and appropriate hand hygiene could have an important role in reducing transmission of A(H1N1) virus.

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**Type: Poster Presentation**

2009 Influenza A (H1N1) first outbreak in a Spanish military training center

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**Background:** The Defense Institute for Preventive Medicine and the Coordination Centre of Sanitarian Alerts and Emergencies (CCAES) were notified the 19th May 2009 of an outbreak of influenza like symptoms (ILS) in a military training center. The Spanish Army Academy in Madrid (Spain) received cadets during the whole year and approximation of 200 recruits monthly for military service distributed in instruction cycles.

The objective of this study is to describe the first outbreak of 2009 influenza A (H1N1) in Spain in a military training center and to analyze the adopted measures to control the virus transmission.

**Methods:** Epidemiological and clinical data were collected with an epidemiological interview to the patients with ILS. During the interview samples of sera and nasopharyngeal swabs were obtained and later processed in the Microbiology National Center by real-time reverse transcriptase-polymerase chain reaction (RT-PCR).

**Results:** Among the 1464 staff and students, 106 suspected cases with ILS were studied.

*Influenza virus A/California/7/2009* was isolated in 31 of the 52 analyzed samples.

Clinical and epidemiological characteristics compatible with 2009 influenza A (H1N1) were present in 76 % (81) of the subjects. Mean age of cases was 22.7 years (range 18-31 years).

Cough (81.5 %), fever (72.8%) and rhinorrea (76.5%) were the most frequent reported symptoms. Total attack rate (AR) was 12.5%. The students of the third cycle suffered 52% of the cases (AR of 21%).

All symptomatic patients were isolated in a separated building (7 days) and oseltamivir prescribed. The persons in contact with people of the cycle affected remained under quarantine without receiving antiviral chemoprophylaxis. The illness was mild in all cases and the symptomatic evolution minor. The outbreak was declared over the 1st June 2009. Neither history of trip out of Spain nor contact with cases out of the military center was found in our cases.

**Conclusion:** The Spanish Epidemiological Surveillance was realized of the probable virus circulation without of history of trips or closed contacts with influenza A confirmed ills.

Early detection of cases benefits the early intervention and adoption of preventive measures in order to contain outbreaks of diseases like the 2009 influenza A (H1N1).

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**Type: Poster Presentation**

Investigation of pandemic influenza A (H1N1) 2009 virus: Isolation and genetic analysis of brazilian strains

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Instituto Adolfo Lutz, São Paulo, Brazil

**Background:** Since April 2009, human cases of respiratory infections originated by novel swine-origin influenza A virus, designated pandemic A(H1N1)v, have been detected worldwide, causing immediate international concern. In Brazil, as of 06 October 2009, there have been 14.229 confirmed cases of infection and 1.167 deaths. Mortality rates of 0.61/100.000 inhabitants were observed.

**Methods:** The virus samples were obtained from clinical specimens from patients who came from the states of São Paulo, Mato Grosso and Distrito Federal. Virus isolation was performed in MDCK cell cultures. Viral RNA was extracted from the infected cells and submitted to reverse transcription-amplification reactions with primers set designed to cover the complete segments of the HA, NA and MP genes. The amplified products were directed sequenced. The complete genes of hemagglutinin (HA), neuraminidase (NA) and matrix (MP) were sequenced.

**Results:** Comparative sequence analysis indicated the presence of point mutations in the HA gene of the Brazilian strains when compared to the reference strain A/California/04/H1N1 (2009). These alterations do not change the all five of the known antigenic sites of the HA protein. The canonical sites for N-linked glycosylation at NxS/T motifs were preserved among strains. Data of the MP sequence analysis revealed that the strains of this study carried the S31N mutation that confers cross-resistance to the adamantane class of anti-influenza drugs. Sequencing of the NA gene showed that the neuraminidase relative drug binding pocket represented by H275 was not altered, yielding the strains sensitive to oseltamivir.

**Conclusion:** These results emphasize the contribution of molecular surveillance, in addition to antigenic characterization to monitor the evolutionary pattern of the pandemic A(H1N1)v, in order to vaccine development, and evaluation of antiviral drugs susceptibility.

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**Type: Poster Presentation**

An analysis of critically ill patients with the novel influenza A (H1N1) virus in Japan

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**Background:** Japan identified its first case of novel influenza A (H1N1) on 9 May 2009, and its incidence has increased steadily. As of 26 October, 34 deaths have been reported. The aim of this study was to summarize the clinical findings of critically ill patients in Japan.

**Methods:** Critically ill patients are defined as confirmed or probable cases who meet one of the following conditions: a case who (1) was diagnosed with influenza-associated encephalopathy, (2) was admitted to an intensive care unit (ICU), (3) was intubated or (4) died. We analyzed a total of 120 cases, including 23 deaths, who developed the disease by 6 October 2009.

**Results:** Of the 120 cases, those aged < 20 years accounted for 71.7%. All 11 cases aged 65 and older resulted in death, while the proportion of deaths among cases aged < 20 years and adults aged < 65 years was smaller (5.2% and 34.8%, respectively). The mean (SD) and median times from onset to hospitalization were 62.8 (40.2) and 48.0 hours, respectively. The mean (SD) and median times from onset to death for the 23 fatal cases were 160.4 (116.5) and 120.0 hours, respectively. The timing of antiviral administration did not appear to significantly affect the clinical course among critically ill patients.

Of the 120 cases, 57 (47.5%) had at least one underlying medical condition. Of the 86 cases aged < 20 years, 27 (31.4%) had one or more comorbidity, with asthma being the most common (22.9%). Similarly, of the 34 adult cases, 30 (88.2%) had comorbidities and chronic respiratory disease (23.5%) was the most common.

**Conclusion:** First, age below 20 were more frequently reported as critically ill than adults. As the epidemic progresses further, the age-composition of severe cases may change. Second, the clinical courses appeared to have been rapid after onset. The time from onset to death in Japan was shorter than those reported elsewhere. Third, approximately half of the cases were accompanied by at least one underlying condition, and this proportion was particularly high among adults.

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**Type: Poster Presentation**

Clinical, radiological features and outcome in children with confirmed infection by 2009 influenza A H1N1, admitted to a reference hospital of Curitiba, Brazilian South region

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**Background:** Since 16 July, when pandemic 2009 A H1N1 virus was considered widespread in Brazil, a substantial number of children with acute respiratory symptoms (ARS) demanded medical services. The impact of this outbreak was significant; especially in Parana, São Paulo and Rio Grande do Sul states (South region). We describe clinical and radiological characteristics of 32 children attended at Hospital Pequeno Principe, a pediatric reference center.

**Methods:** After review medical charts from 133 children attended because of ARS, from 24 July till 16 Oct, we collected data from 32 patients hospitalized for at least 24 hours because of influenza-like illness, and who had rRT-PCR positive test for 2009 A H1N1 influenza virus.

**Results:** The median age was 4.05y (range, 3m-14y1m): 51.61% were < 5 y, and 54.83% males. All patients received oseltamivir, on average 2.25 days (range 1– 22d) after the onset of illness and 87.5% had received antibiotics at the time of hospitalization. The median time of hospitalization was 5.75 days (range 3- 22) and 12.9% need intensive care. Rx exam was performed in 22 children and was suggestive of bacterial infection in 7 (31.81%). Samples for blood culture were taken from 11 children (only 1 positive for CNS). At least one underlying medical condition was found in 35% of children and the most common was congenital heart disease. Two children dead (CFR = 6.25%): both were > 2 y, had underlying conditions (1 cardiac and other pulmonary chronic disease), demanded medical help 5 days after the onset of disease and have not been immunized against pneumococcal, as recommended by National Program of Immunization for at-risk children.

**Conclusion:** These data suggest that early treatment with oseltamivir and antibiotics can benefit children with ARD and should start early in serious cases and immunocompromised children. About 1/3 of children had radiologic condensation suggestive of co-infection by *S.pneumoniae*. The low positivity rate of blood culture do not exclude infection by this bacteria, because of antibiotic use and low sensitivity of this test. It is necessary to divulgate recommendations for immunization against *S. pneumoniae*, that is offered free of charge for at-risk children in Reference Centers for Immunobiologics, but is underused.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Novel influenza A 2009 - A comparison of intensive care unit vs non-intensive care unit patients

**C. Boehringer**

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**Background:** Novel 2009 influenza A cases were first noted in California in mid April. To our knowledge there are no studies to date, which have reviewed duration of illness, fever, treatments and outcomes. We reviewed patient demographics and clinical characteristics in ICU vs non-ICU patients.

**Methods:** From June 2009 to October 2009, 41 patients were admitted to our 620 bed teaching hospital and 80 bed community hospital with confirmed H1N1 by rRT-PCR. 16 patients admitted to ICU- and 25 to non-ICU beds. Data was analyzed using multi-logistic regression analysis.

**Results:** 51.2% Hispanic, 19.5% Caucasian, 17.1% Asian and 12.2% African-American.

Seventeen males; 24 females, 8 were pregnant. The mean BMI 33.4 ICU vs 32.3 non-ICU,  $p = 0.76$ . Mean age for ICU was 43.7 vs 39.6 non-ICU,  $p = 0.46$ . There were 4 deaths.

Fever ( $>37.8$  C) was noted in 75% ICU and 72% non-ICU patients (mean  $38.5 \pm 0.62$  vs  $38.9 \pm 0.75$  respectively). Duration of fever was 4.5 days ( $\pm 4.8$ ) vs 2.2 ( $\pm 2.5$ )  $p = 0.028$ . Of ICU patients 43.8% had leukocytosis (mean 19.6). 3 of 25 (12%) floor patients had leukocytosis, mean 8.9 ( $p = 0.009$ , [95% CI, 1.062, 1.541])). Mean WBC in pregnancy was  $9.11 \pm 2.3$ .

Duration of illness in ICU patients, from onset of symptoms to day of discharge was 19.93 days ( $SD \pm 10.5$ ) vs of 9.4 days ( $SD \pm 4.99$ ) non-ICU ( $p = 0.00013$ ).

Mean days on mechanical ventilation were 14.81 ( $SD \pm 12.71$ ). Days to ER presentation for ICU vs non-ICU patients  $3.4 \pm 3.0$  vs  $2.72 \pm 1.9$  ( $p = 0.236$ ).

Antivirals were received by 15 patients (93.7%); 62% received coverage with 2 antivirals and 62% received steroids.

Treatment for non-ICU patients was 63%. Three pregnant women did not receive treatment ICU treatment with Oseltamivir was begun a mean of 2.8 days into hospitalization and in non-ICU patients, 3.5 days after hospital admit ( $p = 0.52$ ).

Duration of treatment was  $9.8 \pm 4.3$  days and  $5.5$  days  $\pm 0.92$  ICU and non-ICU respectively ( $p = 0.0005$ )

Secondary bacterial infection was found in 2 ICU patients by mini-BAL. One patient with Streptococcus pneumoniae, 1 patient with MRSA and Haemophilus Influenzae (12.5%)

**Conclusion:** Overall mortality was 9.75%, and 18.75% for the ICU. No pregnancy related deaths. All deaths were in ICU patients. A significant increase in length of illness, duration of fever and leukocytosis was seen in ICU patients. No difference was noted in the onset of treatment between the 2 groups but longer treatments in ICU patients were seen. Time of onset of symptoms to ER presentation did not impact outcome. There was no difference in the BMI or age of those in the ICU vs non-ICU.

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Impact of educational measures about influenza A (H1N1) directed to healthcare workers and patients

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Hospital Alemán, Buenos Aires, Argentina

**Background:** The aim of this report is to describe the impact of educational measures during the influenza A(H1N1) pandemic in an acute-care hospital in Buenos Aires.

**Methods:** This is a retrospective descriptive study based on own data and data from the National Health System during epidemiological weeks 20 to 30 (17th May-1st August).

**Results:** Because of the influenza epidemic a crisis committee was organized under the leadership of the Infectious Diseases Department and constituted by the heads of the Internal Medicine and Pediatric Departments, the Emergency Room Coordinator, and the head of nurses. They published a daily report and outlined common guidelines about diagnostic and treatment proceedings, prevention measures, and overall hospital performance. A call center with specially trained physicians answering the phone decreased patients and community anxiety, advising asymptomatic patients or patients without risk to stay at home. Visits to hospital patients were restricted to 2 hours per day and suppressed for pregnant women. Alcohol for hand hygiene was offered at hospital entrances. Respiratory masks were given to symptomatic patients until physician's evaluation.

Of 1520 employees, 1083 in contact with patients, only two servants became infected, thus reflecting overall community risk. The hospital cares for 47000 members, 439 (0.93%) were studied because of influenza A(H1N1) suspicion, media age 15 years, 48% men. Forty-one required admission and 3 died.

Data	Suspected influenza	Confirmed Influenza	Deaths	Deaths/suspected influenza		Deaths/confirmed influenza	
<b>Buenos Aires City</b>	22502	930 (4.13%)	27	0.12%	p<0.01	2.90%	p=0.89
<b>Hospital Alemán</b>	439	48 (10.93%)	3	0.68%		6.25%	

There is a statistically significant difference between the number of patients in which we confirmed diagnosis compared with the overall data in the rest of the city. Also the mortality rate was similar in both populations if only confirmed influenza-patients were regarded, but much higher in ours if the suspected patients were taken in account.

**Conclusion:** The suspicion of influenza in our population was much better established leading to a greater diagnosis accuracy, certainly due to the impact of the educational efforts we did.

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How was the telephone consultation service perceived by the officers who provided it in Japan during swine-origin influenza A (H1N1) outbreak? A qualitative study

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**Background:** For the preparation of pandemic influenza, Japan's government requested to set up telephone consultation service at each prefectural and city government. During the initial period of swine origin influenza A (H1N1) outbreak in Japan, the telephone consultation service received a great number of calls. However, the effectiveness of this system had not been evaluated. This study reports the result of a qualitative study, which explored officers who provided the telephone consultation system during influenza outbreak.

**Methods:** A qualitative study, using semi-structured interviews (n=3), was conducted to the officers of a major city government in Japan, who provided the service. Theory construction was conducted with a use of M-GTA and SCQRM (structure construction qualitative research method).

**Results:** Many difficulties were expressed by the officers during the outbreak. The uncertainty of the disease characteristics, rapidly changing situation, fear of bad outcome of patients due to wrong triage, and miscommunications among health care workers were among the difficulties extracted. While inefficiency was one of the problems extracted, lessening health care burden by triaging patients was one of the achievement they felt the telephone service provided. Development of a practical manual made their provision of service easier.

**Conclusion:** Various aspects of the telephone consultation service during swine origin influenza A outbreak were extracted through the interview. Perception of the officers helped to understand the effectiveness and shortcomings of the telephone consultation service, which may lead to further improvement of it.

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**Type: Poster Presentation**

Epidemiological profile of the A (H1N1) pandemic influenza in the state of Sao Paulo, Brazil, for the April-September 2009 timeframe

**T. R. M. P. Carvalhanas**, A. L. F. Yu, G. D. Freitas, A. C. G. Pellini, A. F. Ribeiro, B. L. Liphhaus  
State Secretary of Health of Sao Paulo, Sao Paulo, SP, Brazil

**Background:** On April 2009, the emergence of a new influenza virus strain set the stage for the first pandemic in the 21st century. The speed of global dissemination of the new virus has no precedents in recorded history. We have analyzed the epidemiological profile of the A/H1N1 pandemic influenza virus – the reported number of cases and deaths, confirmed by lab tests. The geographical scope of the analysis comprised the state of Sao Paulo, Brazil, during the Autumn-Winter season (April to September).

**Methods:** Epidemiological data was obtained from the National System of Acute Notifications (Sinan Influenza on line), which aggregates reported cases in a dedicated system, and are analyzed with the Epi Info/Win and Excel software. Initial definition of suspected cases was similar to that of the flu syndrome (fever, cough – associated or not with headaches, myalgia, and arthralgia). After confirmed evidence of transmission, the definition for acute respiratory syndrome (ARS) – fever, cough and respiratory distress was used. Laboratory confirmation tests were processed at Adolfo Lutz Institute in São Paulo, using real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) in nasal or oropharyngeal specimens.

**Results:** There were 25,082 reported cases till September 2009, from these, 53% had ARS criteria, 29% influenza like illness. Among the 13,651 cases with ARS, 28% were positive for Influenza A(H1N1) and 4% for seasonal flu. The majority of reported and confirmed cases were notified in July and August, declining in September. Infants  $\leq$  24 months and young adults 20-29 years of age were the groups more affected. Case fatality rates were greater in children  $\leq$  2 years and adults (20-59 y).

Cases tested positive for A(H1N1) varied from 20-35% within this period. High risk groups included children  $\leq$  24 months and young adults, patients with chronic pulmonary disease, smokers, patients with metabolic disease and pregnant women.

**Conclusion:** The characteristics and evolution of the cases were similar to those occurring in the northern hemisphere, with predominant circulation of the pandemic influenza virus. These findings can provide elements to define strategies for prevention and control of A (H1N1) outbreak.

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**Type: Poster Presentation**

H1N1 Pandemic: From imported cases in July 2009 to sustained transmission in school clusters in September, Uganda, 2009

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**Background:** In Uganda, since the confirmation of the first case of Influenza A (H1N1) on July 1, 2009, Influenza-like illnesses (ILI), were reported in school clusters in September. The clusters were investigated to confirm ILI and determine the causative strains.

**Methods:** Clinical records were reviewed and specimens collected for laboratory testing. Epidemiological analysis of data was done for descriptive and analytical reporting. The WHO ILI case definition was adapted as any person with fever, cough, headache, runny nose, and sore throat in the last 3 to 5 days. A total of 543 specimens of throat and nasal swabs from different clusters and individuals were collected for laboratory testing and epidemiological analysis. Case based surveillance was administered to all students in the first school cluster (Kitabi Seminary), with ILI from beginning of September 2009 to assess the clinical and possible exposure history. Line lists were used to collect demographic and clinical information from the other clusters.

**Results:** Pandemic influenza A (H1N1) 2009 in was confirmed in 13/18 school clusters. The index case in the first cluster with a sustained H1N1 transmission was a student who came with the infection from the holiday. In the school clusters, the number with ILI symptoms ranged from 50- 430. The major signs and symptoms from the first cluster were cough 341/403(84.6%), headache 330/403(81.9%), sneezing 288/403(71.5%), general body weakness 283/403(70.5%), fever 269/403(66.7%), runny nose 208/403(51.6%), sore throat 104/403(25.8%), and others 126/403(31.3%). The attack rate as determined from first cluster by dormitory of residence ranged from 47.1 - 89.1 per 100 population, with a mean of 76.2 per 100 population. The nested case control analysis from the laboratory database revealed a significant association with confirmed cases of H1N1 and having fever OR 1.68(CI: 1.16-2.45), and cough OR 1.86(CI: 1.2-2.86).

**Conclusion:** The H1N1 pandemic in Uganda has since the confirmation of the first case, reached high levels of person to person transmission resulting in clustered epidemics. Observations from the case control analysis reveal a more likelihood of finding an H1N1 positive laboratory result from a patient with fever and cough compared from the other Influenza-like illnesses.

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Factors associated to death in patients with confirmed diagnosis for influenza A/H1N1. State of Sao Paulo, Brazil, April to October, 2009

**A. Ribeiro**, A. C. G. Pellini, T. R. M. P. Carvalhanas, B. D. L. Liphhaus, A. L. F. L. F. Yu, G. D. Freitas, M. D. C. S. T. Timenetsky

State Secretary of Health of Sao Paulo, Sao Paulo, SP, Brazil

**Background:** In April, 2009, a new subtype of influenza A/H1N1 virus emerged from the American continent, spreading worldwide. Until the end of October, 2009, WHO reported more than 440.000 influenza A/H1N1 laboratory-confirmed cases, registering more than 5.700 deaths. Studies point out that population groups who develop higher severity and mortality are: children less than two years of age, young adults, patients with chronic diseases and pregnant women.

**Methods:** An exploratory analysis was performed to identify factors associated with death among confirmed cases of influenza A/H1N1, considering the residents and other people treated in healthcare facilities in SP, from EW 16 to 41. Data were collected from the *Information System of Notifiable Hazards – Influenza Online*. Association between remarkable expositions and death caused by influenza A/H1N1 was investigated by unadjusted odds ratio estimation, with 95% confidence intervals, employing the EpiInfo software.

**Results:** In SP, since EW 16, 31.589 influenza A/H1N1 suspected cases were reported, from which 5.242 (16,6%) were confirmed and fulfilled the criteria for Severe Acute Respiratory Disease. Among these, 372 cases resulted in death, 3.619 were discharged from hospital and 1.251 are still under investigation. The following variables showed association with death caused by influenza A/H1N1: chronic cardiopathy OR=3,00 (IC95%=2,05-4,40); chronic renal failure OR=3,85 (IC95%=2,02-7,33); immunosuppression OR=4,93 (IC95%=3,35–7,25); tobacco abuse OR=1,52 (IC95%=1,03-2,26); chronic metabolic disease OR=6,36 (IC95%=4,27–9,48); diarrhea OR=1,56 (IC95%=1,16-2,10); residence in the metropolitan area of Greater Sao Paulo in relation to the interior of state OR=1,37 (IC95%=1,11-1,70). Variables which did not present association with death were: lack of previous vaccination against influenza OR=1,65 (IC95%=0,93-2,93) or pneumococcus OR=1,22 (IC95%=0,44-3,39); chronic pneumopathy OR=1,14 (IC95%=0,80-1,61); haemoglobinopathy OR=1,74 (IC95%=0,60-5,04).

**Conclusion:** Widespread knowledge of influenza A/H1N1 epidemiology, its complications and risk factors for death is extremely important to support the implementation of strategies to prevent and control this disease in high-risk groups. Comparison of data found in the assessed population with other similar studies is recommended.

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H1N1 Epidemic: Our experience at PGIMER Delhi, India

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**Background:** Patients infected with the H1N1 strain of the influenza A virus frequently develop rapidly progressive lower respiratory tract disease resulting in respiratory failure. We describe the clinical characteristics of patients who expired in ICU at our hospital in Delhi, India. We did the study to see cause and pattern of death in H1N1.

**Methods:** Using guidelines developed by the Indian health ministry, all individuals with flu-like symptoms were screened by a physician into three management categories- Category A (out-patient treatment and home isolation), Category B (hospital isolation ward) and Category C (intensive care units) based on clinical presentation. Of the 236 patients with confirmed H1N1 evaluated between 8th August to 27th October 2009 at our facility, 26 patients expired. Data was collected from the hospital records for patients with H1N1, who expired in the ICU. Influenza A (H1N1) virus infection was confirmed in specimens using TaqMan real-time reverse transcriptase-polymerase-chain-reaction (*CDC Protocol*).

**Results:** Median age was 29 years (Range: 12 - 74); >80% of patients were < 40 years. Three (11.5%) were diabetic, 2 (7.7%) pregnant, 5(19.2%) hypertensive, 2(7.7%) had pre-existing respiratory disease and 14 (53.9%) had no pre-existing medical conditions. 24 (92.3%) had primary viral pneumonitis and 2 (7.7%) had exacerbation of structural respiratory disease. 14 (53.9%) developed acute respiratory distress syndrome (ARDS), 4(15.4%) developed multi organ dysfunction syndrome (MODS), 3 (11.5%) developed acute renal failure (ARF), 2(7.7%) developed myocarditis/Congestive heart failure (CHF) and 3(11.5%) developed disseminated intravascular coagulopathy (DIC).All 26 required mechanical ventilation. Oseltamivir administration delay ranged from 3 to 10 days after illness onset, 52.78% received high-dose (300mg/day) treatment, and treatment duration ranged from 1 to 10 days (mean  $3.0 \pm 2.5$ days).

**Conclusion:** Over a 12 week period, H1N1 virus infection caused ICU deaths in 26 adult patients with a high case fatality rate of 26/236 (11.0%). Deaths resulted from organ failures including ARDS, MODS, ARF, DIC and CHF. Clinicians should be aware of the severe organ complications of H1N1, particularly in pregnant and previously healthy young adults

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Deaths in women during fertile years and pregnant women as confirmed for pandemic influenza A (H1N1) in the state of São Paulo

**A. Ribeiro**, G. D. Freitas, A. C. G. Pellini, T. R. M. P. Carvalhanas, A. L. F. Yu, B. D. L. Liphaut, M. D. C. S. T. Timenetsky

State Secretary of Health of Sao Paulo, Sao Paulo, SP, Brazil

**Background:** Since 16 July, the new influenza virus A (H1N1) was considered widespread in Brazil. Some groups had more serious evolution, with higher hospitalization and mortality rates. The risks for complications and deaths were higher in children under two years of age, young adults, patients of chronic diseases and pregnant women.

**Methods:** Analysis was performed to identify factors associated with death among confirmed cases of influenza A/H1N1 in women during their fertile years (WFY:10-49 years of age) and included pregnant women, both with rRT-PCR positive for influenza A (H1N1). Data were collected from the national bank for influenza A (H1N1) reporting system, during the epidemiologic weeks (EW) 16 and 41, 2009. Software employed: Epiinfo.

**Results:** In this period (EW16- 41), we identified 31,589 suspicious cases of influenza A (H1N1); from these, 9,350 (29.6%) were confirmed, 27% (N=2,548) in WFY and 26% (n=668) in pregnant women. The case fatality rate in pregnant women was 7.5% (N=50). The proportion of gestation in this group is 26%, higher proportion when compared with population. The first pregnant who died was registered in the EW 26 and the last one, in EW 39, most of cases were registered during WE 28 and 29. Risk factors associated were: chronic diseases: cardiopathies OR 2,5 IC95%1,2-5,3; immunosuppression OR 2,7 IC95%=1,5-5,0; metabolic diseases OR 4,9 IC95% 2,5-9,7 and renal diseases OR 3,2 IC95%

1,2-8,5. Among the women during their fertile years, pregnancy was not associated to the deaths OR 0,9 IC95% 0,7-1,3. In pregnant women there was an association to death with the second and third trimester of pregnancy OR 4,3 IC95%=1,3-14,1.

**Conclusion:** These results confirmed that pregnant women should be included in at-risk for more severe disease. For death, the risk is more important after second trimester. It is essential to alert people and doctors that pregnant women should have better control of healthy conditions, and start immediately antiviral if they develop symptoms of influenza like illness. This group should also be prioritized for influenza vaccination

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The impact of the “novel” influenza A (N1H1) pandemic in Norway, as monitored by recorded fatal cases - end of October 2009

**V. Hasseltvedt**

Sykehuset Innlandet Trust, Lillehammer, Norway

**Background:** This paper deals the with impact of the pandemic of “novel” influenza A (N1H1), in Norway, as documented by experiences from the end of April 2009 to the end of October 2009, as monitored by recorded fatal cases, during the six months up to October 25, 2009.

From the 1980s the excess mortality from “seasonal flu” has been estimated ranging from 1000 to 1500 cases during the time span of the outbreak. The duration of “seasonal flu”, caused by influenza virus A has been estimated to be approximately 10 weeks. The Norwegian projections given by The Directorate of Health, on April 27, 2009 (source- URL: dagsavisen.no) gave a scenario of: up to a possible 13 000 deaths - and up to 1.2 million infections – caused by “novel” influenza virus A (H1N1).

**Methods:** Data on Norway’s preparedness and surveillance - available from bodies and sites (URLs) comprise some of the following, The Norwegian Public Health Institute (fhi.no), The Directorate of Health (helsedirektoratet.no) – including the general information given at The Norwegian Governmental Official site - (pandemi.no).

The projections and scenarios given by these governmental bodies have been compared with data from Norwegian sources – as well as from - promedmail.org and WHO data, as of October 25, 2009 - from who.int/csr.

**Results:** From the end of April 2009 to the end October 25, 2009 13 deaths were recorded. Many of these individuals had predisposing illnesses/ conditions, which may have contributed to a fatal outcome. The known impact has been 13 recorded deaths, so far, - not 13 000 deaths - i.e. one thousandth – in contrast to the worst-case scenario.

**Conclusion:** The documentation above highlights uncertainties concerning projections made in an early epidemic/pandemic phase. It also might give reasons for caution – when extrapolating small data sets – in order to give short/intermediate term projections, to health professionals, as well as to the public in general.

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A survey in Taiwan 2009 for the public perception and the willingness to be vaccinated of pandemic influenza A (H1N1)

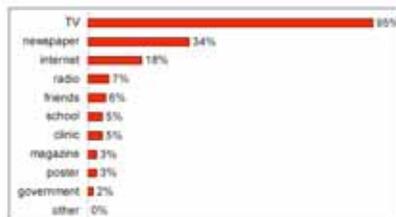
**W.-S. Jhao**, T.-H. Wang, L.-L. Ho, H.-P. Huang, C.-M. Chen, Y.-P. Chou, M.-C. Peng  
 Taiwan CDC, Taipei, Taiwan, R.O.C.

**Background:** Taiwan established H1N1 Central Epidemic Command Center on April 27, 2009 while swine flu outbreak developed in Mexico. The first wave communication was focusing on border quarantine and travel alert. The second wave communication drifted to self prevention and the treatment after the first death on July 30. Until October 26, there are 423 H1N1 hospitalized cases and 26 deaths with fatality rate round 0.11%.

**Methods:** Telephone survey targeting people aged 18 and older knowing H1N1 flu sampling by the Computer Assisted Telephone Interview (CATI) was conducted during October 1-3 2009, Questionnaires were developed under the theoretical framework of the KAP model. After excluding 50 cases of respondents who haven't heard of H1N1 flu, 1,123 valid interviews have been successfully collected at 95% confidence level with sampling error is  $\pm 2.99\%$ .

**Results:** Our survey explored that the perceptions of the respondents' to H1N1 flu such as the transmission routes, treatment and vaccine for prevention have all reached to 70%. The perception of the preventive measures such as hands-washing, seeing doctor immediately after the symptoms develop reached to above 90%. The main channel of H1N1 information was TV (95%), followed by newspaper (34%) and internet (18%). 95% of the respondents have prepared some materials for H1N1 evention such as face mask(85%), thermometer (78%), or handkerchief or tissue paper (77%). Almost 60% worried that the H1N1 outbreak will be more serious during fall and winter in Taiwan. More than 50% worried about contracting H1N1 flu. 58% would like to receive H1N1 shot and 25% won't mostly due to safety concern of vaccination (45%). 17% haven't made their decision yet.

Table1 Where people get their information?



**Table1 Where people get their information?**

**Conclusion:** Most people in Taiwan have good awareness and right perception to this novel influenza, the media played a critical role for the public awareness. The worrisome about the epidemic on autumn and winter commonly existed and drove the residents doing something such as stockpiling the face masks. 37% complained of the exaggerations of the media reports of H1N1. Striking the balance of awareness and the panic of the public is the challenge for the public communication of the H1N1.

Table2 Important attitude analysis of demographic variables

		Worrying H1N1 would be more serious during fall and winter	Worrying themselves may get H1N1 flu	Willing to accept H1N1 flu vaccine
Gender	Male (n=542)	52% **	50% *	64% **
	Female (n=581)	61%	57%	53%
Age	Less than 29 (n=246)	62% **	58%	58%
	Older than 29 (n=877)	55%	52%	58%
Education	Under than university (n=624)	58% ***	53%	59%
	Up than university (n=479)	55%	54%	56%

\* : p<0.05 \*\* : p<0.01 \*\*\* : p<0.001

**Table2 Important attitude analysis of demographic variables**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

The outcomes of H1N1 09 screening at the 17th World Transplant Games

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**Background:** The 17th World Transplant Games occurred on the Gold Coast, Australia August 22 to 30, 2009, at the end of the winter season in the southern hemisphere. Due to concerns related to the virus and after 4 countries withdrew altogether, a mandatory screening process and influenza clinic was instituted to assist in the health and safety of the participants. This review outlines the procedure and outcomes of this process.

**Methods:** Screening consisted of a medical questionnaire and tympanic temperature scan, conducted by 4 nurses and 2 doctors. Positive symptoms and/or a temperature >37.5 C (99.5.F) were referred for physician assessment. The BinaxNOW test kit (Inverness Medical, Maine USA) was used for the rapid diagnosis of influenza A. Screening attendance was cross referenced with the registration database, and individual team managers helped reinforce screening compliance and isolation processes.

**Results:** A total of 2125 individuals presented for screening over a 2.5 day period, arriving from over 45 countries. Only 8 registered athletes and supporters failed to attend for screening however 29.4% of the volunteer group failed to attend screening and 7.5% of volunteers did not register for the Games altogether.

Only 0.5% of attendees required further medical evaluation. Of these, 68 people were deemed to have had a mild viral illness of either respiratory or gastrointestinal origin. 31 people were swabbed nasally and all returned a negative result with the BinaxNOW Rapid Influenza test, 7 were clinically deemed non infectious, 14 were placed in home isolation for 48 hours, treated conservatively and medically reassessed, 9 were prescribed influenza prophylaxis and 7 convention centre staff were instructed to take a medical leave of absence until symptoms resolved.

Separate to this process, just fewer than 10% of participants were prescribed influenza prophylaxis by their regular physicians. This practice varied by country and by registration status.

**Conclusion:** The screening process was limited by time constraints and volunteer compliance. No documented cases of H1N1 09 were identified during the Games; however 25 conservatively treated upper respiratory viral illnesses were managed in the clinic.

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Epidemiology of severe pediatric patients with novel influenza A (H1N1) in Korea

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**Background:** Since the first outbreak of novel influenza A (H1N1) in May 2009, the virus has been spread throughout local communities. More than 4,000 diagnosed cases are being reported daily as of November 2009. One of the major infection routes is the educational institutions, so children and teenagers have high risk of viral exposure. Korea Centers for Disease Control and Prevention (KCDC) is operating nationwide monitoring system for severe hospitalization cases.

The objective of this study is to highlight demographics, infection risk factors and clinical courses.

**Methods:** Influenza A (H1N1) patients who were hospitalized in ICU or had pneumonia in needs of intubation were categorized as severe pediatric patients. Between June and October, total of 22 cases under the age of 18 were identified as severe patients. After the medical chart review, we had an interview with the doctor in charge. All the patients were laboratory-confirmed influenza A (H1N1) virus infection by means of real-time PCR. Based on the Advisory Committee on Immunization Practices, the patients with high-risk medical conditions were defined as having higher risk for influenza complications.

**Results:** Among the reported 22 severe cases, 15 were male and 7 were female. Ages ranged from 2 months to 18 years old (median 7, standard deviation 5.4). Fourteen patients (63%) had high-risk medical conditions such as 1) age less than 59 months (6 cases), 2) chronic respiratory disease (3 asthma cases), 3) neuro-developmental disorder (3 cases), 4) congenital heart disease (1 case) and 5) leukemia (1 case). Total of 7 patients have expired. Patients took anti-viral agent (Tamiflu®) average 2 days after onset. Thirteen patients received ventilator care, 7 did not and 2 were unsure. Viral pneumonia was the most common complication (17 cases, 77%) and 3 patients exacerbated into acute respiratory distress syndrome. Initial symptoms were fever and cough (18 cases, 81% each). There were 11 leukocytosis, 3 leucopenia and 3 thrombocytopenia cases on complete blood count.

**Conclusion:** Half of patients with high-risk medical conditions have expired. Considering current situations, we need to maintain high-risk medical conditions category and to have continuous tracking for severe pediatric patients with influenza A (H1N1).

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Epidemiologic characteristics of deaths associated with 2009 pandemic influenza A (H1N1) virus infection in Korea

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**Background:** The 2009 pandemic influenza A(H1N1) virus has emerged to cause the first pandemic of the 21st century. Clinical and epidemiologic features of fatal cases associated with 2009 influenza A(H1N1) infection is a important information to manage the disease

**Methods:** Nationwide enhanced public health surveillance of Korea residents who were died with laboratory evidence of pandemic 2009 influenza A(H1N1) infection reported to the Korea Centers for Disease Control and Prevention between August 15 and November 05, 2009

**Results:** During the study period there were 51 cases of death due to pandemic 2009 influenza A(H1N1) infection reported in Korea. Among the 51 cases, the median age was 55 years (range, 2 months-83 years) ; 27(52.9%) were male and 24(47.1%) were female. The median time form symptom onset to diagnosis was 3days(range : 1-16days) and from symptom onset to death was 6days. Antiviral drugs were administered to 42patients(82.4%). 24patients(57.1%) of them start antiviral medication before diagnosis and others start antiviral medications as soon as the influenza confirmed. 42 patients(82.4%) had risk factors for seasonal influenza complications such as 1) age less than 59months were 4 patients, 2) age more than 65years were 22 patients(19 cases of these had chronic medical conditions), 3) chronic medical conditions were 16 patients(malignant tumor, DM, chronic lung disease and chronic renal disease). 46 patients(90.2%) of those had pulmonary complication like pneumonia or acute respiratory distress syndrome. Evidence of coinfection was found in specimens from 19.6%(10/51) patients, including 6 caused by bacteria and 2 caused by fungus

**Conclusion:** Fatal cases from 2009 influenza A(H1N1) in Korea occurred in high-risk patients and was associated with viral pneumonia and severe acute respiratory distress syndrome. Considering current situation, we need to manage high-risk patients actively.

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The effect of influenza-like illness symptoms and laboratory confirmed influenza A on hand hygiene and other health habits among US University students

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University of Michigan School of Public Health, Ann Arbor, MI, USA

**Background:** Although hand hygiene and other non-pharmaceutical interventions have been used for mitigating influenza, the effect of influenza illness on adherence with these measures is unknown.

**Methods:** Data analyzed came from a cluster randomized intervention trial held at the University during the 2007-2008 flu season. Immediate and sustained effects on hand hygiene and reduction in social contacts from clinically verified ILI and seasonal influenza A were examined using lagged regression to estimate beta ( $\beta$ ) coefficients and 95% confidence intervals. Separate analyses were conducted for comparing (1) clinically verified ILI cases, non-clinically verified ILI cases, and healthy ILI-free controls (no reported ILI either clinically or on surveys); and (2) influenza A cases, clinically verified or survey reported ILI cases not positive or tested for influenza, and healthy ILI-free controls. Clinical ILI and influenza cases were further dichotomized by ILI symptom severity (ss) based on their mean value.

**Results:** During the week of illness verification, clinically verified ILI cases reported washing hands less than participants with non-clinically verified ILI and healthy controls (i.e. non-clinical ILI cases) ( $\beta=-1.04$ ,  $p=0.03$ ; high ss cases vs. low ss/non-clinical ILI cases:  $\beta=-1.55$ ,  $p=0.02$ ; low ss cases vs. high ss/non-clinical ILI cases:  $\beta=-0.52$ ,  $p=0.56$ ). Clinical ILI cases also reported using hand sanitizer more, spending less time handwashing, and spending fewer hours in their own residence hall rooms during the week of illness verification (all  $p>0.05$ ). Comparing influenza A cases to ILI only cases and controls (i.e. non-flu cases) during the week of viral confirmation, flu cases reported spending fewer hours in their own residence hall rooms ( $\beta=-2.56$ ,  $p=0.01$ ; high ss cases vs. low ss/non-flu cases:  $\beta=-3.52$ ,  $p=0.03$ ; low ss cases vs. high ss/non-flu cases:  $\beta=-1.60$ ,  $p=0.56$ ). However, flu cases reportedly had better hand hygiene than non-flu cases (all  $p>0.05$ ). No sustained effects were observed.

**Conclusion:** Although adherence to non-pharmaceutical interventions varied by clinical ILI and seasonal influenza A occurrence, the temporal changes were not statistically significant. Emphasis on the importance of complying with non-pharmaceutical recommendations such as hand hygiene and voluntary reduction in social contacts is needed to potentially mitigate disease spread among university students on campus.

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The progressive expansion of the Novel A (H1N1) v epidemic in the EpiSouth region (Mediterranean and Balkans)

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<sup>1</sup>Institut de Veille Sanitaire (InVS), Saint Maurice, France, <sup>2</sup>Istituto Superiore di Sanita, Rome, Italy

**Background:** EpiSouth is a network covering 26 Mediterranean and Balkan countries. Since April 2009, all continents have been progressively affected by the A(H1N1)v influenza pandemic.

**Methods:** In the scope of the project, EpiSouth countries shared, on a voluntary basis, information regarding their confirmed cases, case definitions and cases management strategies. Data concerning confirmed cases were analysed on a weekly basis.

**Results:** The first confirmed case was reported on the 27th of April in Spain. As of 06 July 2009, 2,577 confirmed cases were reported by 24/26 countries. The most affected country was Spain (776 cases) followed by Israel (681) and France (330). The pandemic spread within the four EpiSouth sub-regions was slightly different: the number of cases started to increase markedly first among EpiSouth EU countries (week 19), followed by Middle-East (week 23), and finally North Africa and Balkans (Week 27).

These different dynamics can be partly explained by the historical or socio-economical links existing between countries. The more rapidly affected EpiSouth countries (e.g. Spain, Israel or France) are those with close links (e.g. numerous direct daily flights with the Americas) while countries with less direct or frequent connections could delay longer the implementation of a local cycle of transmission. Later, population movements within EpiSouth countries also contributed to a further pandemic spread (e.g. cases exported from France to Algeria, Slovenia and Tunisia and from Spain to Lebanon and Serbia). The third phase was linked to relations with neighbouring areas e.g. Saudi Arabia exported cases to several EpiSouth countries.

**Conclusion:** While all countries were faced with the same difficulties regarding implementation of control measures, the ongoing information exchange between countries has proven its importance.

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**Type: Poster Presentation**

An outbreak of influenza A pandemic (H1N1) 2009 in a residential home for the disabled in Hong Kong and detection of the first local case of oseltamivir-resistant infection

**W. H. A. Ieung, L. T. T. LOH**

Centre for Health Protection, Hong Kong, China

**Background:** In Hong Kong, outbreaks of pandemic influenza A (H1N1) virus infection occurred in institutions since June 2009. The Centre for Health Protection (CHP) of the Department of Health carried out epidemiological investigation and provided oseltamivir chemoprophylaxis for residents of residential home for the physically and mentally disabled for confirmed influenza outbreaks. We described the epidemiological findings of an outbreak with a case of oseltamivir resistant infection detected.

**Methods:** We calculated the effectiveness of seasonal influenza vaccination 2008-2009 which comprised A/Brisbane/59/2007 (H1N1)-like virus, A/Brisbane/10/2007 (H3N2)-like virus and B/Florida/4/2006-like virus in protection against pandemic influenza A (H1N1) 2009, and the effectiveness of oseltamivir chemoprophylaxis. Thirty-four respiratory specimens (15 nasopharyngeal aspirates, 15 nasopharyngeal swabs and 4 throat swabs) were taken from 34 residents for realtime RT-PCR testing for pandemic influenza A (H1N1) 2009. All of the 21 positive samples were further tested for antiviral resistance.

**Results:** Seasonal influenza vaccination did not confer protection against pandemic influenza A (H1N1) virus (OR 2.23, 0.70 to 7.00;  $p > 0.05$ ), but oseltamivir prophylaxis was found to be effective in preventing disease (OR 0.31, 0.10 to 0.98;  $p < 0.05$ ). Overall compliance with oseltamivir chemoprophylaxis was satisfactory (94.5%). Two staff members who were offered oseltamivir reported early discontinuation due to side effects while two others did not start the medication at all. Oseltamivir resistance in influenza A (H1N1) virus infection was detected in one of the residents who had been given oseltamivir prophylaxis for 6 days. There was no evidence of spread of the resistant strain in the outbreak.

**Conclusion:** Oseltamivir chemoprophylaxis was effective in reducing the transmission of pandemic influenza A (H1N1) virus infection in long-term care facilities during outbreak. Clinicians, microbiologists and public health physicians should be alerted to the possibility of emergence of oseltamivir-resistant viruses in patients who have received chemoprophylaxis.

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Evaluation of direct immunofluorescent assay (DFA) and rapid antigen test (RAT) for diagnosis of new pandemic influenza A H1N1 2009 (FLU AH1N1) during first wave in Santiago, Chile

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**Background:** Since May 17th 2009 (epidemiological week 20th), the new strain of influenza A H1N1 was detected in respiratory samples of symptomatic patients in Santiago, Chile. The circulation of the virus lasted 11 weeks, with a peak between weeks 25-27th. The objective of our study was to evaluate the performance of influenza tests for diagnosis of FLU AH1N1.

**Methods:** Nasopharyngeal swabs were taken from in and outpatients with influenza like illness (ILI), between June 1st and July 19th of 2009 (weeks 23-29th) and the results of DFA and RAT were compared using RT-PCR FLU AH1N1 (Light mix Kit Influenza A virus M2 and Light Mix Kit FLU A swine H10 of TIB MOLBIOL) as gold standard. We analyzed sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of DFA (D3 Ultra 8 DFA Respiratory & Identification Kit à de Diagnostic Hybrid) and RAT (QuickVeuÒ of Biomerieux). Results were compared by age group and over three different periods of the outbreak: increasing, peak and decreasing.

**Results:** 510 patients had RT-PCR for FLU AH1N1 with simultaneous DFA, 385 with RAT and 48 with both tests. Average age with DFA was 25,8 years (1 month-108 years, 53% females) and with RAT 32,9 years (2 months-108 years, 51% females), ( $p < 0,0001$ ). Comparing periods of the outbreak, DFA sensitivity was 58%, 77% and 81% in ascending, peak and descending period, respectively ( $p < 0,001$ ) and specificity was 90%, 83% and 91% respectively ( $p > 0,05$ ). Evaluating RAT, sensitivity was 41%, 61% and 67% ( $p < 0,001$ ) and specificity was 87%, 96% y 92% ( $p > 0,05$ ) in different periods.

DFA and RAT for diagnosis of FLU AH1N1

	DFA	RAT	DFA+RAT
Sensitivity	75*	59	60
Specificity	87*	94	94
PPV	80	88	82
NPV	84	74*	84

Sensitivity and Specificity of DFA and RAT in different age groups

	< 1 year	1-4 years	5-14 years	15-54 years	≥ 55 years
DFA sensitivity	77	72	87*	71	35*
DFA specificity	94	87	74*	87	92
RAT sensitivity	66	54	60	62	45*
RAT specificity	83	95	94	92	96

\* Significantly different,  $p < 0.001$

**Conclusion:** DFA had better sensitivity than RAT for diagnosis of FLU A H1N1. Sensitivity varies with age and periods of the outbreak; it is worse in ≥55 years old patients and at the beginning of the outbreak. Using both tests simultaneously doesn't improve sensitivity. It's important to consider the age of patients and the relative period of the wave to decide the exam to perform.

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Influenza A (H1N1) pandemic in Argentina. Experience in two private general hospitals during the outbreak (June 2009)

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**Background:** On June 14th 2009 the first confirmed case of Influenza AH1N1 was hospitalized in our institution, four days after the WHO declared the pandemic.

**Methods:** We retrospectively compared the clinical and radiological findings between the 2 groups of adult patients tested positive or negative for H1N1 RT-- PCR assay (nasal swab).

**Results:** A total of 73 adults were hospitalized with presumptive diagnosis of Influenza AH1N1, from June 14th to October 18th 2009. Tests were positive for 21 patients and negative for 52. The mean age was 43 years (range 16-85), male/female ratio: 13/8 in the positive results group and 56 years (range 17- 92) male/female ratio: 29/23 in the negative. There were no significant differences in age (p: 0.14) and sex (p: 0.29). Among the 21 positive cases 15 (71%) had preexisting medical conditions, obesity being the most frequent 6 (28%). Among the 52 negative adults 38 (73%) had preexisting medical conditions, HTA being the most frequent 9 (17%). The following symptoms were present at admission in both groups (positive/negative): fever 95%/ 83%, myalgia 76 %/ 35%, coughing 67%/ 65% and shortness of breath 38%/ 31%.

Among the 21 positive cases, both CT scan and chest X ray were obtained in 15, 5 patients only had chest X rays. There was only 1 patient showing normal images. Of the 52 negative cases, 42 had both CT scan and chest X-ray, 4 had only chest X-ray and 6 had no diagnostic images. Only 3 patients showed normal images.

Table 1. Radiological findings

<b>Radiological Pattern</b>	<b>n positive A H1N1(%)</b>	<b>n negative A H1N1(%)</b>	<b>P-value</b>
Interstitial	7/20 (35)	11/43 (25)	(p: 0.21)
Bilateral	16/20 (80)	26/43 (61)	(p: 0.27)
Pleural Effusion	5/20 (25)	11/43 (24)	(p: 0.61)
Lymphadenopathy	11/14 (79)	34/40 (85)	(p: 0.42)
Peripheral localization	4/20 (20)	12/43 (28)	(p: 0.36)

**Conclusion:** No significant differences were found in age and clinical presentation at admission, although mean age was inferior and myalgia was more frequent in the confirmed A H1N1 group. There were no significant differences in radiological findings, which could be attributed to the small size of our study population.

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Epidemiologic and clinical finding of A H1N1 2009 pandemic influenza in the Dominican Republic

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**Background:** The influenza virus belongs to *Orthomyxoviridae* family and is classified into three types of virus A, B and C. These viruses have two major surface glycoprotein: hemagglutinin (H) and neuraminidase (A) which helps the ranking of multiple subtypes.

One of the most important characteristics is their ability to mutation and antigenic variation. Influenza A has been linked to catastrophic pandemic. The last of these is called Swine flue (influenza A H1N1), which since its inception has caused great alarm, high morbidity and mortality especially in pregnant women. This is a sample of the features of the disease in Dominican Republic.

Dominican Republic is a tropical country located in the Caribbean that receive substantial annual number of tourists, which makes influenza outbreaks throughout the year: both seasonal peaks in the northern and southern hemisphere.

**Methods:** The Epidemiology National Program starts a surveillance since the epidemic arise at level 5, at epidemiological week 16.

Diagnosis confirmation was made with rt-PCR for A H1N1 to all symptomatic respiratory cases.

**Results:** Until the 40th epidemiology week, we have 2,664 suspicious cases, 997 (37%) hospitalized, 441 confirmed cases, from this 191 (45%) hospitalized, and 22 (5%) death, 76 (17%) cases were pregnant women with 16% mortality.

Clinical manifestations were: fever (94%), cough (89%), nasal congestion (76%), headache (71%), muscle pain (67%), sore throat (60%), dyspnoea (45%), nausea (25%), rales (21%), vomiting (18%) and diarrhoea (11%).

Pregnancy was one of the more important risk factor associated with mortality.

Necropsy findings where: edema, hemorrhage and necrosis in lung tissues, frequently associate with intravascular thrombosis.

High mortality was cause due a delay in search of medical attention and no suspected diagnosis from health cares personel.

**Conclusion:** It must be increased the Primary Care Services to obtain an early diagnosis of the disease, and began the antiviral treatment in high risk populations, when this pathology is suspected, mostly in pregnant women.

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Prolonged prophylaxis for A H1N1 Influenza pandemic in Oncohematologic patients - Adherence, security and efficiency.

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**Background:** Since 1 May to 31 October 2009, 1,241,612 cases of a kind of Influenza disease were notified in Argentina. 24,504 virologic samples were processed, confirming that 10,248 were cases of AH1N1 Influenza, there were 12,471 cases that required hospitalization and 600 confirmed deaths.

Oncohematologic patients were in the high risk group to this infection. As a preventive strategy a prolonged prophylaxis with oseltamivir was administered during 30 days.

Our objective was value the adherence, security and efficiency of the prolonged prophylaxis against AH1N1 Influenza with oseltamivir in an oncohematologic patients cohort.

**Methods:** From July to September 2009, a prospective study was performed from a cohort of 45 oncohematologic patients above 19 years old, that received a 75 mg daily dose of oseltamivir during 30 days. Then, by an anonymous survey, age was consulted, as well as sex, oncohematologic pathology, treatment performance, adverse effects that patients presented, doses that were used, whether and why medication was interrupted, concomitant medication and whether the patient had to consult the doctor during that period of time.

**Results:** Nor Influenza Syndromes were registered in the cohort, neither Influenza cases during the administration of such prophylaxis. Medium age was 54 years (range 19-87), 22 females and 23 males. Basal diseases were Lymphomas in 42, 2 % of the cases; Acute Leukemias in 24 %; Multiple Myeloma in 17, 8 %; Hodgkin disease in 11,1 % and Myelodysplasia in 6,7 %.

From 45 patients, 24 (53, 3 %) presented adverse effects, 60 % with digestive symptoms, 18 % neurologic and 15 % fatigue.

In 34 patients (75, 5 %) a 100 % adherence was achieved. From those patients that did not finish the treatment, 5 said that it was because they had a digestive intolerance, 3 did not give their reasons and another one because he/she began late.

No serious adverse effects requiring hospitalization were objectived.

There were 32 patients that were already receiving other medication during antiviral prophylaxis, and only 6 patients had to consult their doctor during such period of time.

**Conclusion:** Daily prophylaxis efficiency with oseltamivir was successful in 100 % of the cases. The adherence to oseltamivir was partial (75,5%) mainly because of digestive intolerance. No serious adverse effects were detected.

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Homologous and heterologous immune responses to naturally-acquired influenza virus infection

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**Background:** Humoral antibody responses, typically measured by hemagglutination inhibition (HAI) and viral neutralization, are common measures of immunity to influenza virus infection. An antibody titer of greater than 1:40 is thought to correlate to around 50% protection to infection, while a 4-fold rise in antibody titers between two times is evidence of infection during that interval. Infection from a specific strain of influenza can elicit an antibody response against other strains of influenza, which may correlate with protection.

**Methods:** Subjects presenting with influenza like illness (ILI) were recruited from general outpatient clinics throughout Hong Kong. Those subjects who tested positive for influenza by a rapid diagnostic test, and their household members, were followed up with home visits to determine secondary infections in the household. Serum samples taken at baseline and convalescence were tested for HAI specific to pandemic H1N1, seasonal H1N1 and seasonal H3N2. Nasal and throat swabs (NTS) were collected from each individual and tested by RT-PCR to confirm active infections.

**Results:** There were 18 pandemic H1N1, 5 seasonal H1N1, and 24 seasonal H3N2 virus positive cases confirmed by RT-PCR. Among those positive for influenza virus, 39 were adults ( $\geq 16$  years) and 8 were children. The proportion of individuals with a homologous antibody titer of  $\geq 1:40$  rose from 17%, 20% and 4% to 78%, 100% and 38% after infection with the pandemic H1N1, seasonal H1N1 and seasonal H3N2 viruses respectively. Heterologous responses were strongest between pandemic H1N1 and seasonal H1N1. In individuals with RT-PCR confirmed seasonal H1N1 infection, the proportion with an antibody titer  $\geq 1:40$  for pandemic H1N1 increased from 20% to 40%. In the opposite direction, the proportion of those confirmed with pandemic H1N1 with an antibody titer  $\geq 1:40$  for seasonal H1N1 increased from 33% to 44%. Low levels of cross-strain HAI titer rise to  $\geq 1:40$  were detected in other variations.

**Conclusion:** We identified low baseline immunity prior to naturally-acquired infection with seasonal or pandemic strains of influenza in the 2009 summer season in Hong Kong. This suggests that a proportion of those infected with either seasonal H1N1 or H3N2 viruses could gain protection against the novel pandemic H1N1 strain and vice-versa.

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The role of schools as social networks in transmission of Influenza A (H1N1 2009): The Mbulu District, Northern Tanzania, November 2009 experience

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**Background:** Novel influenza A H1N1 virus is the latest emerging viral disease with a pandemic phase 6 status. Since July 4th when the first case was confirmed in Tanzania, there have been several sporadic cases across several regions. A cluster of cases were identified at a school in Mbulu district on September 16th 2009, Northern Tanzania. Within a week, 94 cases were confirmed (72 in the school and 22 within the community). This prompted immediate outbreak investigation, risk factor analysis and establishment of control measures. We describe the steps taken to curtail this health event in Tanzania.

**Methods:** An unmatched case control study was done. A case was defined as any person with acute respiratory illness from Mbulu district with laboratory confirmed influenza A (H1N1) virus or who is epidemiologically linked to a confirmed case. Cases were actively searched and identified; specimens were collected and sent to the National Influenza Centre in Dar es Salaam for rt-PCR confirmatory testing for Influenza A (H1N1). Data analysis was done using Epi info 3.5.1

**Results:** The index case was a student of the school and from September 16, through October 5, a total of 99 were confirmed with Influenza A H1N1. The mean age of cases was 16 years (SD=8.4); 78% of patients were less than 20 years. Twenty two percent were identified from the community following a graduation activity at the school on September 26, 2009. The most common presenting symptoms were fever (90%), cough (46%), headache (40%), and Joint pains (30%). Significant transmission occurred in the class and dormitory where the first case was identified (attack rate = 23.8%). Being present in a graduation gathering was found to be a risk factor for developing the disease at the community level (OR=1.4, 95%CI= 1.2–6.0)

**Conclusion:** Following the outbreaks and the subsequent community spread, Tanzanian FELTP residents and MoHSW were co-opted to investigate, control and prevent future spread. The epidemic was subsequently controlled and surveillance reinforced. Early identification and institutionalization of control measures are vital tools in shifting epidemic curves to the left.

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Co-infection between the pandemic influenza virus A H1N1 and seasonal influenza A virus in a patient presenting severe acute respiratory disease

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**Background:** With the pandemic circulation of the new influenza A/H1N1 virus in 2009, became mandatory to implement the national preparedness plan to improve both health care and laboratory settings activities. However, it is also important a continuous surveillance is essential to promote a better comprehension of mechanisms of transmission, viral virulence, and treatment efficacy. The aim of this study is to describe the clinical, laboratorial, and epidemiological aspects of a fatal case of severe acute respiratory disease (SARD) with simultaneous identification of both pandemic and seasonal influenza A virus.

**Methods:** Retrospective and descriptive study based on information collected in medical records and notification file about a patient hospitalized in Campinas, State of São Paulo, during the first influenza A/H1N1 epidemic period in Brazil.

**Results:** A white female patient, 25 years old, with antecedent of obesity and diabetes, was admitted in July/2009 with 2 days history of fever, cough, sore throat, myalgia, and malaise. On admission, chest X-ray showed bilateral, diffuse, and interstitial-alveolar opacities. Despite the started treatment with oseltamivir, ceftriaxone, and azithromycin, she presented with tachypnoea, tachycardia, cyanosis, and hypoxemia in the day-3 of disease. In day-4, her respiratory pattern progressed to severe respiratory distress, lung hemorrhage, and shock; invasive ventilatory support and vasoactive drugs were introduced. Her outcome was death in the 8-day of disease. The specific RT-PCR performed by Instituto Adolfo Lutz – a public health laboratory reference for laboratorial diagnostic of pandemic influenza virus in Brazil – identified seasonal influenza virus in respiratory secretion and pandemic A/H1N1 pandemic virus in lung specimen collected *post mortem*.

**Conclusion:** In the present case, it is not possible to postulate the real impact of simultaneous infection predicting the poor clinical evolution. However, it reinforces the importance of a continuous and sensitive epidemiological and laboratorial surveillance of respiratory syndromes, during epidemic and interepidemic period, as a strategy to detect early changes in the epidemiological pattern and to know what respiratory virus are circulating, including possible emerging new influenza virus strains eventually more virulent or antiviral resistant.

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Response to pandemic influenza in the English and Dutch Caribbean

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**Background:** In June 2009, the first cases of pandemic influenza occurred in the English Caribbean, just prior to the WHO declaration of pandemic level 6. Since then the virus has spread rapidly throughout the sub-region.

**Methods:** In response to the pandemic, at the sub-regional level: alerts, updates and guidelines were developed and distributed; a Situation Room was activated with two 24/7 emergency phone lines; a multidisciplinary Flu Team was formed; the laboratory testing algorithm for influenza was revised; there was a rapid increase in testing capacity; specimen collection kits, laboratory supplies and reagents were sent to countries; and prepaid courier accounts were set-up for shipment of specimens. Countries revised and implemented their pandemic influenza plans.

**Results:** As of November 18, 2009, there were 1,334 laboratory cases of pandemic (H1N1), including 298 hospitalized cases and 18 deaths identified in 20 of 21 English and Dutch Caribbean countries [Table 1]. Of the 18 deaths; 10 had underlying medical conditions, 9 were obese and 3 were pregnant. Pandemic (H1N1) is the predominant circulating influenza virus type, with seasonal influenza viruses (H1N1, H3N2 and influenza B) also circulating, but at much lower levels. Most (> 85%) influenza cases had symptoms of upper respiratory tract infections; approximately 20% of cases had gastrointestinal symptoms; and approximately 40% had other symptoms such as body pain, myalgia and/or headache. The age groups most affected by pandemic influenza were those aged 0-14 years and 20-49 years. The least affected age group was those aged >60 years.

The second wave of pandemic influenza appears to have peaked in the English and Dutch Caribbean.

**Conclusion:** The Caribbean Community responded well to a rapidly changing pandemic influenza situation. The major lessons learnt during this pandemic were the importance of: continuous communication at all levels; efficient and timely surveillance with collaborative clinical, epidemiological and laboratory input; and adequate intersectoral planning, with evaluation and adaptation to meet the changing needs of the disease situation.

With another wave expected early in 2010, countries will need to continue to be adequately prepared to deal with the expected increase in influenza cases.

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Enhanced influenza survey: Khartoum state survey in selected paediatric hospitals

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**Background:** On July 2009, the first influenza A (H1N1) virus laboratory confirmed cases were reported in Sudan. It was brought by a family coming from the UK. The situation continued static, except for another three cases coming from abroad. Two months later specifically after the return of pilgrims from omra (visit to holy Muslim places in Saudi Arabia); we started to detect clusters of H1N1 within the community with no history of travelling abroad or contact with abroad comer. An increase acute respiratory diseases incidence in Khartoum state hospitals above the base line was also reported. The National Public Health Laboratory conducted a survey aiming to determine the burden of H1N1 virus incidence among Sudanese children with acute respiratory illness attending emergency clinics in four pediatrics hospitals in Khartoum state.

**Methods:** Pre-survey orientation about influenza pandemic and expected bacterial pneumonia was held. Training of personnel in specimen collection and preservation. From 5th to 21st of November 2009, a total of 115 nasopharyngeal swabs were collected from children selected according to the WHO criteria of case definition of influenza-like illness. Specimens were sent to the National Public Health Laboratory for real-time reverse-transcriptase–polymerase-chain-reaction confirmatory testing for H1N1.

**Results:** H1N1 was confirmed in 51 patients (44.4%), another 5 patients were flu A (untypable) positive. Male to female ratio 26:25. 4 patients were under one year of age, 10 patients were within the range from 1-5 years and 24 patients above 5 years of age. The signs and symptoms of the disease were mild and rarely required more than 24 hours hospitalization. One case of fatality was reported.

**Conclusion:** H1N1 outbreak (>40%) among Sudanese children have been confirmed with predominance of incidence in school aged children (above 5 years).

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Assessing H1N1 (2009) mitigation strategies under epidemiologic and programmatic uncertainty

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**Background:** Decision-makers faced substantial uncertainties during the H1N1 (2009) pandemic. Uncertainties were both epidemiologic (e.g. unknowns about likely attack rate and severity) as well as programmatic (e.g., unknowns about when vaccines would be available). Simulation models can be used to assess the effectiveness of mitigation strategies, but model projections may change according to assumptions about epidemiologic and programmatic variables.

**Methods:** We developed a simulation model of a pandemic (H1N1) 2009 outbreak in a medium-sized Canadian city using demographic and epidemiologic influenza pandemic data. Simulated individuals were allocated into homes, schools, workplaces and communities, and the contact patterns and resulting spread of influenza were modeled. We projected the attack rate under different combinations of vaccination, school closure, antiviral drug strategies, and "trigger" thresholds and under various levels of pre-existing immunity. To assess the impact of epidemiologic and program uncertainty, we used combinatorial uncertainty analysis in which all possible scenarios combinations are simulated, and the results stratified according to questions of interest. This permitted us to identify the general features of public health response programs that resulted in the lowest attack rates.

**Results:** Delays in vaccination of 30 days or more reduced the effectiveness of vaccination in lowering the attack rate. However, pre-existing immunity in 15% or more of the population kept the attack rates low, even if the whole population was not vaccinated or vaccination was delayed. School closure was effective in reducing the attack rate, especially if applied early in the outbreak, but this is not necessary if vaccine is available early or if pre-existing immunity is strong. These results are robust under the combinatorial uncertainty analysis. For a baseline scenario of 5% pre-existing immunity and no school closure, the attack rates under scenarios of (i) no vaccination, vaccination of 30% beginning (ii) 30 days or (iii) 60 days after the outbreak were (i) 21.7%, (ii) 7.6%, and (iii) 12.5% respectively.

**Conclusion:** Early action, especially rapid vaccine deployment, is disproportionately effective in reducing the attack rate. Combinatorial uncertainty analysis can be useful for assessing the impacts of policies when decisions must be made in an environment of uncertainty.

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Providing guidance during the swine flu outbreak in 2009: An evaluation study of the National Resource for Infection Control (NRIC)

**S. Wiseman**<sup>1</sup>, P. Kostkova<sup>2</sup>, E. de Quincey<sup>2</sup>, G. Jawaheer<sup>2</sup>

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**Background:** Over 40 000 professionals monthly access the evidence provided by the National Resource for Infection Control (NRIC, [www.nric.org.uk](http://www.nric.org.uk)) - a digital library for infection prevention and control, was launched in May 2005. The project funded by the Department of Health (UK) is endorsed by NeLI ([www.neli.org.uk](http://www.neli.org.uk)) and its success has been in its unique ability to provide the best available evidence published within the last 5 years (where possible) on investigation, management, prevention, control and treatment of, healthcare associated infection, and infectious diseases. The user base is coming from the UK, US and many other non-English speaking countries. The key added value is the quality appraisal of posted documents conducted in collaboration with major professional societies.

**Methods:** During the swine flu outbreak from April until August 2009, we conducted a wide evaluation of user searches and needs and access of key resources to better understand user concerns.

We conducted a number of evaluation searches that will be presented on at this conference. Most importantly, page views for resources related to swine flu peaked in late August, as illustrated on Figure 1.

**Results:** Further, important revelation was investigation of the swine flu searches along the timeline of the outbreak clearly demonstrating the raise and increase of the keyword "pandemic" (graph in red) after 11th June 2009 and the keyword "influenza" (graph in green) after 23rd July 2009 while the keyword "pandemic flu" (graph in blue) initially popular decreased. Figure 2 illustrates the popularity of the three most frequent keywords.

Direct access to influenza resources on NRIC was also encouraged by the placement of a dedicated link on the Home Page (green line on Figure 2). Further timelines, traffic and information needs analysis revealed an increase in interest in evidence around pandemic influenza.

**Conclusion:** Having learning these lessons we have updated the NRIC Home page in October 2009 and are in the process of collecting more results for the autumn second wave. A comparative study of this unique evaluation and user navigation behaviour, user demographics as well as searches from popular search engines will be presented at the conference.

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Severe acute respiratory disease caused by pandemic influenza A H1N1 virus. A case series of hospitalized patients in Southeastern Brazil during the 2009 epidemic

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**Background:** During the pandemic influenza A H1N1 (FluAH1N1) period in 2009, it was observed a significant number of suspected cases presenting severe acute respiratory disease (SARD). In this context, all health care settings have adapted their structure – including infection control strategies, emergency and intensive care unities organization, treatment protocols – to increase their capacity of pandemic response. The main objective of this study is to describe the main clinical and epidemiological features of a case serie of severe acute respiratory disease caused by pandemic FluAH1n1 in a metropolitan region.

**Methods:** Retrospective and descriptive study based on information of medical records and epidemiological files of SARD hospitalized cases caused FluAH1N1 in 5 hospitals in the metropolitan region of Campinas, São Paulo State, during the first epidemic period in Brazil.

**Results:** Between July/6 and October/30 there were evaluated 254 cases of SARD; of them, 49 have been confirmed as FluAH1N1 infection (19.3%) and 14 (5,5%) as seasonal influenza strain. Of the 49 confirmed FluAH1N1 cases, 28 were female (57.1%) and the median age was 26 years (range: 0-75 years). The median time from onset of illness and hospital admission was 2 days (range, 1 to 5). Nine cases (18.3%) were admitted in intensive care unity and 5 (10.2%) required mechanical ventilation. The most important clinical features were fever and cough(98%), dyspnoea (77.5%), and malaise (83.6%). X-ray abnormalities were present in 75.5% of patients; leucocytes count was elevated in 5 (10.2%) and reduced in 6 (12.2%) patients. Pre-existing conditions were observed in 28 patients (55.1%); chronic respiratory disease (51.8%) and obesity (22.2%) were the most frequent underlying medical conditions. There were 4 deaths associated with FluAH1N1 (lethality 8.1%), one of them with simultaneous seasonal influenza strain virus infection.

**Conclusion:** As observed in other case series, it has been observed a higher frequency of SARD in female gender, younger adults, and in patients with underlying medical conditions. The identification of seasonal influenza virus strains in SARD patients reinforces the potential impact of seasonal influenza on morbidity and mortality annually and the importance of a continuous epidemiological and laboratorial surveillance of respiratory syndromes.

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Is the 2009 influenza A (H1N1) virus uncovering health disparities in Miami?

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**Background:** Since last April 2009 when the first cases of 2009 H1N1 influenza were diagnosed in the United States, the Miami-Dade County has been the hardest hit by this disease among Florida counties. This outbreak has also uncovered large disparities in the outcome of this disease among ethnic and racial groups in our community. Sixty-two percent of the 2,400,000 Miami-Dade County residents are of Hispanic origin, eighteen percent are Black Non-Hispanic, and White Non-Hispanic respectively. Among County residents, twenty-eight percent have no health insurance, and seventeen percent live below the poverty level.

**Methods:** Information was retrieved from the enhanced public health surveillance database of Miami-Dade County residents who were hospitalized or died due to laboratory-confirmed 2009 H1N1 influenza infection reported to the Miami-Dade County Health Department between April 26th and November 30th, 2009.

**Results:** During this period, a total of 32 patients died, and 423 were admitted at Miami-Dade hospitals due to this disease. The hospitalization rate among Black Non-Hispanic residents (26.7 per 100,000) was more than three times higher than the one observed among White Non-Hispanics (7.3 per 100,000). Black Non-Hispanic residents (1.9 per 100,000) were more than twice likely to die due to this disease than Non-Hispanic Whites (0.7 per 100,000). Miami-Dade had more pronounced disparities among racial/ethnic groups than the ones observed in other areas of the State of Florida.

**Conclusion:** The current 2009 H1N1 influenza outbreak has hit more severely the minority population in Miami-Dade, underscoring the need to address the social and environmental factors leading to increasing health disparities observed on this community.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Comparative evaluation of ARDS patients with and without H1N1 infection at a tertiary care referral center

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**Background:** The recent emergence of H1N1 as pandemic has raised concerns in its critical care management. The management in our ICU is primarily based on guidelines of sepsis and ARDS. So we compare our experience of Acute lung Injury patients referred to our centre with and without H1N1 infection.

**Methods:** All patients admitted to our centre over a period of 3 months were studied. Patients with Acute lung Injury and ARDS and clinical suspicion of H1N1 were shifted to the ICU specifically assigned for management of such patients. The demographic profile, presenting features, outcome and parameters of oxygenation and ventilation were recorded and compared.

**Results:** The clinical features were comparable in the two groups – H1N1 and nonH1N1 group, at the time of presentation in our centre. A total of 40 patients were shifted to our ICU with the clinical suspicion of H1N1 infection and having respiratory compromise requiring ventilatory support. Out of these 26 patients were tested positive for H1N1 and rest were negative. The mean age was 32.8 and 31.8 years and male: female ratio of 16: 10 and 6:8 in patients with and without H1N1 infection respectively. 12 patients in H1N1 group and 3 patients in nonH1N1 group had associated comorbidities. The mean duration of symptom prior to need of ventilator support was 6.4 and 5.9 days respectively in the two groups. The mean PaO<sub>2</sub> at the time of ICU admission was 48.6 and 52.7 mm Hg in the two groups respectively. The H1N1 group had 100% mortality while it was 71.4% in nonH1N1 group. The mean duration of stay in ICU was 3.4 and 5.5 days respectively in two groups. The acute renal failure was seen in 8 patients in H1N1 group as compared to none in nonH1N1 group. 42% and 36% of the patients required inotropic support in the two groups respectively.

**Conclusion:** The H1N1 positive patients has more florid and severe course with high mortality as compared to nonH1N1 patients with similar demographic profile, initial clinical symptoms and respiratory compromise requiring ventilatory support.

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**Session: Influenza**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Comparison between three not pharmacological strategies aimed to prevent the dissemination of the A/H1N1 influenza virus in Colombia

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**Background:** Influenza by the new A H1N1/09 virus is an emerging disease characterized by high transmissibility, rapid capacity to spread, high pandemic potential and seriousness of its complications, particularly in population with risk factors already established. The countries have been preparing for the impact of mitigation in case of appearance of a new pandemic through the adoption of different strategies of which the social distancing is one of the most used strategies. Social distancing strategies have lasted in an arbitrary manner between 7 and 21 days, and have a potential impact on the spread of the epidemic virus and at the same time an economic impact on individuals, the productive sector or insurers, depending on the time of license and various forms of recruitment. In the literature there are different models that show the impact generated by this measure in different populations but not in our population.

**Methods:** Our study was a simulation of discret events with the Arena Professional Edition Software Version 11, starting from the existence of a first case infected with the virus, taking as susceptible population the total population of Bogota, to compare 3 strategies of social distancing in workers with wages: 1. Only hygiene measures in the work site (washing hands and mask); 2. Unable to work for 3 days; and 3. Unable to work for 7 days. We calculated the costs of productivity lost with each of the strategies through the approach of human capital.

**Results:** The most cost-effective strategy for our population was to give incapacity during three days. For this strategy we calculated a total of 1,862,331 of infected patients and a mortality rate of 1.0%, the lost of productivity calculated was 6 days, and the cost was 155 dollars patient

**Conclusion:** The strategy of social distancing more cost-effective in preventing the spread of the virus influenza A H1N1 in the Colombian population is unable to work for 3 days.

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**Session: Malaria & Blood-borne Parasites**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

A study of acute myocardial infarction in a hospital cohort of malaria: 4 years retrospective analysis

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**Background:** Malaria, a protozoal disease, caused by genus plasmodium, is prevalent in about 100 countries worldwide & is a major cause of morbidity & mortality especially in sub Saharan Africa, Southeast Asia & Latin-America. Myocardial infarction has not been recognized as a complication of malaria though there have been reports of myocardial involvement in experimental studies and in postmortem findings. The objective of the present study was to analyze the association of acute myocardial infarction (AMI) in malaria patients

**Methods:** Retrospective observational study of 38,919 in-patients of Dr. TMA Pai Rotary Hospital was done from the year 1995 to 1998. Analysis had been started from 1995 as malaria resurged in Mangalore city from 1995 onwards. A year wise categorizations of patients were done & occurrence of AMI among patients with malaria was compared with the occurrence of AMI among all other in-patients for each year & cumulatively. Diagnosis of malaria cases had been established by QBC test. Diagnosis of myocardial infarction had been established by following standard ECG changes and cardiac biomarkers profile. After tabulation, p value has determined by applying standard Gaussian test. p value <0.05 was considered to be significant.

**Results:** AMI was found in 2.47 % (9/365), 1.29 % (6/465), 0.96 % (4/418), 1.06 % (3/283) of malaria patients in the year 1995, 1996, 1997, 1998 respectively and 0.87 % (96/11005), 0.87 % (97/11113), 0.87 % (75/8646), 0.71 % (47/6624) of non-malarial in-patients during the corresponding period. The occurrence of AMI gradually decreased in malaria patients as the years progressed - from 2.47% in 1995, when Mangalore experienced a resurgence of malaria, to 1.06 % in 1998.

**Conclusion:** Compilation of 4-year data has shown a higher occurrence of AMI among all malaria patients (1.44%) compared to AMI among all in-patients other than due to malaria (0.82%), (p<0.05). These findings suggest that AMI should be regarded as an important clinical complication of malaria. This is of importance since some of the anti malarial drugs also depress cardiovascular function.

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**Session: Malaria & Blood-borne Parasites**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Severe malaria in East Kalimantan, Indonesia

**C. gunawan**<sup>1</sup>, P. D. Nasution<sup>2</sup>, A. R. Magdaleni<sup>2</sup>

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**Background:**

Malaria is endemic in many provinces in Indonesia, especially in Eastern Region, where 35 % of population live in areas with risk of getting infected by malaria. Mortality of severe malaria reported in Indonesia is 10 – 30 %. Abdul Wahab Sjahranie General Hospital, Samarinda is the top referral hospital in East Kalimantan Province that manage many severe malaria patients sent from some districts around Samarinda.

**Objectives.** To know the features of severe malaria patients treated at Abdul Wahab Sjahranie General Hospital Samarinda.

**Methods:** An observational study was performed on severe malaria patients treated at the Department of Internal Medicine of Abdul Wahab Sjahranie General Hospital, Samarinda during 2 years (January 2007 – December 2008). Severe malaria was diagnosed based on WHO criteria (positive microscopic examination of *Plasmodium falciparum* with one or more complications). Anti malarial drug given was quinine infusion 10 mg/ kg BW/ 8 hours for at least 48 hours, and then continued with sulfate quinine tablet if patients could take oral medicines until 7th day.

**Results:**

There were 47 severe malaria patients treated in this hospital during 2 years, consisting of 43 males (91.5 %) and 4 females (8.5 %). All patients worked or lived in the forest areas out of Samarinda. Patients ages` were 13 - 63 years, most of them were 20 – 40 years (57.5 %). The most common complications found were jaundice (72.3 %), cerebral malaria (40.4 %), acute renal failure (31.9 %), while severe anemia was only 6.4 %. Most patients had 1 complication (63.8 %), while patients with 2 complications were 21.3 %, and patients with 3 or more complications were 14.9 %. Mortality of patients with 1 complication, 2 complications, 3 or more complications were 6.7 %, 10.0 %, 71.4 %, respectively. Overall mortality rate was 17.0 %.

**Conclusion:** Almost all severe malaria patients treated in Samarinda are males (91.5 %) in productive ages (20 – 40 years). Jaundice, cerebral malaria and acute renal failure are the most common complications found. Mortality is related to the number of complications found, whereas patients with 3 or more complications have very high mortality rate (above 70 %). Overall mortality rate of severe malaria cases is 17.0 %.

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**Type: Poster Presentation**

Performance of four rapid diagnostic tests for diagnosis of falciparum and non-falciparum malaria in endemic areas of Gondar region, Northern Ethiopia

**A. K. Gelaglie**

Addis Ababa University, Medical Faculty, Addis Ababa, Ethiopia

**Background:** Malaria remains a major public health problem in Ethiopia, despite decades of a sustained national control program. One of the major obstacles to this control program is the lack of accurate and rapid diagnostic service in most resource poor settings where malaria is endemic. Very recently, efforts have been made to develop and implement various formats of malaria RDTs.

**Methods:** In view of this, the performance of the OptiMAL-IT, Paracheck-Pf, CareStart™ malaria pLDH 4 line test and CareStart™ malaria pLDH/HRP II combo test were investigated in comparison with microscopic examination of thick and thin blood film in malaria endemic areas of Gondar region. In order to evaluate these assays, the sensitivity, specificity, PPV and NPV values of each RDT were calculated taking microscopy results as the gold standard in a total of 588 febrile patients.

**Results:** Paracheck-Pf was the most sensitive (100%) assay for the diagnosis of *P. falciparum* in comparison with OptiMAL-IT (98.1%), CareStart™ malaria pLDH 4 line test (98.1%) and CareStart™ malaria pLDH/HRP II combo test (96.2%). However, OptiMAL-IT was the most specific (99.1%) as compared to Paracheck-Pf (97.9%), CareStart™ malaria pLDH/HRP II combo test (96.4%) and CareStart™ malaria pLDH 4 line test (93.8%) for falciparum malaria diagnosis. For the diagnosis of *P. vivax*, both CareStart™ assays had better sensitivity (94.4% for CareStart™ malaria pLDH 4 line test and 94.2% for CareStart™ malaria pLDH/HRP II combo test) as compared to OptiMAL-IT 88.2%. But OptiMAL-IT gave the higher specificity (99.8%) than CareStart™ malaria pLDH 4 line test (98.1%) and CareStart™

**Conclusion:** Although microscopy remains the gold standard for malaria diagnosis, OptiMAL-IT, Paracheck-Pf, CareStart™ malaria pLDH/HRP II combo test and CareStart™ malaria pLDH 4 line test may prove a useful screening for malaria control in Ethiopia where microscopic examination is not in place. Finally, further studies on RDT performance is recommended to be undertaken in multisite study fields, in monitoring drug therapy and with respect to molecular analysis.

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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Clinical presentation and outcome of severe falciparum malaria in Eastern Nepal

**S. K. Sharma**<sup>1</sup>, B. Khanal<sup>1</sup>, D. Manandhar<sup>2</sup>, S. Rijal<sup>1</sup>

<sup>1</sup>BP Koirala Institute of Health Sciences, Dharan, Nepal, <sup>2</sup>Nepal Medical college, Kathmandu, Nepal

**Background:** Malaria is endemic in 65 out of 75 districts of Nepal and >70% of the total population are at risk of the disease. The clinical presentations of severe and complicated malaria vary.

**Methods:** Clinical profile, biochemical parameters and outcome in 138 adult patients of malaria requiring hospital admission in BP Koirala Institute of Health Sciences hospital, a tertiary care hospital in eastern Nepal during April 2002 to April 2005 were studied.

**Results:** Mean age of the patients was 33 ±16 yrs with majority (n=88) being in age group of 15 to 34 years. 67% of the patients were from terai belt (southern plain area). Mean duration of febrile illness was 13±9 days at the time of presentation and 54% patients had recent history of travel to India. Hepatic dysfunction (39%), anemia (30%), hypotension (18%), metabolic acidosis (16%), convulsion (11%), hypoglycemia (8%) and 22% (n=30) had acute renal failure according to WHO criteria. Three or more complication was present in 32%. Apart from antimalarial therapy, dialysis support and mechanical ventilation was provided to 22 and 25 patients respectively. All the patients who died (23%) had ≥3 complications.

**Conclusion:** Death from complicated malaria is high. Delayed in diagnosis leading to multiple complications might have contributed to high mortality.

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**Type: Poster Presentation**

APACHE III score as a prognostic marker in severe malaria in a tertiary care hospital from south India

**K. Nataraj**<sup>1</sup>, M. Prabhu<sup>2</sup>, M. Sangar<sup>2</sup>, B. Ramachandran<sup>2</sup>

<sup>1</sup>NRS Medical College, Kolkata, West Bengal, India, <sup>2</sup>KMC Manipal, Manipal, Karnataka, India

**Background:** Malaria is a most common parasitic infection with transmission in 103 countries affecting > 1 billion people and causing between 1 and 3 million deaths each year. Falciparum malaria causing severe malaria is one of the commonest infections with high mortality in India in spite of potent chemotherapy. APACHE III is the most recent version of the scoring system developed by Knaus and colleagues. So far no studies are available using APACHE III in severe malaria in Indian setting

**Aims:** Prospective clinical study of APACHE III score as a predictor of mortality in patients with severe Malaria.

**Methods:** 41 patients with diagnosis of severe Malaria admitted in Kasturba Hospital Manipal, tertiary care hospital during the period of May 2006 to June 2008

**Statistics:** SPSS 15.3 was used to perform statistics, depending on the normalcy of distribution curve and skew deviation mean or median was compared using Independent T test or Non parametric t test such as Mann Whitney's were used respectively.

**Results:** During 2 year study period (May 2006- June 2008) 41 patients with Severe Malaria were admitted in different ICUs of Kasturba Hospital, of whom 6 were females and 35 were males. 11 patients succumbed to their illness and 30 survived. There was no significant difference in age and parasite index in patients who survived and succumbed to illness. Clinical parameters like tachycardia, hypotension and hypoxia were significantly seen in patients who succumbed to illness. Neurological obtundation was seen in all 11 dead patients at presentation is another independent variable predictive of severe malaria. Among lab parameters thrombocytopenia, raised BUN, Creatinine, low blood sugars were significant predictive factors for mortality. Most patients 10 out of 11 had lung involvement with ARDS required ventilation. Mean APACHE III scoring among alive patients was  $50.94 \pm 17.25$  and among dead patients was  $100.18 \pm 26.86$ . The scores were compared by using Independent T test and the scores were highly statistically significant ( $p < 0.005$ )

**Conclusion:** 1. APACHE III is very good prognostic marker in predicting mortality in severe malaria

2. Among individual clinical parameters tachycardia, hypotension, hypoxia oliguria, altered sensorium were associated with significant mortality

3. Among lab parameters raised BUN, Serum creatinine, Hypoglycemia thrombocytopenia and chest X ray shadows (ARDS) predict independently high mortality and need for ICU monitoring and aggressive treatment.

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**Type: Poster Presentation**

Poor impact of the primary health care (PHC) on malaria control in rural communities of Southeast Nigeria

**O. Odikamoro**

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**Background:** Malaria is a disease of poverty and low socio-economic status and these conditions abound in developing countries. This means that the successful implementation of malaria control programmes requires a certain level of basic health services. In countries where malaria is a serious impediment to socio-economic progress, emphasis should be on malaria control rather than eradication. This will reduce the burden of the disease and pave the way for the speeding up of socio-economic development which may in the long run contribute to a future eradication of malaria. The resurgence of malaria is a global phenomenon. Most studies on malaria have dealt with clinical, laboratorial and entomological aspects. Few studies have dealt with human factors.

**Methods:** 2400 volunteers from all age- groups from selected endemic rural communities of southeast Nigeria were screened for malaria parasitaemia from June 2008 to August 2009, using the thin and thick smear microscopy. Following this, 16 close-ended questionnaire items were generated and administered on 600 adults, mainly heads of households to assess their knowledge, attitudes, and practices on the various aspects of malaria transmission, management and control, thus evaluate the impact of the Primary Health Care on malaria control in the study areas.

**Results:** The results showed the following percentage infection in the various age-groups: 0-11 months (24%); 1year-2years (43%); 2-4 years (80%); 5-9 years (74%); 10-14 years (71%); 15 years and above (68%). A total of 1440 persons were positive for *Plasmodium falciparum* parasitaemia, representing an average infection of 60%. In the questionnaires, 93% of the respondents did not associate malaria with mosquito bite; 60% were not carrying out any form of vector control; only 7% had adequate knowledge of the management of fever, and 15% had any knowledge of government action towards the control of malaria.

**Conclusion:** The study revealed poor implementation of the Primary Health Care as it concerns malaria control at the peripheral level. The remoteness of some rural communities and lack of easy accessibility makes it difficult for health programmes to get to the grassroots. There is the need to train a large number of Village-Based Health Volunteers, who should step up community mobilization and health education campaigns. Parents should know how to prevent and manage malaria fever in their children.

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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Malaria parasitemia in surgical patients in University of Calabar Teaching Hospital, Nigeria  
**N. Usoro**, E. E. Anwan, I. O. Uchejuru, O. J. Bam  
University of Calabar Teaching Hospital, Calabar, Cross River State, Nigeria

**Background:** Malaria is endemic in Nigeria. Surgical patients in our environment are predisposed to perioperative anemia and postoperative pyrexia due to malaria. We carried out a prospective study of malaria parasitemia in surgical patients to evaluate the need for routine malaria parasite (MP) test for surgical patients in an endemic area.

**Methods:** Fifty adult elective surgical patients were tested preoperatively for MP by Giemsa staining of thick blood smear. Pre-operative hematocrit was also recorded and patients were followed up for postoperative pyrexia.

**Results:** Forty-four patients (88%) were positive for MP. Twenty-one of these (47.7%) were anemic with mean hematocrit of 33.3%. None of the 6 patients who were MP negative was anemic (mean hematocrit 42%). Thirty-six patients had surgery and 20 of these had postoperative pyrexia for which 15 required antimalarial medication for resolution. All 15 had tested positive for MP.

**Conclusion:** There is significant malaria parasitemia among surgical patients in our environment. This contributes to the perioperative anemia and postoperative pyrexia and therefore the morbidity of our surgical patients. Routine preoperative MP test would facilitate prompt diagnosis and treatment of malaria, anemia, and postoperative pyrexia in this group of patients, thus reducing morbidity.

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**Type: Poster Presentation**

Improving estimates of malaria intervention coverage from household surveys using GPS data

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<sup>1</sup>Blue Raster, LLC, Arlington, VA, USA, <sup>2</sup>ICF Macro, Calverton, MD, USA

**Background:** Standard indicators of malaria prevention interventions, such as ownership and use of insecticide-treated mosquito nets (ITNs), are restricted to the population at risk of getting malaria. Household survey measurements of those indicators, however, are typically reported at the national level, including areas with little or no malaria transmission. Moreover, many surveys are not conducted during the high malaria transmission season. For these reasons, estimates of coverage by malaria interventions are often biased downward.

This paper quantifies the effect of these biases by examining two coverage measures for universal coverage: 1) household possession of at least one ITN (HP1+) and 2) at least one ITN for every two usual residents (HP1/2), by

A) Using GPS data to correlate these measures with levels of malaria endemicity and seasonality, and

B) Recalculating coverage estimates based on the population at risk, taking into account the timing of the survey and endemicity in the area.

**Methods:** Data come from 15 recent sub-Saharan Demographic and Health Surveys (DHS) or Malaria Indicator Surveys (MIS). GPS location of household cluster is linked with Malaria Atlas Project maps to evaluate the malaria transmission zone and season.

**Results:** The percentage of HP1+ households varies from 3.4% in Ethiopia to 53.3% in Zambia, and the percentage of HP1/2 households varies from 0.4% in Ethiopia to 18% in Zambia. If the estimates are restricted to households located in endemic areas, coverage is considerably higher. For example, HP1/2 universal coverage is 6% nationwide in Namibia, but ranges from 2% in areas with no malaria to 11% in highly endemic areas (with parasitemia >40%). Rates of ITN use also differ substantially: in Uganda, 11% of the population in highly endemic areas interviewed during the malaria season slept under ITNs, compared to only 3% in areas with no malaria.

**Conclusion:** Estimates of ITN ownership and use based on household surveys are strongly affected by the location of survey clusters and the month of the interview. Taking these factors into account is recommended to improve the monitoring of progress toward achieving national goals and to inform in-country programmatic decision-making.

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Assessment of chloroquine resistance of Plasmodium in patients attending malaria clinic in a government general hospital, Kurnool; Strategies to prevent chloroquine resistance

**N. Mullaguri**

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**Background:** Prevalence of chloroquine resistant malaria is on a rise and our area is one of the declared endemic zones for malaria. Recent mortality trends of the disease have increased considerably seeking immediate modification in the treatment guidelines to decrease the complications and thus the mortality of the disease. We have attributed the present condition to the chloroquine resistance, the drug which is used to treat the disease in this area for so long. Even the effective surveillance system fails in decreasing the mortality figures by following the prescribed treatment guidelines. Hence, we have undertaken this project to assess the drug resistance and to state new treatment guidelines in the areas where chloroquine resistant malaria is rampant.

**Methods:** 250 patients are taken as sample in this project. After diagnosing them as Malaria by peripheral smear and IgM antibody detection tests, the patients are prescribed chloroquine tablets as per the treatment guidelines in this region for 3 days closely watching them for complications. The number of patients cured of the disease are noted and the number of uncured cases are assessed for the continuation of symptoms. The percentage of cured to uncured is calculated and this serves as an evaluation tool for chloroquine resistance. The Uncured subjects are prescribed Tablet Artesunate for 3 days.

**Results:** 106 patients are not cured after Standard chloroquine treatment and prescribed Artesunate treatment.

144 patients are cured after the chloroquine treatment.

% of cured patients=57.6%

% of uncured patients=42.4%

The ratio of Uncured to Cured=0.736

the ratio >0.5

Full details will be submitted in the conference.

**Conclusion:** As the Ratio of Cured to Uncured is greater than 0.5 in this area, We want to intervene in the modifications of the standard treatment guidelines by introducing Artesunate instead of Chloroquine for the Patients suffering from Malaria in Our region.

Any Endemic region with the ratio of Uncured to cured >0.5 should modify the treatment guidelines to decrease the complication rates and thus the mortality caused by this disease.

For the regions with the ratio less than 0.5, Co-prescription of Artesunate is advised instead of relying only on Chloroquine.

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**Type: Poster Presentation**

Blood microfilarial-stage specific gene expression profile of *Brugia malayi*

**S. Nuchprayoon**<sup>1</sup>, S. Sungpradit<sup>2</sup>, A. E. Jedljicka<sup>3</sup>, J. Bailey<sup>3</sup>, A. L. Scott<sup>3</sup>

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**Background:** Lymphatic filariasis, a mosquito-borne disease, is mainly caused by the nematodes *Wuchereria bancrofti* and *Brugia malayi*. The adult worms reside in the lymphatic vessels where they cause damage and the female release an abundance of offsprings (microfilariae; Mf) into the host's circulatory system. The Mf stage is associated with disease transmission, complex disease pathology, and host immunomodulation. Potential targets from the Mf stage for drug and vaccine development were investigated in order to reduce Mf density, improve disease morbidity, and prevent disease transmission.

**Methods:** The Filarial Nematode Oligonucleotide Array-Version 2 (BmV2array) slides comprising 18,153 oligonucleotide elements in duplicate that represent expressed genes and predicted ORFs from *B. malayi*, *Onchocerca volvulus*, *Wuchereria bancrofti*, and *Wolbachia*, were used to investigate gene expression changes in triplicate. 300,000 *B. malayi* Mf cultured *in vitro* to identify potential therapeutic targets.

**Results:** By 48 hours, significant increase of Mf gene expression was found in succinate dehydrogenase, malate dehydrogenase, NADH dehydrogenase, and cytochrome, which are important in glycolysis/gluconeogenesis, citrate cycle, ubiquinone biosynthesis, and oxidative phosphorylation, respectively. Furthermore, expression of immunomodulatory genes (e.g., macrophage inhibitory factor, transforming growth factor beta, serpin, and glutathione peroxidase), cathepsin-like cysteine protease, microfilarial sheath protein, motility genes (e.g., actin, myosin, tropomyosin, tubulin, and calmodulin), and ribosomal RNA were also found up-regulated.

**Conclusion:** Microarray analysis is a valuable screening tool for identifying stage specific *B. malayi* Mf genes and related metabolic pathways. The roles of these genes as a target for developing novel antifilarial drugs or vaccines should be verified.

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**Session: Malaria & Blood-borne Parasites**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Nitric oxide synthase immunity in the malaria non-vector *Anopheles culicifacies* species B: a putative transmission blocking *Plasmodium vivax* immune responsive mechanism for refractoriness

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**Background:** Innate immune-related anti-parasite defenses mounted by *Anopheles* may suppress the growth of *Plasmodium* in mosquitoes. Nitric oxide (NO) produced by the action of an inducible NO synthase (NOS) and its gene elements kill malaria parasite *in vitro* and may be central to the anti-parasitic arsenal of this mosquito.

**Methods:** In the present study, we have identified and characterized the expression of *Anopheles culicifacies* nitric oxide synthase (AcNOS) in non refractory (species A) and refractory (species B) in order to elucidate a plausible mechanism of refractoriness in terms of NOS physiologies. Understanding the difference between vector and non-vector mosquitoes can facilitate development of novel malaria control strategies

**Results:** The specific activity of AcNOS and circulating levels of nitrite/nitrate in mid-guts and haemolymph, end products of NO synthesis, were found to be significantly higher in the refractory species B as compared to non-refractory species A soon after invasion of the mid-gut by *Plasmodium vivax* at the beginning and during the course of blood feeding. Dietary feeding of a NOS inhibitor, L-NAME significantly decreased AcNOS enzyme activity and increased the parasite numbers (oocysts) in infected mosquitoes, confirming that *An. culicifacies* species B limits *Plasmodium* development via a NO mechanism. Amplification of the AcNOS gene fragment (200bp) and sequence analysis show the highest level of homology to other characterized inducible NOS genes. Increased levels of mRNAs (encoding iNOS) were observed at 7 days and 9-14 days after ingestion of an infected blood meal using semiquantitative RT-PCR analyses in the refractory species. Nitric oxide synthase (NOS) gene elements inhibitory to growth of malaria parasite *in vitro* and *An. culicifacies* NOS gene (AcNOS) is transcriptionally activated by the malaria parasite *Plasmodium vivax* in refractory mosquitoes

**Conclusion:** Our studies have revealed that AcNOS may be used as an additional effector gene to block the development of the malaria parasite in *An. culicifacies* mosquitoes. Our studies are important for understanding of innate immune-related anti-parasite defenses of the mosquito, parasite-vector interactions and may relate to/elucidate the mechanism of refractoriness and fight against the disease.

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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Malaria vector studies in the Republic of Korea: Vector parasite rates and habitat distribution  
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<sup>1</sup>65th Medical Brigade, Seoul, Korea, Republic of, <sup>2</sup>168th Multifunctional Medical Battalion, Seoul, Korea, Republic of, <sup>3</sup>Walter Reed Army Institute of Research, Suitland, MD, USA

**Background:** In 1993, vivax malaria reemerged along the demilitarized zone (DMZ) of the Republic of Korea (ROK) and rapidly increased to more than 4,000 cases by 2000. Although it was presumed that malaria would rapidly spread throughout Korea, malaria transmission remained concentrated near the DMZ. In 2005, two new species of Anopheles mosquitoes were identified, with studies indicating that Anopheles pullus and An. kleini were likely the primary vectors, while An. sinensis s.s., was a secondary vector. New evidence also suggests that An. belenrae is a potential vector. Preliminary studies indicate that, although An. pullus, An. kleini, and An. belenrae are found throughout Korea, population densities are highest near the DMZ and possibly accounting for the high rates of transmission in this area.

**Methods:** More than 5,000 larvae were collected from selected habitats near Warrior Base (approximately 3 Km south of the DMZ), labeled, placed in 100% ethanol, and shipped to the Walter Reed Biosystematics Unit where they were identified by PCR to species. Additionally, >7,000 adult anopheline mosquitoes were collected by light traps, Mosquito Magnets, and resting collections at selected sites in northern Gyeonggi and Gangwon Provinces (1-30 Km south of the DMZ). The head and thorax of individual specimens were identified to species by PCR and sporozoites, and malaria infected mosquitoes identified by single step and semi-nested multiplex-PCR.

**Results:** Larvae were identified to species from selected habitats and include Anopheles sinensis s.s., An. pullus, An. kleini, An. belenrae, An. lesteri, and An. sineroides. Rice paddies were the predominant habitat sampled. From adult collections, Plasmodium vivax was identified in An. belenrae, An. kleini, An. pullus, and An. sinensis s.s. We discuss the potential role of these vector species in maintaining malaria in the ROK.

**Conclusion:** The identification of potential malaria vectors, their role in malaria transmission, and their distributions, including population density, are important in understanding the dynamics of transmission and epidemiology of human cases in the ROK. Studies to determine the distributions of Anopheles spp. and their relative population densities over their range are needed.

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**Session: Malaria & Blood-borne Parasites**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Cardiac function and haemodynamics in African children with severe malaria

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**Background:** Mortality from severe malaria remains unacceptably high in sub-Saharan Africa. Several markers of cardiovascular compromise and metabolic acidosis correlate with mortality. The role of cardiac dysfunction in the pathogenesis of severe childhood malaria remains unknown.

In this study we aimed to investigate cardiac function and haemodynamic status of children admitted with severe malaria, assessing changes over time and response to fluid resuscitation.

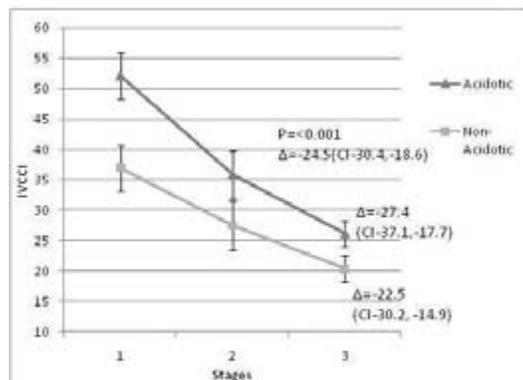
**Methods:** Setting: High Dependency Unit, Kilifi District Hospital, Kenya.

We examined thirty children admitted with severe malaria using portable echocardiography to assess their cardiac function and haemodynamic status on admission (day 0), day 1, and discharge. We compared haemodynamic parameters in two study groups: children presenting with metabolic acidosis (base deficit > 8) and children without acidosis. Acidotic patients received fluid resuscitation with either Dextran 70 or Hetastarch at admission.

**Results:**

Several markers of haemodynamic compromise were noted on admission including severe tachycardia, low stroke volume index (SVI) and high inferior vena cava collapsibility index (IVCCI) that improved with subsequent readings (fig 1). Overall, cardiac function assessed by ejection fraction ( $63.1\% \pm 5.2$  vs.  $71.9\% \pm 2.8$   $P < 0.001$ ) and left myocardial performance index (LMPI) ( $0.32 \pm 0.16$  vs.  $0.25 \pm 0.08$   $P = 0.03$ ) was mildly abnormal on admission compared to discharge. Acidotic patients had worse haemodynamic indicators, with significantly higher IVCCI on day 0 than non-acidotic patients ( $52.1 \pm 21.9$  vs.  $37.7 \pm 15.4$   $P = 0.03$ ); plus lower SVIs and worse cardiac function with higher LMPI ( $0.38 \pm 0.18$  vs.  $0.26 \pm 0.11$   $P = 0.05$ ). SVI increased post first fluid bolus in 80% of acidotic children, from an average of  $36.7$  ml/m<sup>2</sup> (95% CI: 30.9- 42.5) to  $41.5$  ml/m<sup>2</sup> (95% CI: 37.19- 45.8,  $P = 0.007$ ).

Inferior Vena Cava collapsibility index in severe malaria by acidosis over time



**Inferior Vena Cava collapsibility Index in severe malaria by acidosis over time**

**Conclusion:** Children with severe malaria have evidence of intravascular volume depletion and associated mild cardiac dysfunction which are more marked in those with metabolic acidosis. By optimizing cardiac output, this might aid microvascular flow and tissue perfusion with the aim of impacting on the metabolic derangement and associated high mortality in these children.

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**Type: Poster Presentation**

Transfusion-associated Babesia infections: Reports received by the FDA 1997 to 2008  
**D. Gubernot**, K. C. Lee, G. B. Conley, L. G. Holness, S. O'Callaghan, S. Cannon, E. Cowan, H. Nakhasi, R. P. Wise  
Food and Drug Administration, Rockville, MD, USA

**Background:** Babesiosis is a known transfusion-transmitted disease risk and there is no FDA-licensed test for donor screening. Approximately 80 transfusion-transmitted cases have been reported 1979 through 2008. This research evaluated the Babesia-related transfusion events reported to the Food and Drug Administration (FDA) with particular focus on numbers and characteristics of transfusion-related babesiosis fatality reports.

**Methods:** Data were collected from FDA's Blood Collection and Transfusion Fatality Reporting and Biological Product Deviation Reports (BPDRs) surveillance systems.

**Results:** From 2005 through 2008, the FDA received 10 transfusion-related babesiosis fatality reports after only one prior report in 1998. Recipients presented with symptoms 2.5 to 9 weeks after transfusion of implicated red blood cell units. In recent years, FDA has also seen an increase in Babesia-related BPDRs involving donors with post donation illness and reports of potential transfusion-transmitted disease. Most reports submitted to the FDA implicate *B. microti*.

**Conclusion:** The reports received by the FDA indicate Babesia infection may be a rare but increasing risk to the blood supply. Without a licensed screening test to prevent these transmissions, enhanced clinician awareness of the possibility of babesiosis in febrile, recent transfusion recipients may facilitate prompt diagnosis, improved prognosis, and more timely investigations to interdict infected units. Prompt reporting of babesiosis donor and transfusion-related events assists the FDA in assessing the scope of this risk and developing appropriate public health control measures.

Disclaimer: The findings and conclusions in this abstract have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Clinical and epidemiological study of patients with malaria treated at the hospital central de Las Fuerzas Armadas in Dominican Republic

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Hospital Central de Las Fuerzas Armadas, Santo Domingo, Dominican Republic

**Background:** As a tropical country, Dominican Republic is a malaria endemic area and although it has reduced its incidence, continues to be in the differential diagnosis of febrile illnesses.

**Methods:** This is a retrospective study to assess the epidemiological and clinical characteristics of patients diagnosed with Malaria admitted from January 2008 to November 2009, at the hospital, a tertiary-care center, and regional reference, that serves to the military population, relatives of these and the civilian population as social action. Inclusion criteria were suspected diagnosis of Malaria at the time of admission and confirmed by the presence of asexual forms in thin blood smears or thick made at the time of initial evaluation.

**Results:** Of 93 febrile patients admitted with suspected malaria, this diagnosis was confirmed in 13 (13.9%), 11 (84.6%) were men. The mean age found was 32 Years (range 15-62). While 46.1 % were found within Santo Domingo and Distrito Nacional, the distribution by region was: South (7.7 %), North (7.7%), the border region (38.5%). All were symptomatic at diagnosis. The typical clinical presentation observed in most cases: fever (98.4%), headache (85.6%), chills (46.6%), myalgia (34.5%) and jaundice (10.9 %). All were managed within the hospital, required standard of this military institution. All cases were autochthonous and *P. falciparum* were the only parasite found in all the patients, responding satisfactory to chloroquine and primaquine treatment. Death occurred in 1 (7.7%). The average time between the start of clinical setting and the diagnosis was 6.5 days for patients who were transfer from other parts of the country and 2.8 days ( $p < 0.05$ ) for those who came seeking medical care directly in the hospital.

**Conclusion:** Attention should be given to febrile patients, and test to investigate malaria must be included as a routine, regardless of the provenance, but especially those living in the border region with Haiti, the interval between the clinical setting and the diagnosis has a prognosis value. The *P. falciparum* is the unique species prevalent in Dominican Republic, and chloroquine and primaquine continues to be therapeutic option of choice.

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**Session: Malaria & Blood-borne Parasites**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Artemisinin combination therapies: Public and private market and policy surveys in Burundi and Sierra Leone

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<sup>1</sup>Komfo Anokye Teaching Hospital, KUMASI, Ghana, <sup>2</sup>National Malaria Control Program, BUJUMBURA, Burundi, <sup>3</sup>Ministry of Health, FREETOWN, Sierra Leone, <sup>4</sup>Drugs for Neglected Diseases Initiative, Geneva, Switzerland

**Background:** Access to Artemisinin-based Combination Therapies (ACTs) is still limited in Africa, even though ACTs are recommended by the WHO since 2002. In order to better understand the situation where access has traditionally been limited, policy analyses and market surveys on antimalarial availability and accessibility have been conducted in selected countries where the ACT, artesunate and amodiaquine (AS+AQ), has been adopted as first line.

**Methods:** The analyses described here were conducted in the public and private sectors of Burundi and Sierra Leone using internationally standardized methodologies. Data were obtained by reviewing publications; interviewing relevant health stakeholders; and visiting hospitals, health centres, pharmacies, and drug sale outlets.

**Results:** Patients are not accessing ACTs due to systemic barriers varying between countries. In Burundi, quinine was the most common over-the-counter antimalarial found in all sectors despite not being first line and being more expensive than ACTs. Chloroquine (CQ), which has been banned from importation, was not found. Public sector cost of AS+AQ (0.16 USD) was found to be 1/5 the cost in the private sector ( $p=0.00021$ ). In Sierra Leone, no antimalarial importation policy exists, the average cost of AS+AQ was approximately three times the cost of CQ. Although antimalarials like AS+AQ are not expected to be sold in the public sector, AS+AQ was found in three public sector outlets at a higher cost (1.88 USD) than either the mission/NGO cost or the private-sector cost ( $p<0.0001$ ).

**Conclusion:** Having on-the-ground data best informs the malaria community - including the partnership who made the fixed-dose AS/AQ available in 2007 - on how to overcome access barriers to the widespread adoption and proper use of ACTs. In Burundi, the paucity and cost of ACTs in the private sector must be addressed. In Sierra Leone, a specific antimalarial importation policy could allow for uniformity and improve adherence to nationally recommended standards.

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**Session: Malaria & Blood-borne Parasites**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Role of ultrasonography in malaria pregnancy

**M. Brock**<sup>1</sup>, F. E. M. Espinosa<sup>2</sup>, J. R. D. T. Leao<sup>3</sup>

<sup>1</sup>University of Amazon State (UEA), Manaus, AM, Brazil, <sup>2</sup>Fundação de Medicina Tropical do Amazonas, Manaus, Brazil, <sup>3</sup>Universidade do Estado do Amazonas - Fundação de Medicina Tropical do Amazonas, Manaus, AM, Brazil

**Background:** Malaria acquired during pregnancy is one of the major causes of poor maternal and birth outcomes in tropical areas endemic for this disease. Malaria during pregnancy induces deterioration of placental function, resulting in transient fetal hypoxia and can induce growth restriction. Ultrasound assessment of intrauterine growth, fetal and Placental abnormalities is often used as an important clinical tool to identify these pathological findings.

**Methods:** Fetal biometric measurements, placental and amniotic fluid evaluation were obtained and thereafter at 4 week intervals from pregnant women enrolled in a longitudinal study. The study population comprised 162 singleton gestations with ultrasound derived gestational age in the first trimester. Estimated fetal biometric parameters and weight were calculated at each ultrasound examination using the Hadlock algorithm. Placental thickness were estimated using Lubschenko data and amniotic fluid was evaluated based in Phelan study.

Were included on these study pregnant women with any Malaria infection in treatment.

Were excluded of these study pregnant women without treatment, with any pathology that can affect fetal growth, and multiple gestations.

**Results:** Fetal growth restriction occurred in 7 patients (4%) . Placental thickness was increased in 4 patients (2,4%) and decreased in 1(1,2%), 3 patients had oligodramnios. 1 fetus (1,2%) had bilateral cleft lip detected by ultrasound scan.

**Conclusion:** These data are against the normal populations data were the incidence of fetal growth restriction is 3% and fetus malformation 1-2%. This study shows that in a well treated population, the fetal growth can be the same as the population without Malaria. The ultrasonography is a good method of fetal, placenta and amniotic fluid evaluation and should be used to follow pathologic pregnancy.

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**Session: Malaria & Blood-borne Parasites**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Treatment failure of Artemether-lumefantrine in Falciparum Malaria in Brazilian Amazon area  
M. Quiroga<sup>1</sup>, A. Lara<sup>1</sup>, **M. Mascheretti**<sup>2</sup>, P. Abati<sup>1</sup>, M. Boulos<sup>3</sup>

<sup>1</sup>Tropical Medical Center, an accord between the Infectious Diseases Department Hospital das Clínicas University of Sao Paulo School of Medicine and the Health Department of Santarém, Pará, Brazil., Santarem, Brazil, <sup>2</sup>Centro de Vigilância Epidemiológica CVE/CCD/Secretaria de Estado de Saúde de São Paulo, Sao Paulo, Brazil, <sup>3</sup>University of Sao Paulo, Sao Paulo01246, Brazil

**Background:** According to Pan-American Health Organization (PAHO) around 11 million of Brazilians live in regions under moderate risk of malaria transmission and 1.5 million under high risk. The Ministry of Health in Brazil registered 314,678 malaria cases (Pf: 46,093, Pv: 265,530, Pf+Pv: 2,975, P.m: 78, Po: 2) all over the country in 2008, 99.5% of them in Amazonian area. Para state was responsible for 69,212 cases in 2008 (Pf: 10,746, Pv: 57,686, Pv+Pf: 753, P.m: 27) Artemisinin combination therapies have been establish as first choice treatment for falciparum malaria as an effective public health of Brazilian Ministry of Health since 2008.

**Methods:** Descriptive study. The goal of this study is to describe the epidemiological and clinical profile of treatment failure of Artemether-lumefantrine in two cases of Falciparum Malaria in Santarem, Para state, Brazil in 2009. Cases were admitted at the Municipal Hospital of Santarem, case records were retrospectively reviewed.

**Results:** Case 1: male, 52 years old, gold miner worker in Para state, admitted in August, 2009 with symptoms of fever, headache, nausea, vomit and fatigue. Clinical manifestation as anemia and painful hepatomegaly was observed. Peripheral blood smear was positive for malaria *P. falciparum* and *P. vivax*. Patient received specific treatment with Artemether-lumefantrine and primaquine with no side effects and clinical improvement. The cure control blood smear on day 3 was negative. On day 12 microscopy revealed *P. falciparum* parasitemia.

Case 2: male, 43 years old, gold miner worker in Para state, admitted in October, 2009 with symptoms of fever, headache, malaise and fatigue. Clinical manifestation as mucocutaneous pallor and hepatomegaly was observed. Peripheral blood smear was positive for malaria *P. falciparum* (8,000 trophozoito/mm<sup>3</sup>). Patient received specific treatment with Artemether-lumefantrine with no side effects and clinical improvement. Seventeen days after the malaria diagnosis re-started symptoms of fever, headache and diarrhea, peripheral blood smear was positive for *P. falciparum* (9,000 trophozoito/mm<sup>3</sup>). Both cases were successfully re treated with fixed combination of artemether-lumefantrine.

**Conclusion:** Treatment failure with artemisinin combination therapies has been sporadically reported. Further studies are necessary to investigated artemisinin resistance.

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**Final Abstract Number: 30.001**

**Session: Mycology, Fungal Infections and Antifungal Drugs**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

*Candida* species in the lower respiratory tract of healthy individuals

**H. J. Salzer**<sup>1</sup>, R. B. Raggam<sup>2</sup>, I. Zollner-Schwetz<sup>1</sup>, M. Hoenigl<sup>1</sup>, T. Valentin<sup>1</sup>, R. Krause<sup>1</sup>

<sup>1</sup>Medical University of Graz, Graz, Austria, <sup>2</sup>University Hospital Graz, Graz, Austria

**Background:** *Candida* species (spp.) are frequently isolated from respiratory tract secretions such as endotracheal aspirates (EA) in patients with pulmonary infiltrates. Since the prevalence of *Candida* spp. in the lower respiratory tract is unknown in healthy individuals the pathogenetic relevance of *Candida* spp. in the lower respiratory tract is difficult to assess.

**Methods:** A total of 71 healthy individuals without clinical, laboratory or radiological evidence of respiratory tract infections (temperature >38°C, elevated CRP >8mg/dl, leukocytosis >11400, elevated neutrophils) or any other lung diseases (e.g. COPD, asthma bronchiale, sarcoidosis, interstitial lung disease, malignant diseases of the lung) undergoing general anaesthesia due to elective surgery were enrolled. Exclusion criteria included antifungal therapy within 8 weeks and antimicrobial therapy within 4 weeks prior to study inclusion (with the exception of antimicrobial surgical prophylaxis), immunosuppressive therapy, active haematological diseases and HIV positivity. The presence of *Candida* spp. in the lower respiratory tract was investigated in EA samples cultivated on *Candida* CHROMagar. The *Candida* colonies were differentiated as *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis* or others according to the color of colonies and by API Aux test. For bacterial growth EA samples were further cultivated on selective agars. Bacterial cultures are counted and identified by routine microbiological procedures. In addition, oral swabs were obtained and cultured on *Candida* CHROMagar.

**Results:** A total of 88 microorganisms were isolated from 41 of the 71 EA samples containing 1 yeast (*C. krusei*), 62 gram-positive and 25 gram-negative bacterial species including the indigenous oral flora. In 30 EA samples neither bacteria nor fungi were cultured. A total of 24 *Candida* spp. were isolated from 21 of the 71 oral swabs containing 18 *C. albicans*, 2 *C. glabrata* and 1 *C. krusei* isolates. In addition, *C. krusei* isolated from both EA sample and oral cavity was originated within the same patient.

**Conclusion:** The colonization of the lower respiratory tract with *Candida* spp. in healthy individuals seems to be uncommon, despite a relative frequent *Candida* spp. colonization of the oral cavity.

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**Final Abstract Number: 30.002**

**Session: Mycology, Fungal Infections and Antifungal Drugs**

**Date: Wednesday, March 10, 2010**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Influence of gender, age, previous hospitalization and hospital stay on *Candida*-colonization of patients in a non-surgical ICU

**A. Gloeckner**<sup>1</sup>, P. Abel<sup>2</sup>

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**Background:** The incidence of systemic fungal infections is increasing and their mortality remains high despite all advances in therapy. Accurate diagnostic measures are still difficult to obtain and evaluate in order to determine the need for antifungal treatment. Moreover, *Candida* species differ substantially in their antimycotic susceptibility. Thus knowledge of species to be expected is crucial for not missing the diagnosis and making the right decision how to treat.

**Methods:** In a prospective study over 30 month we analysed samples from 411 patients (160 women and 251 men, mean age 63.6 years, mean APACHE-II-Score 20.8) admitted to our ICU. Swabs from nostril, throat and anus and specimens of tracheal secretions and urine were taken and cultured on CHROM- or CANDID- Agar at 36°C. The hospitalization history of the patients was investigated.

**Results:** Positive results were found in 43% of all 1868 investigated samples. Concerning the distribution of species, we found *Candida albicans* in 69%, *Candida glabrata* in 35% and *Candida tropicalis* in 8% of all positive specimens. The colonization index was higher in women than in men (mean 0.47 vs. 0.39,  $p < 0.01$ ) with no significant influence of age. This difference was due to a higher rate of colonization of anus and urine in women (both  $p < 0.001$ ). The species distribution showed no difference between sexes, but the rate of non-albicans species rises significantly with age (in females  $p < 0.05$ , in males  $p < 0.01$ ). A hospital stay longer than 7 days before admission to the ICU was linked with a higher rate of colonization (52% vs. 38%) but not with a significant change in species distribution. Patients who had been hospitalized within two years before the current hospital admission, showed a higher rate of colonization (42% vs. 36%) and a higher proportion of non-albicans species (48% vs. 34%).

**Conclusion:** 1. Women are more frequently colonized with yeasts than men, particularly in urine and anal swabs. 2. With advancing age, the proportion of non-albicans species detected is increasing. 3. A longer duration of hospital stay is associated with a higher colonization rate. 4. Repeated hospitalizations are linked with a higher rate of non-albicans species.

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**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Mixed fungal colonization in non-surgical intensive care patients

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**Background:** Knowledge of risk factors is important for properly applying and evaluating diagnostic tests to derive therapeutic conclusions. Colonization with fungi has been identified as an independent risk factor for invasive mycosis. In addition to colonization with a single species, the combination of different fungal species may be of interest.

**Methods:** In a prospective study we analyzed samples taken from 411 patients after admission to our ICU. Swabs from nostril, throat and anus and specimens of tracheal secretions and urine were taken and cultured on CHROM- Agar.

**Results:** Positive results were found in 798 (42.7%) of all 1868 samples. Of these, 618 were positive for a single species, 158 for two species, and 22 for three species. Concerning distribution of species, we found *Candida albicans* in 69.3%, *Candida glabrata* in 34.8% and *Candida tropicalis* in 8.1% of all positive specimens. In 90 cases, cultures grew *Candida albicans* together with *Candida glabrata*, in 23 cases, *Candida albicans* together with *Candida tropicalis*, in 12 cases, *Candida albicans* together with *Candida glabrata* and *Candida tropicalis*. Most frequently, a mixed colonization was detected from throat swabs (74 mixed, out of 281 positive cultures, 26.3%), followed by tracheal secretions (35 mixed, out of 153 positive cultures, 22.9%) and anal swabs (48 mixed, out of 235 positive cultures, 20.4%). In contrast, a mixed colonization was significantly less frequent in nasal swabs (18 mixed, out of 136 positive cultures, 13.2%) and in urine (5 mixed, out of 56 positive cultures, 8.9%).

**Conclusion:** A large proportion of samples showed growth of yeasts. Out of culturally positive, in 22.6% were found more than one species. Colonization with more than one species was found to be significantly more frequent in throat, trachea and anus compared to nose and urine.

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**Type: Poster Presentation**

Recent trends of Candida epidemiology in cancer and non-cancer patients

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**Background:** In recent years a shift towards candida non albicans has been reported from candidemia trials. A species shift in candidemia is important, since newer guidelines favor upfront echinocandins containing an economic burden. This has to be balanced with medical needs. Trends of epidemiology outside of controlled trials are therefore of particular interest. We analyzed all candida isolates from five Munich teaching hospitals (3500 beds). The objective was to compare all candida isolates and all candidemia eps in 2008 and 09 with the previous 2ys (data in brackets).

**Methods:** Between 01/08 and 10/09 a total of 15258 candida isolates were detected. No routine azole prophylaxis was given beside high risk cancer pts. There was no hint for a seasonal cluster during the study periods.

**Results:** While 64,20% (64.9%) were *C. albicans*, 7,5 % (7.8%) were *C. glabrata* ahead of *C. tropicalis* with 4,7 (4.6%), while in 1,74% *C. krusei* was detected. 384 isolates were obtained from two hemato-oncology units with *C. albicans* 52, 3% (80.5%) ahead of *C. glabrata* 8,1 % (7.8%), *C. tropicalis* 5,4 % (4.7%) and *C. krusei* (1.9%). A total of 148 isolates were detected from blood cultures. *C. albicans* was found to be less common in candidemia 57,5 % (58.9%), but dominated far ahead of *C. glabrata* 17,1 % (20.9%), *C. tropicalis* 6,8 % (7.0%), *C. parapsilosis* 4,8% (5.4%) and *C. krusei* 2,7% (3.1%). 28.6% of candidemia eps was by *C. glabrata* in cancer pts in 2008 and 09.

**Conclusion:** Although a shift towards *C. non albicans* has been described elsewhere, our study indicated *C. albicans* remains the leading species. No further shift to *C. glabrata* and *C. tropicalis* has been observed within the last 4 years. If candida is found, *C. glabrata* is detected about 2.3-fold more often and accounts for 17% of candidemia eps, with an even 3.8-fold higher risk in cancer pts. Echinocandins, newer azoles and lipid AmB therefore seem to be justified for upfront candidemia Rx, in particular cancer and those pts with an unstable clinical condition.

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**Type: Poster Presentation**

Anti-Saccharomyces cerevisiae (ASCA) antibody levels in a subgroup of patients with ulcerative colitis, Crohn's disease, GI Behcet, and GI tuberculosis: Correlations with disease duration, activity, and extension

**M. Aslan**<sup>1</sup>, B. Kocazeybek<sup>1</sup>, A. Celik<sup>2</sup>, Y. Erzin<sup>3</sup>, I. Hatemi<sup>2</sup>, G. Hatemi<sup>2</sup>, H. Yazici<sup>2</sup>

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**Background:** Clinical utility of serological markers in inflammatory bowel disease (IBD) diagnosis and differentiating is controversial. Recently ASCA has been found to have some correlation with the complication and recurrent surgery rate. Our aim was to seek for correlations between ASCA levels and disease duration, extension, activity, CRP levels, and use of immunosuppressive therapy.

**Methods:** A total of 41 consecutive patients (16 UC, 20CD, 3 GI BD, and 2 GI Tb; 34 women, 7 male) were analyzed regarding ASCA IgG levels with anti-ASCA IgG ELISA kit (Euroimmune, Lübeck, Germany), the cut-off value being 15 U/ml. Disease activity was assessed using SEO for UC and CDAI for CD, GI BD, and GI Tb patients, respectively. Additionally, a simplified endoscopic extension score was used by dividing the colon into six equal units and accepting ileal involvement as an additional unit in an ordinal manner. SPSS 15 for Windows is used for data collection and are expressed as means, with SD of the mean calculated when appropriate. Correlations were sought using Pearson's and Spearman's correlation coefficient and multivariate analysis was performed by using a stepwise regression model.  $p < 0.05$  was regarded as significance.

**Results:** The mean age ( $\pm$ SD) of patients was  $37.31 \pm 10.65$  years, 83% of them being female, and 14 out of 41 (34%) patients were in an active phase of the disease. ASCA IgG levels significantly were correlated with symptom duration x disease extension score factorial ( $r = 0.481$ ,  $p = 0.001$ ), disease duration x disease extension score factorial ( $r = 0.468$ ,  $p = 0.002$ ), and SES-CD ( $r = 0.480$ ,  $p = 0.001$ ). No correlations were noted between ASCA and CRP levels and clinical activity. On age-sex adjusted stepwise regression analysis, symptom duration, disease duration, disease extension, and SES-CD entered into the model, disease extension score was found to be the only independent predictor of ASCA IgG levels ( $R^2 = 0.1$ ,  $p = 0.044$ ).

**Conclusion:** Although the aetiopathogenesis of inflammatory bowel disease remains unsolved, a serologic anti-microbial response exists one of them being ASCA. Disease and symptom duration, disease extension but clinical activity have significant correlations with ASCA levels pointing out to the importance of sustained immunological stimuli as a triggering process. These results might provide new insights into the mechanisms of epithelial responses to antigens and ideas for therapies.

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**Session: Mycology, Fungal Infections and Antifungal Drugs**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Clinical utility of perinuclear antineutrophil cytoplasmic antibodies and anti-Saccharomyces Cerevisiae antibodies for discriminating specific intestinal inflammations

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**Background:** The role of perinuclear antineutrophil cytoplasmic antibodies (pANCA) and anti-Saccharomyces cerevisiae antibodies (ASCA) assessment in inflammatory bowel disease (IBD) diagnosis and differentiating is still imprecise and controversial

**Methods:** The aim of the study was to determine the accuracy of pANCA and ASCA in patients with specific intestinal inflammations, namely UC, CD, GI Behçet (GI-BD), GI tuberculosis (GI-TBC) which are under the same inflammatory bowel registry, compared to tree control groups; namely, Celiac disease, irritable bowel syndrome (IBS) patients and healthy controls (HC). A total of 493 subjects ( 102 with UC, 63 with CD, 13 with GI BD, 10 with GI Tb, 130 with IBS, 10 with Celiac disease, and 165 HC) firstly admitted to our weekly IBD outpatient practice of a tertiary referral center were analyzed regarding pANCA and ASCA Ig A-G via immunofluorescent assay (IFA) with commercially available IFA kits (Euroimmune, Lübeck, Germany).

**Results:** The prevalence of any pANCA or ASCA positivity and age and sex of patients are summarized in Table 1. In UC patients the prevalence of pANCA was 42.2%, which was significantly higher than in CD-4.8% (p=0.000). ASCA was found significantly more often in CD-54% than in UC patients-4.8% (p=0.000). The prevalence of ASCA in BD patients-15.4% disclosed a significant difference compared to CD patients (p=0.014), but the prevalence of ASCA in TBC patients showed no significant difference compared to CD or BD patients.

**TABLE 1:**

Prevalence of pANCA and ASCA in different subgroups. Marked values (p<.05)				
	pANCA(+)	ASCA(+)	age	male(%)
<b>IBS ( n=130)</b>	1(0.8%)	4(3.07%)	40.84(SD 12.69)*	42.3(a)
<b>HC ( n=165)</b>	1(0.6%)	7(4.2%)	35.07(SD 10.49)*,**	40(b)
<b>UC ( n= 102)</b>	43 (42.2%)	11(10.8%)	40.72(SD 13.44)**	50(c)
<b>CD (n=63)</b>	3 (4.8%)	34(54%)	37.56(SD 12.65)	38.1(d)
<b>GI-BD (n=13)</b>	0	2(15.4%)	32.11(SD 8.89)	61(e)
<b>Celiac Disease ( n= 10)</b>	0	4(40%)	36.77(SD 7.94)	0(a;b;c;d;e;f)
<b>GI-TBC ( n= 10)</b>	1(10%)	3(30%)	35.75 (SD 9.96)	70(f)

**Conclusion:** Our results confirm that in clinical practice ASCA is not specific enough to be a useful tool in differential diagnosis of any specific inflammation. However, it may have some value in screening of normal population for any bowel inflammation. pANCA may have a better clinical value in the discrimination of UC from other intestinal inflammations.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Comparative studies on the in-vitro activity of pentamycin against non-*albicans Candida* species and *Saccharomyces cerevisiae* in 161 clinical isolates

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**Background:** Pentamycin is a broad-spectrum polyene macrolide and the available intravaginal formulation (FemiFect®, 3 mg vaginal tablets, Lumavita AG, Basel, Switzerland) is effective in the treatment of vaginal trichomoniasis, candidiasis sustained by *Candida albicans* and mixed infections (Clin. Ter. 92: 137-142, 1980; Internet Journal of Gynecology and Obstetrics 11(1), 2009). Because yeasts other than *C. albicans* are frequently isolated in recurrent or mixed forms of vaginal mycoses, it is important to assess the activity of pentamycin against these species, which also exhibit reduced susceptibility to conventional antimycotic drugs. Therefore, the objective of this study was to compare the in-vitro activity of pentamycin with that of nystatin, amphotericin B, and fluconazole against strains of non-*albicans* candidal species and strains of *Saccharomyces cerevisiae* isolated from medical samples.

**Methods:** Two collections of clinical isolates included in total 40 strains of *C. glabrata*, 41 strains of *C. parapsilosis*, 30 strains of *C. tropicalis*, 30 strains of *C. krusei* and 20 strains of *S. cerevisiae*. In-vitro susceptibility testing was performed using the broth microdilution method developed by the Clinical and Laboratory Standards Institute (CLSI), document M27-A2. The minimal inhibitory concentration (MIC) of each tested drug was read visually after 24 hours and 48 hours of incubation.

**Results:** The MIC at which 90% of strains of each yeast species were inhibited (MIC<sub>90</sub>) after 48 hours of incubation was 1-4 µg/ml for pentamycin, 1-2 µg/ml for nystatin, 0.5-1 µg/ml for amphotericin B and 1-32 µg/ml for fluconazole. Some strains of *C. glabrata* and *S. cerevisiae*, and most strains of *C. krusei* were categorized as susceptible-dose dependent or resistant to fluconazole, according to the CLSI document (MIC >8 µg/ml). At concentrations ≤4 µg/ml, pentamycin inhibited completely the growth of 100% of strains of all species tested, including those strains less susceptible to fluconazole (MIC range: 16-32 µg/ml).

**Conclusion:** Pentamycin exhibited potent in-vitro fungicidal activity against the yeasts that are frequently isolated in recurrent forms of vaginal mycoses and mixed infections, like *C. glabrata* and *S. cerevisiae* (J. Clin. Microbiol. 43: 2155-62, 2005; Mycoses 49: 471-5, 2006). Pentamycin was also highly active against the strains of non-*albicans* candidal species that were susceptible-dose dependent or resistant to fluconazole.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Mycotic keratitis in Mashhad, Iran: Predisposing factors, etiologic agents & clinical manifestation  
**S. FATA**<sup>1</sup>, A. DERAKHSHAN<sup>2</sup>, A. BOLOORIAN<sup>3</sup>, M. Sedaghat<sup>1</sup>, H. Khakshoor<sup>2</sup>, F. Berenji<sup>1</sup>, M. J. Najafzadeh<sup>4</sup>, M. Afzal aghee<sup>1</sup>, M. Meshkat<sup>5</sup>, A. FATA<sup>1</sup>

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**Background:** Mycotic Keratitis is a suppurative, usually ulcerative, and sight threatening infection of cornea that sometimes lead to loss of vision. The peak of incidence is observed in the tropical and subtropical regions. To investigate the etiological agent, predisposing factors and treatment follow up of mycotic keratitis; this study was undertaken over a period of 2 years in Mashhad.

**Methods:** In this prospective, cross sectional study, among 466 individuals suffering from keratitis, 65 patients, highly suspected to mycotic keratitis were examined by direct fresh smear (KOH 10%) and culture in Mycology Media. The results were analyzed by SPSS method.

**Results:** Among 65 clinically suspected individuals, the results of direct smear and culture of 44 patients were positive.

21 patients were male (47.1%) and 24 were female (52.3%). The patients were between 8 to 84 years old. More than 40% of them were farmers. Trauma was the most common predisposing factors in more than 47 % cases due to vegetable material.

Molds and Yeasts were isolated from 86.4% and 13.6% of the samples respectively. *Fusarium spp.* were the most frequent isolates (44.4%). Others included *Aspergillus spp.* (21.8%), *Acremonium spp.* (8.3%), *Penicillium spp.* (5.6%) and *Candida albicans* (13.9%). Tearing and redness were the most common symptoms observed (93.2%). Amphotricin B was used in patients with *Aspergillus* and *Candida* Keratitis, but for *Fusarium* and other molds keratitis, Natamycin was used for 6 to 12 weeks. In one patient with *Fusarium* Keratitis, medical therapy was failed and therapeutic penetrating keratoplasty was performed. Six months follow up showed that prognosis was good in all of the patients.

**Conclusion:** In relation to the last decade, the incidence of Mycotic Keratitis is increased in Mashhad. Keratitis due to the molds is more prevalent than yeast keratitis. Entrance of foreign body (Plant particles) is the most common predisposing factor. However, it needs long term therapy but the prognosis is good.

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**Type: Poster Presentation**

Assessment of a novel region of the 28S rRNA operon for identification of clinically significant Mucormycota

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**Background:** Mucormycosis is a frequently lethal invasive infection in high risk individuals including transplant recipients and diabetic individuals. Members of these taxa are difficult to identify by traditional methods in the clinical microbiology laboratory and *in vitro* differences in antifungal susceptibility within the genera have been noted. Today, comparative sequences based methods are considered the gold standards for rapid, accurate and objective species identification of fungi. Although the rRNA regions including the 18S, ITS, 5.8S and D1-D2 hypervariable region of the 28S have been evaluated as targets, none have proven to be useful as a "universal" locus for broad fungal identification. Recently, Fredricks et al., analyzed a hitherto unexplored region of the 28S rRNA gene for species identification of a wide range of fungal genera. In the present study, we explored the utility of this target region for specific and discriminatory identification of Mucormycota genera.

**Methods:** A total of 100 isolates representing 10 different medically important genera of Mucormycota from the culture collections of the Centers for Disease Control and Prevention (CDC), Atlanta, GA, and the National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Peoria, IL were used in this study. Genomic DNA was extracted using Qiagen kit and PCR amplification and sequencing of the extended 28S rRNA was performed as previously described. Sequences were aligned using Clustal W and distance matrices were generated using the Biolumics software.

**Results:** PCR products were generated for all the isolates tested and amplicon size differences were detected among the genera. Accordingly, large regions of insertion/deletion were observed when the sequences were aligned. Distance matrices and phylogenetic analyses revealed appreciable nucleotide differences among the genera tested.

**Conclusion:** In summary, the novel 28S rRNA region evaluated in this study was readily amenable to PCR/sequencing and revealed genus specific nucleotide differences, thus holding promise as a target for discriminatory genus identification of Mucormycota.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Recurrent/resistant fungal infections in females

**N. Como<sup>1</sup>, D. Kraja<sup>2</sup>, N. Gjermeni<sup>3</sup>, M. Qato<sup>3</sup>, A. Kica<sup>3</sup>, K. Duraku<sup>3</sup>**

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**Background:** Recognition of epidemiological, etiological, topographical and treatment aspects. In this study are included 45 cases, 18-35 years-old, during the years 1999–2008 with recurrent (at least 4 episodes of fungal infections in a year)/resistant (which doesn't respond to antifungal therapy) fungal infections.

**Methods:** We have analyzed the group-ages, the origin and the immunity state of the patients. We have determined the etiology, topographic variant of the fungal infections and the real sensibility against antifungal drugs (Ketoconazol, Intraconazol, Fluconazol, Clotrimazol).

**Results:** Epidemiologic:

Group-ages	Number of cases
18-21	1
22-25	8
26-30	11
31-35	25

Where 28 were citizens, and 17 were peasants.

The immune state:

a) immunocompetent-30 cases

b) immune-compromised-15 cases

Diagnosis	Number of cases
HIV	4
colagenosis	4
Diabetes Mellitus	2
Lymphoma	1
Visceral Leishmania	1
Hypogamaglobulinemi	1
Thyrotoxicosis	1
Chronic hepatitis	1

Disease-topographic: Oro-pharyngeal fungal infection-42 cases

Topography	Number of cases
keilitis	6
glositis	9
pharyngitis	5
pharynx-esophagitis	4

Disease-topographic: dermal-16

Topography	Number of cases
axillary	1
submamare	2
inguinal	3
gluteal	3
intradigital	4

Disease-topographic: vulvovaginale -26

Topography	Number of cases
anal-perianal	2
onicomycosis	2

Etiologic: *Candida albicans* -33 cases

*Candida glabrata* -5 cases

*Trichophyton* -6 cases

*Cryptococcus Laurens* -1 case.

Therapeutic: According to mycogram, the clinical response and the subject quality.

-In (19) resistant cases we have:

a) repeated the therapeutic cycle (in 11 cases)

b) extended the local treatment (10 cases),

c) associated the systemic treatment (16 cases),

d) changed the antifungal drugs (13 cases).

We have done diagnose reconfirmation (2 cases from 8 has resulted misdiagnosed).

3 from 5 cases with *Candida glabrata* were resistant against azole.

-In (26) recurrent cases we have applied the treatment with (Fluconazol 100 mg(p.o.) or supp. Cotrimazol 500 mg 1 time/week until 6 month)

In 14 cases, after the treatment of recurrent episodes (fluconazol 100–200mg/day/7–14 days) we have improved the management of immune-compromised disease of subjects in 13 cases.

**Conclusion:** The recurrent/resistant fungal infections are encountered more frequently in group-ages 31-35 years-old, 54% of all cases.

33% were immune-compromised;

67% were immune-competent (women).

6% of all cases were named as idiopathic, the long-term treatment resulted ineffective.

Vulvo-vaginal fungal infections were predominated in 57.7% of cases.

The most frequent etiologic cause was *Candida albicans* (73.3%).

The resistant fungal infections were 42.2% of all cases; recurrent 57.7%.

The treatment in the resistant forms has resulted effective in 84.2% and ineffective in 15.7%; in the recurrent forms 73.1% effective and 26.9% ineffective.

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**Type: Poster Presentation**

Recurrent vulvovaginal candidiasis- where does *Candida albicans* persist?

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**Background:** 5-8% of all women of childbearing age suffer from recurrent vulvovaginal candidiasis (RVVC). In most cases, recurrences are caused by identical *Candida* strains suggesting a site of *C. albicans* persistence in female genitalia. The purpose of this study was (1) to investigate where *C. albicans* persists despite prolonged oral therapy, (2) to determine the clinical symptoms and signs related to *C. albicans* positive vulvar cultures and (3) to evaluate a new therapeutic approach in women with positive interlabial cultures.

**Methods:** A total of 469 women with recurrent vulvovaginal complaints were examined by colposcopy and microscopy of vaginal smears was performed. Swabs were obtained from both vagina and interlabial sulcus of the external vulva and cultured for fungal growth. Women with positive *C. albicans* cultures from the external vulva received 100 mg of oral fluconazole daily for 20 days and topical ciclopiroxolamin cream applied in the interlabial sulcus and perianally for 4 weeks. Follow-up visits were at 3, 6, 9 and 12 months.

**Results:** Of 469 women with chronic vulvovaginal complaints, 139 patients (30%) had positive *C. albicans* cultures. Of these 139 patients, 70% had both *C. albicans* positive vulvar and vaginal cultures, 24% had positive cultures from the vagina only and 6% from the interlabial sulcus only. Pruritus (OR 5.4; 95% CI 2.0 - 14.9), signs of vulvar edema (OR 3.8; 95%CI 1.0-16.8) and fissures (OR 2.4; 95%CI 1.0-5.8) correlated with positive vulvar cultures. Recurrence rates for the combined treatment were 27% at 6 months and 34% at 12 months.

**Conclusion:** Our results point at the stratum corneum of the moist interlabial sulcus rather than the non-keratinizing vaginal epithelium as the site of *C. albicans* persistence and source of endogenous re-infection in patients with recurrent vulvovaginal candidiasis. Based on this hypothesis and analogous with the treatment of nail mycosis, we used a combination of the fungistatic fluconazole and fungicidal ciclopiroxolamin cream. This new approach showed promising results.

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**Session: Mycology, Fungal Infections and Antifungal Drugs**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Delayed diagnosis of disseminated *Histoplasma capsulatum* var. *capsulatum* infection in AIDS patients in a tuberculosis high endemic country

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**Background:** Histoplasmosis is a low endemic mycosis in some parts of South Africa. The final diagnosis of disseminated histoplasmosis (DH) requires pathology and microbiology. Diagnosis and effective treatment is often delayed in resource-constrained settings, because clinical features could be confused with disseminated tuberculosis, and specialized services are limited. To our knowledge, this is the first reported case series of HIV infected patients with DH in Africa.

**Methods:** We report retrospective descriptive data on a series of HIV and DH co-infected patients attending an academic hospital between September 2003 and December 2008 in the Western Cape region.

Clinical and laboratory characteristics of all diagnosed DH were analysed with the statistical package SPSS 15.0.

**Results:** During the study period, 11 cases of DH were diagnosed: the majority of cases occurred in men (82%).

All DH patients presented with skin lesions in addition to other symptoms of which 6 had fever (55%), 9 had constitutional symptoms (weakness, night sweats and weight loss) (82%), 6 had epistaxis (55%), 5 had respiratory symptoms (45%), and 4 had gastrointestinal complaints (36%). The mean CD4 lymphocyte count was 43 cells/ $\mu$ l (IQR: 4-107). The mean CRP level was 166 mg/dl (IQR: 27-306), the LDH was 887 U/l (IQR: 158-3220). Pancytopenia was diagnosed in 3 patients.

Diagnosis was made in all patients by positive histopathology results (100%); only 3 patients had a microbiologically confirmed culture result (27%). The mean time-interval between first symptoms and treatment was 148 days (IQR: 80-305).

Three patients developed paradoxical IRIS features (27%) and three patients unmasking DH after initiation of antiretroviral treatment (ART) (27%). One patient died of DH (9%), due to delayed diagnosis and treatment, after two days of intravenous amphotericin.

**Conclusion:** In a tuberculosis high endemic region, where many AIDS patients with a very low CD4 count are seen in antiretroviral clinics, the follow-up of patients is complex, mostly due to TB related infections.

A high index of suspicion for DH is necessary to detect specific clinical characteristics on time. Patients who present with constitutional symptoms and skin lesions should be checked for DH through pathology and/or microbiology. A presenting epistaxis, and a relative high CRP should increase the likelihood of the diagnosis of DH.

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**Type: Poster Presentation**

Breakthrough *Rhizopus* spp. in an immunocompromised patient receiving caspofungin. Case Report and review

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**Background:** Invasive zygomycosis is a rare opportunistic fungal disease, with high morbidity and mortality rates that affects predominantly immunosuppressed patients. An increase incidence is observed with the widespread use of newer antifungal drugs, such as voriconazole and caspofungin. We report a case of breakthrough invasive zygomycosis in a stem cell transplant recipient on sequential voriconazole and caspofungin treatment, and we review the previous published cases.

**Methods:** Analysis of a clinical case with zygomycosis breakthrough infection and review of previous published cases.

**Results:** To date, seven cases of immunocompromised patient with breakthrough zygomycosis infection receiving caspofungin were published (Table 1)

In September 2007, a 51 year old female patient was admitted for myelodysplastic syndrome RAEB-T, diagnosed a year before. A related allogenic hematopoietic stem cells transplant (HSCT) (HLA 9/10) was performed. On October 12th, the patient developed an hepatic GVHD, treated by prednisone. The clinical evolution was good and the patient was discharged of the hospital on October 29th on prednisone, cyclosporine, penicillin V, valaciclovir, voriconazole, co-trimoxazole and folinic acid. These treatments were stopped 6 weeks later.

On January 3rd, the patient was readmitted presenting a febrile state and aqueous diarrhea. An acute digestive GVHD was diagnosed and large spectrum antimicrobial treatment was initiated. Voriconazole was switched to caspofungin on January 16th, due to liver tests enzymes alteration. After 1 week of treatment, a thoracic CT scan was performed and revealed signs of pulmonary infection. Nasal swab and bronchoalveolar lavage cultures grew with *Rhizopus* spp. The patient experienced an important clinical worsening. Considering the severity of infectious and hematologic prognosis as well as the lack of clinical response, the physician according with the family decided to stop aggressive therapy. The patients died 10 days later. The autopsy finding showed that the death was due to a disseminated angioinvasive mycotic infection.

**Conclusion:** As reported with voriconazole, prolonged caspofungin treatment may be a risk factor for invasive zygomycosis infection in immunocompromised patients. This disease must be always considered in immunocompromised patient receiving caspofungin therapy.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Granulomatous lesions in experimental *Paracoccidioides brasiliensis* infection

**E. Burger**<sup>1</sup>, R. F. S. Molina<sup>2</sup>, J. V. Alves<sup>2</sup>, C. R. P. Pizzo<sup>2</sup>, A. S. Nishikaku<sup>2</sup>

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**Background:** Paracoccidioidomycosis (PCM) is a systemic mycosis, caused by the fungus *Paracoccidioides brasiliensis* (Pb), that affects healthy individuals living in rural areas in Latin America. There are many clinical forms of the disease; severe forms are characterized by the presence of numerous disseminated granulomatous lesions, anergy in cellular immunity and high levels of specific antibodies, in contrast, mild forms have few localized granulomatous lesions, preserved cellular immunity and low levels of specific antibodies. Granuloma formation can be interpreted as a host defense mechanism to destroy or contain Pb and avoid its dissemination.

**Methods:** We infected susceptible (S) and resistant (R) mice with Pb to study the granulomas. We analysed the architecture of the granulomas and associated with presence of morphologically preserved or destroyed Pb, deposition of some extracellular matrix (ECM) components (collagen fibers types I, II, IV, osteopontin, laminin, biglycan, decorin), presence of relevant cytokines to granuloma formation ( $\gamma$ -IFN, TGF- $\beta$ , TNF- $\alpha$ ) and of matrix metalloproteinases (MMP).

**Results:** We detected all the above mentioned elements in the lesions. The thick fibers of collagen type I, (R>S) may be associated with Pb infection containment; the thin reticular fibers of collagen type III may promote the microenvironment for Pb-cell-ECM interactions; the marker of newly formed vessels collagen type IV may promote Pb dissemination and favor the influx of inflammatory cells and the proteoglycans biglycan and decorin, (R>S) may promote fungal containment. The cytokines TNF- $\alpha$  and  $\gamma$ -IFN, this later more observed in R mice may promote macrophage activation, enhancing Pb killing by these cells and the control fungal dissemination; TGF- $\beta$ , (S>R) may promote deactivation and inhibition of Pb killing by macrophages, favoring fungal dissemination and osteopontin may favor infection at its onset (S>R) and promote protection later (R>S). MMP-9 was detected in both S and R mice with active infection, eventually being involved in fungal dissemination.

**Conclusion:** The fate of PCM infection locally depends of the combined effects of ECM components, which can be limiting or permissive to Pb dissemination, and those of cytokines, which can either activate or inactivate phagocytic cells, leading to Pb lysis or survival.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Fibrosis treatment in experimental *Paracoccidioides brasiliensis* infection

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**Background:** Paracoccidioidomycosis (PCM) is a deep mycosis that presents frequent and incapacitating sequelae due to the development of numerous fibrotic granulomatous lesions even after mycological cure, rendering the quality of life of the patients extremely poor.

After characterization of the granulomas developed by susceptible (S) and resistant (R) mice infected *Paracoccidioides brasiliensis* (Pb), we evaluated the effect of drugs that interfere with fibrosis. We treated S mice with drugs that interfere with fibrosis: we used the cytokine  $\gamma$ -IFN because, in addition to its known effect as macrophage activator, it presents direct antifibrotic activity; Tetracycline due to its inhibitory effect on extracellular matrix (ECM) synthesis in addition to its antibacterial effect and the specific COX-2 inhibitors Lumiracoxib and Celecoxib, because these anti-inflammatory drugs increase the expression of collagens types III and IV.

**Methods:** We evaluated *in situ* the presence of some granuloma components such as collagen as well as of its degradation product hydroxyproline. We also determined the local presence of relevant cytokines to granuloma formation and maintenance (TNF- $\alpha$ , TGB- $\beta$ ,  $\gamma$ -IFN, GM-CSF and IL-12) and also of NO, Pb with preserved or altered morphology and the overall architecture of the granulomas.

**Results:** The best indicators of control of PCM as expressed by successful local Pb lysis were the presence of compact granulomas, delimited by a continuous deposit of collagen type 1 arranged in concentric orientation required to contain the fungi, and the production of high concentration of cytokines IL-12 and  $\gamma$ -IFN as well as of NO. The concentration of collagen metabolite *per se* was not an indicator of Pb containment or dissemination.

**Conclusion:** Based on these parameters, we can conclude that therapy with  $\gamma$ -IFN and / or Tetracycline seems promising, reducing the fungal load, increasing the production of NO and of the stimulatory cytokines  $\gamma$ -IFN and IL-12, decreasing that of the inhibitory cytokine TGB- $\beta$  and altering the granulomas architecture towards a compact structure in order to provide Pb containment without excessive fibrosis.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Cost-effectiveness analysis of the therapy for the invasive Candidiasis in Colombia

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**Background:** Candidiasis is a nosocomial infection associated to considerable mortality and high economic impact. The echinocandins are a new class of therapeutic medications that have shown to be effective in treating candidemia and other forms of invasive candidiasis; however, cost of amphotericin B dose is lower. Hypothesis: Is anidulafungin (ANI) cost-effective compared to caspofungin (CAS), and amphotericin B (AMB) in the treatment of invasive candidiasis in non-neutropenic patients hospitalized in Intensive Care Units (ICU) when the fluconazole is not a choice, from a third-party payer perspective.

**Methods:** A decision tree was designed to assess the cost-effectiveness of the three medications and this was validated by 2 critical care specialists and 2 infectologist. The model simulated costs and effectiveness in a 14-week-period. Effectiveness measure was the rate of survival and the main outcome was saved Life Years (LYs). Clinical efficacy and node probabilities were obtained from a published meta-analysis that was identified by systematic literature review. This study estimated the direct costs associated with invasive candidiasis treatment including antifungal drugs, hospitalization, and costs associated with adverse events. Medical costs were extracted from 7 ICUs of 3 major cities and drug costs were taken from a standard colombian costing source. The incremental cost per successfully treated patient was calculated and the one way sensitivity analysis was performed.

**Results:** Patients treated with ANI experienced the higher outcomes (13.7 LYs) followed by AMB (12.1 LYs) and CAS (11.7 LYs). Mean cost per patient was lower with AMB (US\$4,131) followed by ANI (US\$6,001) and CAS (US\$6,444). Based on ICERs, ANI was the dominant therapy compared to CAS and ANI was cost-effective compared to AMB (ICER US\$1.228).

**Conclusion:** Anidulafungin represents the cost-effective treatment of choice when compared to caspofungin and amphotericin B for the invasive candidiasis in Non-neutropenic patients in Colombia.

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**Session: Mycology, Fungal Infections and Antifungal Drugs**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Myocardial infarction caused by aspergillus embolization in a patient with cirrhosis

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**Background:** Patient presented with ST elevation MI and died secondary to multiorgan failure. Autopsy revealed angioinvasive aspergillosis involving several organs.

**Methods:** Case: A 47-year-old African American male with past medical history of alcohol-induced cirrhosis presented with acute midsternal pain of 5 hours duration. Respiratory distress developed and mechanical ventilation was required for airways protection.

**Results:** Initial work up showed markedly elevated cardiac enzymes with CPK 14000 U/L, elevated AST 800 U/L, ALT 210 U/L, total bilirubin 8 mg/dL, direct bilirubin 7 mg/dL, and ST segment elevation on inferior EKG leads. Patient underwent emergent left heart catheterization which revealed patent coronary arteries. Subsequently multiorgan failure resulted in decompensated shock and patient received several vasopressors. Blood, spinal fluid, urine and sputum cultures showed no growth. Patient had negative serology for HIV, acute viral hepatitis, syphilis, dengue fever, tularemia, herpes virus 1&2, CMV, EBV, leptospirosis, Q fever, Lyme disease, brucellosis, and ehrlichiosis. Patient experienced intractable ventricular fibrillation which resulted in death after a 13-day hospital stay. Autopsy report confirmed disseminated angioinvasive aspergillosis involving heart, lungs, bowel, thyroid, kidneys and spleen in addition to complete occlusion of the posterior descending artery with a fungal thrombus and multiple fungal vegetations.

**Conclusion:** Discussion: Aspergillus organisms are ubiquitous and exposure to their conidia must be a frequent event. However, disease due to tissue invasion is uncommon and occurs primarily in the setting of immunosuppression. Risk factors for invasive aspergillosis include prolonged and severe neutropenia, organ transplantation, AIDS and corticosteroid use. Infection of virtually any organ can occur, but most commonly kidney, liver, spleen, and CNS are involved. Aspergillus is second only to Candida as a cause of fungal endocarditis. Patients typically present with fever and embolic phenomena. Blood cultures are rarely positive. Prognosis of Aspergillus endocarditis is poor. Even with combined medical and surgical therapy, the mortality approaches 100 percent. Septic embolization has been occasionally reported as a reason for cerebral, myocardial and pulmonary infarctions. Our report represents rare case of myocardial infarction as a part of aspergillus septic emboli in the setting of hepatic cirrhosis as etiology of the immunocompromised state. Autopsy; however could not identify the original infected source.

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**Session: Mycology, Fungal Infections and Antifungal Drugs**

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**Type: Poster Presentation**

Correlation between HIV-1 viral load and cryptococcal capsular polysaccharide concentration:  
Evaluation in a clinical setting

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**Background:** *In vitro* studies demonstrated that a clinically relevant concentration of cryptococcal polysaccharide enhanced HIV-1 production in peripheral mononuclear cells (Pettoello-Mantovani M, et al. Enhancement of HIV-1 infection by the capsular polysaccharide of *Cryptococcus neoformans*. *Lancet* 1992, 339:21-23). However, the correlation between cryptococcal capsular polysaccharide (CCP) concentrations and HIV-1 viral load has never been assessed in a clinical setting. Considering the immunomodulatory properties of CCP and that extrapulmonary cryptococcosis is one of the most frequent HIV-related opportunistic infections in our region, the evaluation of potential pathophysiological interactions between these pathogens are of importance for development of therapeutic strategies.

**Methods:** Prospective collection of pretreatment blood samples from antiretroviral naive HIV-1 infected patients with culture-confirmed cryptococcal meningitis assisted at the Infectious Diseases Hospital "Francisco Javier Muñiz", Buenos Aires, Argentina (period 2004-2006). Informed consent was obtained from all patients. HIV plasma viral load was measured by reverse transcriptase-polymerase chain reaction using the Cobas Amplicor HIV-1 Monitor Test 1.5 (Roche). CCP antigen concentration was determined by a standard commercial assay (Latex-Cryptococcus Antigen Detection System-IMMY, Immunomycologics). A linear regression analysis was performed with *Statistix* 7.0 software. Florencio Fiorini "Estimulo" Scholarship by Florencio Fiorini Foundation and by Asociacion Medica Argentina was obtained for the development of the study, as part of an investigation of HIV-1 related cryptococcosis.

**Results:** During the study, 37 HIV-1 infected patients with cryptococcal meningitis were enrolled. Data regarding HIV-1 plasma viral load and CCP concentrations were available for 25 patients for the final analysis. Median HIV plasma concentration (copies/mL) was 296000 (range: 1000-750000). Median serum CCP antigen titer was 100 (range: 0-10000). Linear regression analysis found no evidence of a statistical significant correlation between HIV-1 plasma viral load and CCP concentrations ( $r = 0.1522$ ;  $p = 0.4676$ ).

**Conclusion:** In opposition to *in vitro* findings, HIV-1 plasma viral load has no correlation with CCP concentrations. To the best of our knowledge, this is the first study to assess if there is a relationship between the levels of an opportunistic pathogen in blood and HIV-1 viral load in a real life clinical setting

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**Session: Mycology, Fungal Infections and Antifungal Drugs**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Risk factors for candidemia-related mortality in a Neonatal Intensive Care Unit (NICU)

**C. I. Echave**, M. L. PRAINO, M. L. Voza, C. Manso, M. Russman, C. Enfedaque, A. Martinez Burkett, A. Procopio, N. Rivas, E. L. Lopez  
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**Background:** Candidemia is related to significant morbidity and mortality, specially in infants at the NICU. The aim of this study was to identify the risk factors for mortality among these patients  
**Methods:** We retrospectively studied all the patients with positive blood cultures for *Candida* spp. treated in NICU between, January 2003 and December 2008, at the Hospital de Niños "Ricardo Gutierrez" in Buenos Aires, Argentina. We analyzed the clinical baseline characteristics and risk factors for mortality: persistence of positive blood cultures, days to central venous catheter (CVC) removal, underlying diseases, secondary compromised organs and species of *Candida*  
**Results:** During the study period a total of 20 patients were identified. However, 2 patients were excluded, due to insufficient data in the medical records. Ten patients were male (55%); age: median 40 days (range 7-117 ds). All patients were admitted to the NICU, immediately after birth. None of them was less than 28 weeks gestation. The weight at birth was less than 1500g in only one patient. The most frequent underlying disease was surgical pathology of the gastrointestinal tract (9/18, 50%). Most of the patients had risk factors for candidemia as CVC (14/18, 77,7%), parenteral nutrition (15/18, 83%) and previous use of broad spectrum antibiotics (100%). *Candida albicans* resulted the main agent (9/19, 47%). One patient had two different species of *Candida*: *albicans* and *tropicalis*. The global mortality rate was 27.7% (5/18). Mortality was associated with: later removal of catheter, mean 1.9 ds vs. 5.8ds, (p= 0.02) and days to first negative blood culture: mean 4.36 ds vs. 9 ds (p= 0.025), in survivors vs patients who died, respectively  
**Conclusion:** The high mortality rate by *Candida* in our setting was similar to the reported by the literature. *Candida albicans* remained the most common isolated specie. Mortality was statistically significant related to persistent candidemia and the delay to remove CVC.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Analysis of candidemia epidemiological data and antifungigram by distinct methodologies in a large Brazilian teaching hospital

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**Background:** Candidemia results in substantial morbidity and mortality and species distribution and its susceptibility profile should be locally monitored. Antifungigram still a challenge but few laboratories have microdilution techniques in Latin America, in a routine basis, and other methodologies should be evaluated.

**Methods:** Epidemiological data from candidemia episodes were collected during 2006 in a large teaching hospital. Incidence was calculated by 1,000 admission and 1,000 patient days. Minimal inhibitory concentrations (MIC) were determined using Sensititre Yeast-One Y8, and E-test. The following drugs were tested: amphotericin B (AMB); caspofungin (CAS); posaconazole (POS), fluconazole (FLU), Itraconazol (ITRA) and voriconazole (VOR). The disk diffusion method was also performed for FLU and VOR according to CLSI.

**Results:** One hundred and thirty-six cases of candidemia were identified and represented 3.5% of the overall positive blood cultures. Incidence was 1.87 cases per 1,000 admissions and 0.27 cases per 1,000 patient-days. 58.1% patients were male and the median age was 40 years old being cancer the most frequent associated underlying disease. *C. albicans* (52.2%), followed by *C. parapsilosis* (22.1%), *C. tropicalis* (14.8%) and *C. glabrata* (6.6%) were the more frequent species. Among 100 isolates viable for susceptibility tests 100% were susceptible to AMB (MIC 90 = 1.0 mg/L) and CAS (MIC 90 = 0.064 mg/L); 98% to VOR (MIC 90 = 0.008 mg/L); 91% to FLU (MIC90 = 0.5 mg/L); and 66% to ITRA (MIC 90 = 0.125 mg/L). Posaconazole MIC90 was 0.032 mg/mL. The percentage of essential agreement (EA) and categorical agreement (CA) between broth micro dilution and other methodologies were > 93%, except for itraconazole (EA 80%, CA 70%). CA between Sensititre Yeast-One versus disk diffusion and E-test versus disk diffusion for FLU were respectively: (94%; 95%) and for VOR (96%, 98%). Minor errors accounted for the majority of categorical errors.

**Conclusion:** Candidemia by *C. albicans* still the majority of episodes in our Hospital but non-*albicans* *C* species are a growing problem and its susceptibility should be closely monitored even though overall resistance still very low. Disk diffusion and E-test had good CA and may be an alternative methodology for routine Latin America laboratories.

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**Type: Poster Presentation**

Prevalence of Cryptococcal meningitis among HIV seropositive patients in Georgia

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**Background:** Cryptococcal meningitis is a frequently observed opportunistic fungal infection in HIV seropositive patients in Georgia and an important cause of mortality among these patients. This study estimates the prevalence of cryptococcal disease in Georgia in 2003- 2008 among human immunodeficiency virus (HIV)-infected patients who were at risk.

**Methods:** Numerator data were generated by surveying all HIV infected patients in Georgia during 2003- 2008 years. A routine serum cryptococcal antigen screening was performed on 920 HIV-positive/AIDS patients by ELISA (Premier Meridian, Italy) to improve the prognosis of cryptococcal meningitis in HIV-infected patients through earlier diagnosis. The cerebrospinal fluid (CSF) samples were processed for ELISA testing after preliminary microscopic examination, comprising wet mount, Indian ink.

**Results:** Cryptococcal antigen was detected in the sera of 103 (11.2%) of them. Cerebrospinal fluid was obtained from 98 of these 103 patients and the presence of *Cryptococcus neoformans* was demonstrated by direct microscopy in 64 (66%) of them. This represents 7% of the originally screened HIV seropositive group. The incidence of cryptococcal isolation was in the 30-45 age group and predominantly affected were male patients (59 from 64).

**Conclusion:** In Georgia prevalence of Cryptococcal meningitis among HIV seropositive patients is about 7%. We found some relationship between age, gender and prevalence of Cryptococcal meningitis among HIV infected patients. The routine mycological surveillance is required for HIV/AIDS patients to help in an early diagnosis and appropriate therapy.

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**Session: Mycology, Fungal Infections and Antifungal Drugs**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Clinical analysis of 92 patients with Fungaemia - data from national survey in Slovakia

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**Background:** A prospective national survey on fungaemia was done during 2005-2007 in Slovakia. The increasing incidence of fungaemia and candidaemia was documented (2.57 and 2.16/100.000/year, respectively) comparing previous survey. 38% of cases of candidaemia were caused by *C. albicans* and 62% by non-albicans strains.

**Methods:** 92 episodes in 92 patients with fungaemia and available clinical data were analysed according to data from CRFs. Informations about risk factors, antifungal treatment and outcome 4 weeks after the onset of infection were collected.

**Results:** All patients except 16.3 % were adults >18 years old. Majority of patients were hospitalized on ICU (59.7%); 43.4% of patients had cancer and 16.3 % had haematological malignancy. 20 % of patients were neutropenic at the onset of candidaemia. Previous antibacterial treatment (93.4%), inserted central venous catheter (83.6%), total parenteral nutrition (55.4%), surgical procedure (52%) and colonisation with *Candida spp.* were the most common risk factors associated with candidaemia. Fluconazole was the preferred 1st line drug (58.6% of all treated patients) followed by Voriconazole (18.4%) and Amphotericin B (10.8%). 6 patients (6.5%) did not receive any antifungal therapy - 2/6 died and 4/6 survived. Fungaemia-related and candidaemia-related mortality was 33.7% and 27.2 %, respectively. 16/25 (64%) deaths were due to candidaemia caused by non-albicans candida strains and 9/25 (36%) were associated with *C.albicans*.

**Conclusion:** Mortality of patients with candidaemia reflects the epidemiological trend in Slovakia where majority of cases are caused by non-albicans strains. The choice of antifungal therapy should be in concordance with epidemiological data.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

*Cryptococcus*: species distribution and susceptibility profile of isolates in a teaching hospital from São Paulo-Brazil

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**Background:** To describe the percentage of *Cryptococcus neoformans* and *Cryptococcus gattii* encountered in our hospital, a 2500-bed teaching hospital of São Paulo, and to compare minimal inhibitory concentrations to different antifungal drugs.

**Methods:** Consecutive and no duplicate clinical isolates recovered from patients with infections (meningitis, cryptococcaemia, pneumonia and peritonitis) between 2006 and 2008 were included for analysis. Identification to the species level was performed by conventional methods based on growth appearance on Sabouraud dextrose agar at 37°C, production of urease and the presence of a capsule. Results were confirmed using the API 20C (bioMérieux, St Louis, USA). L-canavanine-glycine-bromothymol blue agar (CGB) was used to distinguish *C. gattii* from *C. neoformans*. Minimal inhibitory concentrations (MIC) to amphotericin B, caspofungin, 5-fluorocytosine, posaconazole, fluconazole, itraconazole and voriconazole were determined using Sensititre Yeast-One Y8™ (TREK Diagnostic Systems, Cleveland, USA).

**Results:** In the study period 43 isolates were included. Thirty three (77%) were *C. neoformans* and 10 (23%) were *C. gattii*. *C. neoformans* MIC 50/90 for antifungal drugs were as follow: amphotericin B.(0.5mg/L -1 mg/L,) caspofungin ( 16 mg/L-16 mg/L), posaconazol ( 0.064 mg/L-0.25 mg/L), fluconazole, (4 mg/L-16 mg/L); itraconazol (0.125 mg/L-0.5 mg/L) and voriconazol (0.032 mg/L-0.125 mg/L)

*C. gattii* MIC 50/90 mg/L for antifungal drugs were as follow: amphotericin: (0.5 -1mg/L) ; caspofungin (16 mg/L-32 mg/L); posaconazole ( 0.125 mg/L-0.25 mg/l) ; fluconazole: (8mg/L-16mg/L); itraconazole (0.25 mg/L-0.5 mg/L) and for voriconazole (0.125 mg/L-0.25 mg/L).

Fluconazole MICs equal or higher than 16 mg/L was observed among 40% of *C. gattii* and only 18% among *C. neoformans* isolates.

**Conclusion:** Species identification among *Cryptococcus spp* isolates is an important epidemiological tool and should be done in a routine basis. *C. gattii* showed higher fluconazole MICs and it should be closed monitored.

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**Type: Poster Presentation**

Saccharomyces fungemia associated with esophageal disease identified by D1/D2 Ribosomal RNA gene sequence

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**Background:** We describe two immunocompromised patients with ultimately fatal esophageal pathology and *Saccharomyces cerevisiae* fungemia. Originally misidentified by Vitek automated techniques, both yeast were ultimately identified by D1/D2 LSU rRNA gene sequence. Neither patient had received probiotics.

**Methods:** Case 1: A 49-year-old intoxicated homeless male with a history of chronic alcoholism was admitted for workup of hypoxia. Patient described a chronic cough and a 60 pound weight loss over six months. Examination was notable for cachexia, poor dentition, and inspiratory stidor. CT scan revealed a neck mass compromising the patient's airway. A PET scan demonstrated a hypermetabolic mass in the mediastinum with paratracheal lymph node uptake consistent with metastatic malignant disease. *Zygosaccharomyces bailii* was identified in blood cultures from admission and on hospital day three. Intravenous Amphotericin B treatment was initiated. Subsequent cultures were negative.

**Results:** Case 2: An 87-year-old woman with hiatal hernia was admitted with gastrointestinal obstruction. Examination was remarkable for hypotension and tachycardia (no oral thrush). Esophagogastroduodenoscopy revealed fecal matter in the esophagus and stomach. Post volvulus repair, the patient developed sepsis, secondary to an esophageal perforation. Surgical closure was performed and she was treated with broad spectrum antibiotics and corticosteroids. Blood cultures were positive for *Candida albicans* (two occasions) and *Saccharomyces cerevisiae* (one occasion) fungemia. Fluconazole was added to the antibiotic treatment. Patient never fully recovered and care was withdrawn.

**Conclusion:** Disseminated *Saccharomyces* infection has been reported in immunosuppressed patients treated with probiotics, but disseminated *Saccharomyces cerevisiae* infection associated with underlying esophageal disease is not previously described. *Saccharomyces cerevisiae* (which occasionally colonizes the gastrointestinal tract) is closely related to *Candida albicans*, and may be misidentified by common automated microbiologic systems. The relative resistance of *Saccharomyces cerevisiae* to azole drugs and its ability to cause widespread infections with multi-organ involvement makes rapid, correct identification critical. Using the standard yeast card, the Vitek-2 system identified one yeast as *Saccharomyces cerevisiae* and one as *Zygosaccharomyces bailii*. We found no documented cases of *Zygosaccharomyces* fungemia so we employed DNA sequencing to confirm the Vitek identification. Based on the nucleotide sequence of the D1/D2 LSU ribosomal RNA gene both the fungi were identified as *Saccharomyces cerevisiae*.

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**Type: Poster Presentation**

Determination of antifungal susceptibility in immunocompromised patients

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**Background:** The aim of the study was to compare antifungal sensitivity of yeasts to selected antimycotics isolated from immunocompromised patients with haematological disorders and critically diseased patients in intensive care units with sensitivity of yeasts isolated from immunocompetent individuals.

**Methods:** Within the period between August 2007 and October 2008, we examined 450 samples of clinical material isolated from hospitalized patients with both proven and suspected mycotic infection. Yeasts isolates were identified by Chromagar Candida and AUXACOLOR 2 biochemical test. Susceptibility to antimycotic agents such as fluconazole (FLZ), ketoconazole (KTZ), itraconazole (ITZ), voriconazole (VRZ), flucytosine (5FC), amphotericin B (AMB), and caspofungin (CAF) was determined by microdilution method according to CLSI M27-A2 standard procedure.

**Results:** Among all isolates nine yeast species were identified, the most frequently *C. albicans* strains (n = 151), followed by *C. glabrata* (n = 21), *C. krusei* (n = 11), *S. cerevisiae* (n = 8), *C. parapsilosis* (n = 7), *C. tropicalis* (n = 3), *C. guilliermondii* (n = 3), *C. kefir* (n = 2) and *C. lusitaniae* (n = 2). Among yeasts isolated from immunocompromised patients the incidence of non-albicans *Candida* was 42.6 % while only 17 % among yeasts isolated from immunocompetent patients. Incidence of resistance of commonly susceptible species to the tested antimycotics was rare. The resistance to 5-FC (MIC  $\geq$  32  $\mu\text{g}/\text{mL}$ ) was showed only in case of two *C. albicans* strains. The highest resistance, specifically to azole antimycotics, was observed in *C. glabrata* strains, amongst which, except VRZ (MIC  $\geq$  32  $\mu\text{g}/\text{mL}$ ), resistant strains to FLZ (MIC  $\geq$  64  $\mu\text{g}/\text{mL}$ ), KTZ (MIC  $>$  .125  $\mu\text{g}/\text{mL}$ ) and ITZ (MIC  $\geq$  1  $\mu\text{g}/\text{mL}$ ) were found.

**Conclusion:** Results obtained in the study showed a higher incidence of non-albicans *Candida* yeasts in immunocompromised patients. We did not find significant differences in susceptibility of yeasts isolated from immunocompromised and immunocompetent patients.

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**Session: Mycology, Fungal Infections and Antifungal Drugs**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Candidemia in third level hospitals in Bogotá, Colombia

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**Background:** Candidemia is a serious infectious complication among critical and immunosuppressed patients.

**Methods:** Patients with laboratory confirmed candidemia were prospectively followed. *Candida* isolates were sent to a reference laboratory for identification and susceptibility. Risk factors and clinical data were taken from the medical record. Antifungal use and final outcome were registered.

**Results:** 131 episodes were followed, 61% women, with ages between 9 days and 87 years. 50% of blood cultures were positive after 34 hours (in 44% of the patients blood cultures were positive in more than one bottle). More frequently found risk factors for candidemia were stay in ICU 78%), previous antibiotic use (77%), parenteral nutrition (54%), previous abdominal surgery (40%), previous use of immunosuppressors (29%). *Candida* species identified were *C. albicans* (67%), followed by *C. parapsilosis* (14%) and *C. tropicalis* (10%). Susceptibility to fluconazole were high among *C. albicans* and *C. tropicalis* isolates, but not for the other Non-albicans isolates. Fluconazole was the preferred drug of choice (57%), followed by amphotericin deoxycholate (18%). 18% of the patients never received antifungal therapy. Mortality was high after two weeks of treatment (20%) and was even higher at discharge (36%).

**Conclusion:** Candidemia is a severe complication with high mortality. In Colombia, a high proportion of patients never received antifungal therapy even with laboratory proven infection.

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**Session: Non-tuberculous Mycobacteria**

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**Type: Poster Presentation**

AIDS-associated atypical mycobacteriosis other than *Mycobacterium avium-intracellulare*: a 16-year survey of *Mycobacterium xenopi*, *Mycobacterium kansasii* and *Mycobacterium fortuitum* infections in the setting of HIV disease

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**Background:** A prompt and effective diagnosis and a timely treatment of atypical mycobacteriosis, and especially *Mycobacterium kansasii*, *Mycobacterium xenopi*, and *Mycobacterium fortuitum* disease, remains a serious challenge for clinicians engaged in the management of the immunocompromised host, including HIV disease.

**Methods:** Eighteen, twelve, and three HIV-infected patients with a microbiologically-confirmed *M. kansasii*, *M. xenopi*, and *M. fortuitum* respiratory infection respectively, have been observed in a 16-year period, out of over 4,700 hospitalizations performed because of HIV-associated disorders at our inpatient centre. These episodes were carefully evaluated from an epidemiological, bacteriological, clinical, and therapeutic point of view.

**Results:** In 15 out of the 33 overall episodes (45.5%), a concurrent bacteremia was also retrieved, as a sign of disseminated infection. A rapid and significant reduction of the crude frequency of atypical mycobacteriosis as a major HIV-related complication, occurred shortly after the introduction of potent antiretroviral combinations (cART) in the year 1996. In fact, until early nineties, the lack of potent antiretroviral regimens made frequent the association of this opportunism with full-blown AIDS, a mean CD4+ lymphocyte count of around 20-50 cells/ $\mu$ L, and extremely variable chest X-ray features and systemic presentations. The recent detection of 9 further episodes of atypical mycobacteriosis in the year 2009 was due to a late recognition of a far advanced HIV disease (the so-called "AIDS presenters"), which were already complicated by multiple opportunistic disorders.

**Conclusion:** *M. kansasii*, *M. xenopi*, and *M. fortuitum* respiratory and/or disseminated infection continues to occur, and pose relevant diagnostic problems, including late or missed identification due to slow culture and frequently concurrent opportunistic disease. Serious therapeutic difficulties, due to the unpredictable *in vitro* antimicrobial susceptibility profile of these organisms, and the need to start as soon as possible an effective combination therapy which should not interfere with other medications (especially cART), are also of concern.

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**Final Abstract Number: 31.002**

**Session: Non-tuberculous Mycobacteria**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

FOXO3a Transcription Factor mediates Apoptosis of *Mycobacterium bovis* BCG-Infected Macrophages

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**Background:** Phagocytosis of *Mycobacterium tuberculosis* (Mtb) by macrophages is the first event in the host-pathogen relationship that decides outcome of infection. Apoptosis of infected macrophages occurs during the course of Tuberculosis, and most research indicates that it acts as a host defense mechanism leading to the elimination of the pathogen. However, the mechanisms of this process are not well-defined. Forkhead transcription factors of the Box-O family (FOXOs), major targets of the PI3K/Akt pathway, have been reported to regulate cell-cycle progression, life span and apoptosis. In the present study, we investigated the involvement of FOXO3a transcription factor in the regulation of BCG-mediated apoptosis in PMA-differentiated THP-1 cells.

**Methods:** Human THP-1 cells were treated with phorbol 12-myristate 13-acetate (PMA) to induce maturation of the monocytes to a macrophage-like adherent phenotype before get infected or not with BCG. Cell survival was assessed by annexin V/7-AAD staining and visualization of nuclear condensation by a Giemsa staining. Whole cell proteins, cytoplasmic and nuclear extracts were analyzed by wester blot using specific antibodies. Real-Time PCR was used to analyze Gene's expression profile of infected and non infected cells.

**Results:** Similarly to Mtb, BCG significantly induced macrophage apoptosis when high multiplicity of infection (MOI) was used, while cell death was delayed at a lower MOI. BCG infection was associated with a decrease of both Akt and FOXO3a phosphorylation two hours post infection along with FOXO3a translocation to the nucleus. Moreover, Real-Time PCR analysis of gene's expression profile of the BCG-infected macrophages revealed an up-regulation of two pro-apoptotic FOXO3a targets, the tumour necrosis factor related apoptosis-inducing ligand (TRAIL) and the TNF Receptor-Associated Death Domain (TRADD).

**Conclusion:** FOXO3a plays an important role in BCG-induced cell death of human macrophage through the induction of pro-apototic factors. Understanding the Akt/FOXO3a pathway and its associated death mechanism in macrophages during Mycobacteria infection would lead to identification of potential therapeutic avenues for the treatment tuberculosis.

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**Session: Non-tuberculous Mycobacteria**

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**Type: Poster Presentation**

Hansen's disease in Northeast Brazil

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**Background:** Hansen's disease is an infectious malady with an insidious evolution caused by *Mycobacterium leprae*. This bacteria is transmitted through contact with damaged skin/mucosa or oropharyngeal/nasal secretions of infected patients. Peripheral nerves can be affected by this disease too, which can be incapacitating if left untreated. In spite of the Brazilian government efforts to contain the dissemination of Hanseniasis, it is still an important public health problem, mainly in the poorer areas of the country, including the Northeast region. Knowing the disease epidemiology, making early diagnosis and initiating adequate treatment and follow up are indispensable tools to achieve its control. In order to understand the epidemiology of Hansen's disease in our city, we conducted the present study.

**Methods:** This is a retrospective cohort study of all the patients with Hansen's disease diagnosed and treated in the Dona Libânia Dermatology Reference Center, in Fortaleza, Ceará, Northeast Brazil, in 2008. Patients' medical records were reviewed and the data collected was analyzed utilizing the Epi Info 3.5 program.

**Results:** A total of 273 patients were included in the study and 52% were female. Most prevalent age intervals were 41-60yrs (39,19%) and 20-40yrs (35,16%). Hypochromic or eritematous patches, presence of skin nodules or plaques, neuropathic pain and functional sequelae were the most frequent signs/symptoms found on admission, with prevalence of 81,7%, 59,6%, 23,1% and 13,3% respectively. In 27,8% patients the disease could not be classified. The remaining was classified as follow: 37% borderline, 20,5% tuberculoid, 11,4% lepromatous and 3,3% indeterminate. Only in 16,5% of the patients a household contact was identified as a reservoir of the bacteria.

**Conclusion:** Hansen's disease is still a prevalent disease in our city. Most of the patients were adults, in productive working ages. Some of them presented with functional incapacity or advanced neural damage on admission suggesting an unacceptable delay in the diagnosis. Our findings point to the necessity of a better identification of risk factors, investigation of reservoirs and the use of more sensitive methods for early detection of leprosy in order to achieve adequate control of the disease.

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**Session: Non-tuberculous Mycobacteria**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Identification of immunogenic proteins of *Mycobacterium avium* with diagnostic potential

**E. Amador**, L. Lloret, A. I. Castillo, Y. López

UNAM, Distrito Federal, Mexico

**Background:**

Non-tuberculous mycobacteria (NTM) is the term used to define all the remaining species from *Mycobacterium tuberculosis* complex species (MTC). The major practical distinction between MTC and other mycobacterial pathogens is a difference in their habitats and contagiousness. Members of the *Mycobacterium avium* complex (MAC) may be found in drinking water systems and is now more frequently isolated than MTC and represents the 26% of the total mycobacterial isolates from chronic pulmonary disease of NTM. Since the ubiquitous occurrence of MAC organisms in the environment, and the clinical presentation may be indistinguishable from tuberculosis, the diagnosis of pathogenic agent is important to apply the correct antibacterial treatment. In order to find a diagnostic tool to distinguish between MAC from MTC, we have use an immunoproteomic approach to find immunogenic proteins from *M. avium* and *Mycobacterium bovis* BCG strain México to represent MAC and MTC respectively.

**Methods:** With a panel of 52 serum samples classified into four groups: patients with active pulmonary tuberculosis, patients with chronic pulmonary by NTM, subjects with positive reaction to protein derivative (PPD) who were healthy household contacts and those with negative reaction who were the healthy uninfected controls, ELISA test was performed for titration of IgG2 against 16 mycobacterial strains from three groups: *M. tuberculosis*, *M. bovis* BCG and NTM. The sera with higher titers were used to obtain the immunoproteomes. A standard proteomic analysis of BCG Mexico and *M avium* M7 began with the separation and visualization of the protein mixture using two-dimensional polyacrylamide gel electrophoresis (2-DE) followed by Western blotting for the screening of immunogenic proteins using selected sera.

**Results:** The comparison between the immunodetection of different sera: TB, MNT and PPD+/- against BCG Mexico gave about 80% of unique proteins and 20% of proteins shared among different sera. The immunodetection in *M. avium* gave 90% of unique proteins and 10% of proteins shared among the same sera.

**Conclusion:** The comparative analysis between immunoproteomes of BCG Mexico and *M. avium* allowed the identification of candidate proteins for the differentiation between pulmonary disease caused by NTM and BCG Mexico as a member of MTC.

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**Session: Non-tuberculous Mycobacteria**

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**Type: Poster Presentation**

Characterization of nontuberculous mycobacteria isolated of potable water distribution system and wastewater of Mexico City Metropolitan Area

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UNAM, Mexico City, Mexico

**Background:** Background: Nontuberculous mycobacteria (NTM) are normal inhabitants of the environment and they are found in soil, dust and water including natural and potable water and since there is no evidence of person-to-person transmission, the water is considered the main vehicle for transmission of nontuberculous mycobacteria. Some studies have shown that the rate of infection by nontuberculous mycobacteria is increasing in predisposing hosts as well as healthy persons. On the other hand NTM may alter replication of vaccine *Mycobacterium bovis* Bacillus Calmette and Guerin in animal model and influence in the protection subsequent BCG vaccination. The aim of this study was the isolation and identification of nontuberculous mycobacteria in: drinking water distribution systems in the Mexico City Metropolitan zone.

**Methods:** Water samples were decontaminated by NaOH/SDS and cultured onto Lowenstein Jensen to 37°C/ 30 days. Different colonies were identified by PCR-PRA 65 kDa gen, sequencing and phylogenetic inference.

**Results:** We isolated NTM in both water sources. The most frequently occurring isolates in potable water supply system were *M. nonchromogenicum*, *M. arupense*, *M. peregrinum* and *M. goodnae*. Eleven species were isolated in wastewater and one *Mycobacterium* spp.

**Conclusion:** It is important to know the geographic distribution of nontuberculous mycobacteria presence in drinking and wastewater because represent a risk for the population health.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Mass screening for fever: A comparison of three infrared thermal detection systems and self-reported fever

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**Background:** During the 2003 severe acute respiratory syndrome and 2009 pandemic influenza A (H1N1) outbreaks, infrared thermal detection systems (ITDS) were used at international ports of entry and in hospitals to screen for fever. However, evidence is limited to support the accuracy of ITDS and their benefit over self-reported fever for mass screening. In this study, we compared three different ITDS to self-reported fever.

**Methods:** A cross-sectional study of 2986 patients (age  $\geq 18$  years) was conducted in three hospital emergency departments. Patients were asked if they felt that they had a fever (self-reported fever). We measured patient skin temperatures by using three ITDS (FLIR A20M, OptoTherm Thermoscreen, Wahl HSI2000S) and oral temperatures ( $\geq 100.0^\circ\text{F}$  = confirmed fever) by using digital thermometers. ITDS temperature measurements and self-reported fevers were compared using oral temperatures as a reference. Data were analyzed using simple and multiple linear methods.

**Results:** Of 2873 patients with an oral temperature recorded, 64 (2.2%) had a confirmed fever. Fever was reported by 476 (16.6%) patients and 48 (10.1%) of these were confirmed. Self-reported fever had 75.0% sensitivity and 84.7% specificity. At optimal cutoffs for detecting fever as found in this study, the OptoTherm Thermoscreen and FLIR A20M temperature measurements had greater sensitivity (85.7% and 79.0%) and specificity (91.0% and 92.0%) than self-reported fever. Of the three methods evaluated (ITDS, self report, and a combination in which a signal on either ITDS or self report indicated a fever), ITDS (OptoTherm Thermoscreen and FLIR A20M) had the highest total sensitivity and specificity for fever detection. Correlations between ITDS measurements and oral temperatures were similar for the OptoTherm Thermoscreen ( $r=0.43$ ) and FLIR A20M ( $r=0.42$ ), and significantly lower for Wahl HSI2000S ( $r=0.14$ ,  $p \leq 0.001$  for both comparisons).

**Conclusion:** When compared with oral temperatures, two ITDS (FLIR A20M and OptoTherm Thermoscreen) were reasonably accurate in detecting fever and were better predictors of fever than self report. These findings may be particularly important in the context of travel in which fever may not be reported or cannot be measured using contact thermometers. In such settings, ITDS could provide an objective means for detecting fever as part of a comprehensive public health screening strategy.

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Characteristics of travelers to developing countries: Findings from the 2008 consumer styles survey

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**Background:** Developing countries, while gaining in popularity as travel destinations, may present increased or unfamiliar health risks to travelers from developed countries and require important pre-travel preparation such as seeking medical advice, medications, and vaccinations. Studying the characteristics of travelers to developing countries can help formulate more effective messages for healthy travel.

**Methods:** We analyzed survey data from Porter Novelli ConsumerStyles 2008, a mail survey with a U.S. representative sample, to understand characteristics of overnight stay travelers to developing countries (countries except the United States, Canada, Europe, Japan, Australia, and New Zealand). Odds ratios (OR) and logistic regression were used in the analysis (all p-values<0.05).

**Results:** Among the 10,108 survey respondents, 913 (9%) were travelers. Only 331 (36%) travelers sought pre-travel medical advice from one of the 11 sources listed, 157 (47%) of those 331 sought advice from multiple sources. The top reasons for not getting pre-travel medical advice were the belief that pills/shots were not needed (35%), followed by not thinking about it (27%). A specific question for travelers who visited yellow fever endemic countries (Kenya, Nigeria, Ghana or Senegal) revealed that only 52% (38/73) got yellow fever vaccine. Compared to non-travelers, travelers were more likely to be male (OR=1.24, 95% confidence interval (CI): 1.08-1.42), Hispanic (OR=1.38, CI: 1.15-1.67), over age 55 years (OR=1.26, CI: 1.09-1.45), living in smaller households (<4 members, OR=1.29, CI: 1.12-1.5), from richer families (>\$60k, OR=2.94, CI: 2.54-3.4), and nonwhite (OR=1.19, CI: 1.03-1.37). They were more adventurous (OR=1.34, CI: 1.17-1.54), and in better health (OR=1.82, CI: 1.59-2.09). Most of the 913 travelers traveled for leisure (77%), followed by visiting friends and relatives (17%), and business (10%).

Logistic regression showed that age over 65 years, traveling for business, and volunteering were positively associated with pre-travel advice seeking while being Hispanic was negatively associated.

**Conclusion:** Our study showed that travelers to developing countries were more likely to be male, healthy, adventurous, and have higher income. More than half of the travelers surveyed did not seek any pre-travel medical advice. Messages targeted to Hispanic travelers could improve their awareness of the need to get medical advice before traveling.

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Attitudes towards Avian influenza and sources of media information in travelers to developing countries

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**Background:** Although there is an on-going 2009 H1N1 influenza epidemic, avian influenza virus (A/H5N1) continues to be a significant public health threat. Currently, 442 cases have been confirmed worldwide with 262 deaths, mostly in Asian countries. Risk of disease may be higher in travelers to developing destinations, where these cases occur more frequently. This study investigated travelers to developing countries (TDC) and described their attitudes towards A/H5N1 and defined their sources of media information in order to inform focused avian influenza prevention campaigns for travelers.

**Methods:** Data were analyzed from the 2008 Porter-Novelli ConsumerStyles survey, an annual national mail-in survey that gathers demographic information and media/consumer information about the US population. TDC were defined as persons traveling outside the United States for  $\geq 1$  day anywhere other than Canada, Europe, Japan, Australia, or New Zealand. Odds ratios (OR) and logistic regression were used.

**Results:** Of 10,108 respondents, 913 (9%) reported being TDC; compared to non-TDC, TDC were less likely to be worried about getting ill from A/H5N1 (OR=0.5, CI=0.4-0.8,  $p=0.002$ ). Further, TDC were less likely to have followed news stories about A/H5N1 (OR=0.72, CI=0.56-0.95,  $p=0.02$ ) and were more likely to feel that news media were "exaggerating the dangers" (OR=1.3, CI=1.1-1.5,  $p=0.006$ ), compared to feeling the "news reports are about right." Overall, TDC were more likely to refer to the Internet (OR=1.5, CI=1.3-1.7,  $p<0.0001$ ) for health information than were non-TDC. They were also more likely to read the national news (OR=1.3, CI=1.2-1.5,  $p<0.0001$ ) or travel sections (OR=3.0, CI=2.6-3.4,  $p<0.0001$ ) of the newspaper. TDC were more likely to view travel programs on television (OR=1.6, CI=1.4-1.9,  $p<0.0001$ ). However, for both newspaper and television, the two groups did not differ significantly in reading the health section or watching health shows.

**Conclusion:** Given the initial spread of the 2009 H1N1 virus through travelers and the ongoing threat of A/H5N1, it is important to tailor health messages carefully to best communicate the importance of avian influenza risk to travelers. TDC will likely be better reached via information on the Internet and travel-related media sources.

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Demographics, medical conditions, and use of immunizations and chemoprophylaxis among international travelers within the Global TravEpiNet U.S. National Clinic Network  
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**Background:** International travelers play a significant role in the global spread of infectious diseases. Despite this, data are limited on travel patterns, medical conditions, and medical interventions among international travelers prior to departure.

**Methods:** Global TravEpiNet is a U.S. CDC-sponsored network of U.S. clinics that care for international travelers. We report analysis of health data for 3,159 international travelers seen at U.S. Global TravEpiNet sites in 2009.

**Results:** Females accounted for 56% of travelers. The median age was 33 years; the median duration of travel was 14 days. Thirty-five percent of international travelers were traveling to low-income countries, 46% to low-middle income, 16% to upper-middle income, and 2% to upper-income countries. The main purposes of travel were vacation/leisure (63%), business (20%), extreme-adventure travel (14%), education/research (11%), visiting friends and relatives (10%), non-medical service work (6%), and providing medical care (4%). Two percent of travelers were attending large gatherings. Ten percent were children less than 18 years of age; 4% were less than 5 years of age; and 6% of travelers were over 65 years of age. Sixty-four percent of travelers listed a medical condition; 70% were on daily medication. Ten percent of travelers reported a pre-existing neurologic or psychiatric condition; 7% reported a pre-existing intestinal condition; 2.5% were immunocompromised; and 0.4% of female travelers were pregnant or breastfeeding.

We analyzed vaccine usage for prevention of hepatitis A, yellow fever, and influenza. Eighty-one percent of travelers received immunization against hepatitis A; 7% were considered pre-immune. Of the 38% of travelers visiting countries that included areas endemic for yellow fever, 67% received yellow fever immunization; 18% were considered pre-immune. Yellow fever vaccine was administered to 407 travelers 60 years of age or older. Forty percent of international travelers received influenza vaccine; 30% were considered pre-immune. Of the 2082 travelers traveling to countries that included areas endemic for malaria, 65% received malaria chemoprophylaxis. Of these, 66% received prescriptions for atovaquone-proguanil, 3.5% received doxycycline, and 14% received mefloquine.

**Conclusion:** These data suggest that international travelers range widely in age and frequently have co-morbid medical conditions that heighten the need for pre-travel advice.

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Use and sources of medical information among departing international travelers to low and middle income countries at Logan International Airport-Boston, MA, 2009

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**Background:** International travelers play a significant role in the global spread of infectious diseases, especially travelers to low and middle-income countries (LMICs). Despite this, limited data exist on sources of health information used by these travelers.

**Methods:** To address this, we surveyed 1,254 international travelers who reside in the U.S. and were departing from Boston-Logan International Airport in 2009.

**Results:** Of the 1,254 travelers, 671 (54%) were traveling to LMICs. The mean age of travelers to LMICs was 42 years, and 30% were traveling for more than 2 weeks. Purposes of travel included vacation/holiday (63%), business/work (11%), educational/cultural exchange (6%), performing volunteer work (10%), adventuring (7%), attending a large gathering (2%), providing medical care (3%), receiving medical care (0.5%), and adoption (0.3%). Nineteen percent were traveling as part of a family that included children, and 104 (16%) were born overseas and returning to visit friends or relatives (VFRs).

Among travelers to LMICs, 50% did not seek any medical advice and 74% did not see a healthcare professional prior to travel. For travelers who did not seek medical advice, the most common reasons cited were lack of concern about health issues (60%), not thinking of it (35%), not having enough time (7%), inconvenience (3%), and expense (2%).

A significantly lower percentage of VFRs sought any-source medical advice prior to travel compared with other travelers (37% vs 52%;  $p=0.004$ ). VFRs were less likely than other travelers to use the Internet (12% vs 24%;  $p=0.004$ ), and less likely to see a specialist practitioner prior to travel (2% vs. 15%;  $P<0.001$ ). VFRs and other travelers were equally likely to seek advice from primary care providers prior to travel (21% vs. 17%;  $p=0.32$ ).

**Conclusion:** Our results suggest that half of travelers to LMICs do not seek any healthcare advice prior to their trip, and that most of such travelers do not seek advice from a health care professional. The most common reason these individuals cite for not seeking medical advice is lack of concern about health problems related to travel. These results suggest a need for health outreach and education programs targeting travelers to LMICs.

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Transporting a critically ill patient from the Canadian north - lessons learned from almost a decade of SkyService Medevac experience

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**Background:** Canadian North is vast territorially, yet medical resources are lacking man-power, expertise, equipment and facilities. Transport of seriously ill patients is, hence, a common necessity frequently requiring both ground and air transportation. SkyService Medevac is the major medevac air-transporter in Canada and one of the global leaders in the field.

**Methods:** We reviewed the data related to a total of 988 cases of medical evacuations from 2002 to 2008. The data reveals information regarding demographics, pathology prompting the transport as well as medical expertise required for the transport. We pay special attention to the parameters concerning the neonatal and pediatric population.

**Results:** Of the 988 cases (Table 1) of medical evacuation from Baffin Regional Hospital in Iqaluit, Nunavut, between 2002 and 2008, pediatric population comprised 35.6%, majority of whom were neonates. Almost 17% of the patients were critically ill, intubated and required intensive-care hospitalization. The most common pathologies prompting evacuation were those involving cardiovascular and respiratory systems. There were no in-flight mortalities, while invasive interventions by the medical staff were extremely rare after departure.

Year	Total Pts	Vented	Non Vented	Age <1	Age 1-18	Age >18
2002	111	24	87	19	20	69
2003	169	44	124	40	20	96
2004	100	22	18	21	9	70
2005	104	22	82	19	20	63
2006	119	19	96	29	15	75
2007	164	21	143	25	26	113
2008	221	35	187	52	19	150
<b>Total</b>	988	165	823	205	147	636

**Conclusion:** Safe air transport from any destination is feasible, but required detailed planning, pre-flight preparation and expertise. SkyService Medevac data demonstrate that transport from Canadian North is not only safe, but also economically advantageous.

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Incidence and impact of travelers' diarrhea among foreign backpackers in Southeast Asia

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**Background:** Travelers' diarrhea is the most common disease reported among travelers visiting developing countries, including Southeast Asia, which is visited by large numbers of backpackers each year. Current knowledge of this particular group is limited. This study aimed to determine the incidence and impact of travelers' diarrhea among this group. The secondary objective was to assess their attitudes and practices towards the risk of travelers' diarrhea.

**Methods:** Foreign backpackers in Bangkok, Thailand, were invited to fill out a study questionnaire, in which they were queried about their demographic background, travel characteristics, pre-travel preparations, and actual practices related to the risk of travelers' diarrhea. For backpackers who had experienced diarrhea, the details and impact of each diarrheal episode were also assessed.

**Results:** In the period April-May 2009, 408 completed questionnaires were collected and analyzed. Sixty percent of participants were male; overall, the median age was 26 years. Nearly all backpackers (96.8%) came from developed countries. Their main reason for travel was tourism (88%). The median stay was 30 days. More than half the backpackers (56%) carried some antidiarrheal medication. Antimotility drugs were the most common medication carried by backpackers, followed by oral rehydration salts (ORS), and antibiotics. Although 61% of participants had received information about travelers' diarrhea before the current trip, their practices were far from ideal; 95.7% had bought food from street vendors, 92.5% had drunk beverages with ice-cubes, 34.6% had eaten leftover food from a previous meal, and 27.5% had drunk tap water. Only 23% of backpackers always washed their hands before eating food. In this study, 31% (130/408) of backpackers had experienced diarrhea during their trip. Most cases (88.4%) were mild and recovered spontaneously. However, 8.5% of cases required a visit to a doctor, and 3.1% needed hospitalization; 16.28% had to delay or cancel their trip due to a diarrheal attack.

**Conclusion:** About one third of the foreign backpackers in Southeast Asia had experienced diarrhea during their trip. Their current state of awareness and practices related to the risk of travelers' diarrhea were inadequate and should be improved.

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The health surveillance stations at points of entry in Brazil under the revised International Health Regulations - IHR/2005

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**Background:** The recent revision and update of the International Health Regulations, IHR (2005), provides a new approach to deal with international reaction to public health events and to ensure global health security. Therefore, it is a priority to build, strengthen and to mobilize the necessary resources. The National IHR Focal Point must notify within 24 hours all events which may constitute a public health emergency of international concern. This study aim to assess the effectiveness of Health Surveillance Units at points of entry in Brazil regarding health control of international travelers and epidemiological investigation conducted in accordance with the IHR (2005).

**Methods:** It was analyzed the public health events notified to the Health Surveillance Units at points of entry that occurred in the first year after IHR (2005) entry into force in accordance with the attributes of usefulness, sensitivity, timeliness, and stability, and their relation with Malaria imported cases into Brazil at the same period.

**Results:** Since 1975, Brazil has a broad national epidemiologic surveillance system to reporting infectious and no-infectious diseases and that enables the assessment and control of these events timely. Until 2007, the main activity at points of entry was the supply of the yellow fever vaccine and its verification when an international traveler was arriving from an affected country. At the first year, 26 suspected events of public health concern were reported by points of entry to central office after 4.2 days average, such as unknown death (6 events), chickenpox (5), malaria (4), tuberculosis (2), outbreaks of foodborne illness (2), and one of rubella, hanseniasis, acute fever illness, hepatitis, norovirus, conjunctivitis and accident. At the same time, 203 Malaria imported cases occurred among 30 percent of employees and 60 percent of cases arriving from Africa.

**Conclusion:** The structure of the health surveillance at points of entry in Brazil has changed after the IHR/2005 implementation. The low sensitivity for detection of events in points of entry are in agreement with the relevant literature. There is the necessity of further research on imported cases of notifiable diseases and improvement of the investigation and notification to the central level.

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Varicella (Chickenpox) outbreak in Bhutanese refugee camps in Eastern Nepal

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**Background:** Approximately 100,000 Bhutanese refugees live in seven camps in southeastern Nepal. For those offered resettlement to the USA, Canada, Australia, New Zealand, Denmark, Norway, or Netherlands, the International Organization for Migration (IOM) in Nepal conducts medical screening and arranges travel, moving up to 15,000 refugees annually. Varicella spreads primarily by airborne droplets and patients with infectious varicella are prohibited from the air travel. Varicella vaccine in Nepal is not licensed. In March 2009, an outbreak of varicella was detected at a refugee transit center (TC). Outbreak response was conducted jointly by UNHCR, IOM, and Association of Medical Doctors of Asia (AMDA), implementing partner for refugee camp health services.

**Methods:** A varicella case was defined as an illness with acute onset of diffuse papulovesicular rash without other apparent cause. Refugees received additional health education regarding signs and symptoms of varicella and importance of limiting contacts with sick people. Prior to travel, medical staff screened departing refugees for fever and conducted careful skin inspection. For cases with imminent travel, cases and their family were deferred from travel or travel-related screening for 21 days. Refugees identified with varicella in TC were isolated until lesions crusted.

**Results:** From 25 February to 25 May 2009, 473 cases of Varicella were registered (cumulative incidence 50 per 10,000 refugees). Among the seven camps, incidence ranged from 9 per 10,000 to 124 per 10,000; outbreak duration ranged from 37 to 87 days. The age ranged from 01 month to 30 years, mean 6.5 years. The incidence was the highest among children of 0-4 years (242 per 10,000), and decreased with each successive age group. Eight cases and their families (total 21 refugees) were deferred from travel for 21 days. 14 refugees and their families (total 63 refugees) were deferred from travel-related screening. 3,860 refugees departed during the outbreak period to United States. No refugees were reported to develop varicella during the flight or upon arrival to the final destination.

**Conclusion:** In a setting with endemic varicella transmission, systematic surveillance, combined with isolation of cases, likely decreased transmission and prevented travel related outbreaks of varicella.

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Foreign travel associated with increased sexual risk: A cohort study

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**Background:** An increasing number of people travel abroad for their holidays each year. New sexual relations while abroad may result in the acquisition and introduction of novel strains of sexually transmitted infections (STIs).

**Methods:** We conducted a prospective cohort study to assess the impact of alcohol and drug use, and foreign travel on casual travel sex in students from a British university during the summer break in 2006.

**Results:** Two thirds of students traveled abroad. They were more likely to consume alcohol (RR 1.59, 95% CI 1.17-2.16) and use drugs (RR 1.31, 95% CI 0.88-1.94), particularly Cannabis, and to have new sexual partnerships during holidays. They were also more likely to report sexual relations after holidays (RR 1.29, 95% CI 1.09-1.53). New partnerships were associated with being single, traveling abroad, drinking large amounts of alcohol, having previously had large number of sexual partners. The adjusted relative risk of developing new sexual partnerships with foreign travel was 2.70 (95% CI 1.11-6.61). Testing for a STI after the summer break was associated with both foreign travel (aRR 2.80, 95% CI 1.16-6.74) and younger age.

**Conclusion:** People who travel abroad are more likely to engage in risk taking behavior and to develop new sexual partnerships during their holidays. They are also more sexually active on their return to the UK, increasing the chance of introducing new and resistant strains of STIs in the UK. These individuals are, however, also more likely to be tested for STIs.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Management of an international outbreak of norovirus on board a cruise ship

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**Background:** Managing an outbreak of gastroenteritis on board a cruise ship while minimising disruption to passengers' enjoyment is difficult. This can be more complex with international cruises. We describe epidemiological investigation and control of an outbreak of Norovirus on an international cruise around the British Isles and the Netherlands managed through an international multi-agency incident control team.

**Methods:** A cohort study was conducted using information from lists routinely collected by the cruise ship to assess the possible sources of exposures.

**Results:** A total of 191 of the 1,194 passengers (16%) and 5 crew (1%) became ill with gastrointestinal symptoms. Norovirus was identified through PCR at one of the ports of call. Attack rate was higher among passengers staying in the main deck (RR 3.41, 95% CI 1.47-7.94), which houses both passengers' cabins and leisure facilities (e.g. shops). Also, passengers who went on one of the organised coach tours where there were symptomatic passengers were at increased risk of infection (RR 2.14, CI 1.51-3.03).

An international multi-agency Outbreak Control Group, involving port health authorities and public health agencies in the ports of call, was convened to oversee control measure and advice the incident management team on board the ship. This allowed continuity, ensuring that port health officers inspecting the ship at each port were aware of what had been previously recommended and could monitor progress.

**Conclusion:** Controlling outbreaks on board a cruise can be complex when the ship moves from one country to another and the leadership of the investigation changes. To ensure that an outbreak is appropriately managed, multinational outbreak control groups are needed with one agency taking the lead throughout the outbreak. International agreement between public health authorities in different countries is needed.

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**Type: Poster Presentation**

Medical tourism research: A systematic review

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**Background:** Medical tourism, foreign travel for the purpose of seeking medical treatment, is an important new development in healthcare. Medical tourism is a world-wide, multibillion dollar phenomenon that is expected to grow substantially in the next 5-10 years. To provide a foundation for future investigations, a systematic review was conducted to identify and evaluate current data on medical tourism and associated health impacts.

**Methods:** PubMed, EMBASE and Medline databases and the World Wide Web were searched to identify studies of medical tourism from 1990-2009 containing data.

**Results:** 44 studies were identified. 75% (n=33) were reports on complications associated with medical tourism for: commercial organ transplantation (n=30), cosmetic surgery (n=1), dental care (n=1), and fertility treatments (n=1). There were 9 (20%) surveys of travelers and/or medical tourism providers, and 2 (4%) business studies. Methodologies used were primarily retrospective record reviews (75%) and interview surveys (23%). Studies of commercial organ transplantation reviewed 2506 cases, primarily kidney (92%) and liver (8%) transplants. China and India were the most frequent transplant destinations. Studies' results suggest an apparent increase in "transplant tourism" from 1990-2009, and higher incidence of post-operative tissue rejection and severe infectious complications among transplant tourists compared to other transplant patients. These studies are limited by lack of denominator data for transplant tourism, exclusive focus on complications, survivorship bias, and the possibility that transplant tourists may not be representative of all medical tourists. Business studies calculate widely varying estimates of the frequency of medical tourism, ranging from 60,000-750,000 medical travelers annually. These studies are limited by variability in the definitions and methodologies used to study medical tourism. Survey studies are limited by little or no data on treatment(s) received, reasons for seeking foreign healthcare, or treatment outcomes.

**Conclusion:** Current epidemiological data on medical tourism are limited. Basic questions such as the prevalence of medical tourism and associated complication rates have not been established. Important directions for future research include: developing consistent definitions, and conducting prospective studies of demographics, motivations, treatment outcomes, and cost benefits to better understand the healthcare implications of medical tourism.

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**Type: Poster Presentation**

Preventing enteric fevers in London VFR travellers  
**Y. Knight**, E. Dapaah, B. Walsh  
South West London Health Protection Unit, London, United Kingdom

**Background:** Enteric fevers have recently increased in the UK; 40% of the 288 cases in 2007 were in London. South West London Health Protection Unit saw a doubling of enteric fever cases from 2007 (n=16) to 2008 (n=32). An initiative has been launched in south west London to provide pre-travel health promotion, particularly for travellers visiting friends and relatives (VFR) and of Indian, Pakistani or Bangladeshi ethnicity in whom the majority of UK enteric fever cases occur. An enhanced surveillance questionnaire revealed many VFRs do not seek pre-travel health advice, particularly not from expected sources such as general practitioners or travel clinics. Enteric fever health promotion needs to be targeted at London's VFR travellers and Asian communities.

**Methods:** We present 4 approaches to improving enteric fever health promotion. Using feedback from an enhanced surveillance questionnaire we have developed a travel health information leaflet containing typhoid/paratyphoid prevention advice aimed at VFRs. The leaflet is the first of its kind in the UK, giving food, water, hygiene and immunization advice. We performed two qualitative surveys: i) a targeted reader's group (n=15) to ascertain the accessibility and appropriateness of the leaflet to our target population; ii) in a sample of VFR and Asian Londoners we conducted a focus group (n=8) to identify the perceived barriers to accessing travel health information, and more suitable settings for travel health promotion in this population. All work was performed in collaboration with Leicester NHS, and the Health Protection Agency's Communications and Travel & Migrant Health Sections.

**Results:** Of 30 cases of enteric fever in south west London in 2008, 53% were typhoid, 70% were VFR travellers, and 67% were of Asian ethnicity. Content and style of the typhoid travel health promotion leaflet was found to be accessible to our target audience; concern was raised over potentially inaccessible use of language specific to typhoid and faeces. Perceived barriers included cultural views and notions of the purpose of travel. More appropriate settings for dissemination would include community pharmacies, school nurses and community centres.

**Conclusion:** We have developed an appropriate, innovative travel health promotion leaflet aimed at preventing typhoid/paratyphoid in London VFR travellers which is additionally tailored toward the Asian community.

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**Session: Travel Medicine and Travel Health**

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**Type: Poster Presentation**

International traveler and prevention of diarrheal diseases characterization of the international traveller to whom has been prescribed cholera vaccine at the international vaccination center of Oporto during the years 2007 and 2008

**G. Saldanha**

Public Health, Porto, Portugal

**Background:** Each year more than 50 million people travel from industrialized countries to developing countries. The most common health problem in people who travel to these countries is diarrheal diseases, an important cause of morbidity and mortality. The recent commercialization of a new recombinant vaccine of cholera (the most severe enterotoxic enteropathy) has created new expectations in the protection of travelers. An additional advantage of this vaccine is its ability to induce a protective immune response against labile toxin of enterotoxigenic *E.coli* (the most frequent cause of traveler's diarrhea). The first aim of this study was the determination of prescription rate of cholera vaccine, the period of the year when there were a higher number of prescriptions and the characterization of the international traveler regarding the gender, age, occupation, residence, travel destination, time spent at destination, type of accommodation. In a second phase will be assessed the vaccine efficacy.

**Methods:** Descriptive, quantitative and retrospective study. The sample was the population of Travel Consultation of the International Vaccination Center of Oporto, who has been prescribed cholera vaccine during the years 2007 and 2008. The records of the Center's archive were consulted and Microsoft Office Excel® 2007 was used for data processing.

**Results:** The prescription percentage of cholera vaccine was 6.4%. The great number of prescriptions occurred at 1st trimester of 2007 and 2nd and 3rd trimesters of 2008. Males are the one that prevails in travellers (82%). Most travelers (79%) belong to the age group of 21-50 years. The construction, technical occupations and occupations related to administration includes the majority of travelers. The most common destination is Angola (88%) and in most cases users on business trips (78%). Most users stay in hotel (31%), although a significant proportion also stay with relatives (29%) or on company premises (27%). Most travelers (92%) remains for over a week.

**Conclusion:** The proposed objectives were achieved and the results were consistent with health professional's typical traveller perception - male, working-age, Oporto resident, construction worker, which travels to Angola for professional reasons, staying in hotels or in relatives or business houses, for more than a week.

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**Type: Poster Presentation**

Travelers' diarrhea (TD) incidence in Argentine travelers to high-risk destinations. A telephonic survey at a private ambulatory center for infectious diseases (ID) and travel medicine (TM) in Buenos Aires

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<sup>1</sup>Centros Médicos Dr. Stamboulian, Buenos Aires, Argentina, <sup>2</sup>Centros Medicos Dr. Stamboulian, Buenos Aires, Argentina, <sup>3</sup>Fidec (Fighting Infectious Disease in Emerging Countries)/Funcei (Fundación Centro de Estudios Infectológicos), Buenos Aires, Argentina

**Background:** TD is the most common travel-associated health problem in people visiting high-risk destinations in Latin America, Southeast Asia, the Indian Subcontinent and Africa. Most episodes occur in the first two weeks after arrival. Twenty percent of episodes can limit travelers' activities and 1% can lead to hospitalization. The reported incidence of TD for high-risk destinations is up to 40%.

This telephonic survey was performed as part of a risk evaluation for future studies and consisted in an estimation of TD incidence among travelers seeking pre-travel advice at an ambulatory, private ID and TM center in Buenos Aires.

**Methods:** Medical records of pre-travel consults between October, 2008 and March, 2009 were reviewed. This time-frame corresponds to Argentina's yearly summer vacation. Only travel to high-risk areas such as Latin America, Southeast Asia, Indian Subcontinent and Africa were included. Queries included demographic data, destination, travel duration, new onset of a diarrheic episode, and management. Every patient had received the clinic standard oral and written recommendations delivered as comprehensive handouts with management orientation.

**Results:** A total of 2,020 records were pre-selected. From those, 242 were eligible. Sixty were excluded for different reasons. One hundred eighty phone calls were done. Twenty percent of the 100 that responded, (n=20) presented at least one diarrheal episode, but only in 10% of episodes (n=10) the traditional TD definition was fulfilled. Mean age of travelers was 29 (range: 16-60). Sex distribution was the same (50%).

Travel destinations were Indian Subcontinent (n=10; 50%), Peru and Bolivia (n=8; 40%), Colombia (n=1; 5%), and Southeast Asia (n=1; 5%).

Out of the 20 patients that reported diarrhea, 7 improved without treatment. Thirteen patients decided to take loperamide, among them the 10 patients with TD. Of them, 2 patients also added a quinolone. In all cases the episodes resolved in less than 24 hours.

**Conclusion:** From this series, it seems that with previous, comprehensive pre-travel evaluation and education, only a minor percentage of travelers develop TD and that they can manage it adequately.

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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

First travel medicine center in a public hospital from Buenos Aires province, Argentina. The first 18 months of experience

**M. L. yantorno**, G. de la Parra, M. Lares, K. Aguilera, D. Lizaso, M. Correa, A. Esposto  
HIGA San Martin, La Plata, Argentina

**Background:** Despite the expansion of the Speciality achieved in the last years in the world, its influence in Argentina has been limited and shortened only to a few centers. Although Buenos Aires is the biggest province in our country it did not have a Travel Medicine Center in a Public Hospital.

**Methods:** From March 2008 to August 2009 retrospective, descriptive and longitudinal research has been done with the travellers who consulted the Center before traveling. Demographical characteristics, purpose of travelling, destinations, style and duration have been analysed. It has also been considered what sources suggested the travellers they should consult the center. People who have consulted through e-mail or telephone have been excluded from the research.

**Results:** 193 people were evaluated. Female 110/193 (57%), none of them pregnant. Average age 39,3 years old (6-81); thirteen children under 15 years old (6,7%). Each interview was held on average 27 days until travelling (1-210 days). None of the travellers was advised to come by Travel Agencies. Forty six percent (89/193) of the interviews were held in the months preceding the summer season. Purpose of travelling: holidays 153/193(79,27%), VFR 13/193(6,7%).

Destination: South America 134/193 (69,4%): Bolivia and Peru 43, Brazil 41 and Argentina 27. Asia: 28/193 (14, 5%). Africa: 16/193 (8,2%). Central America and the Caribbean 11/193 (5,7%). Style of travelling: Urban 72/193 (37,3%), Urban-Rural 113/193 (58,54%), Rural: 8/193 (4,1%). Accommodation in hotel 85/193(44%), tent 2/193(1%). The duration of the trip was longer than a month in 51/193 (26, 4%). Travelling to malaria and yellow fever endemic countries 69/193(35,75%) and 76/193 (39,37) respectively. Posttravelling interviews: 5 (two of them had also consulted pre-travelling).

**Conclusion:** Most of the travellers were young people, interviewed before the summer season, due to holiday trips to countries in South America, urban-rural, with a duration of less than 30 days, staying in hotels. They consulted with a reasonable anticipation. There were no interviews with pregnant women and none of the travellers were advised to come by the Travel Agency.

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**Type: Poster Presentation**

Trends in fatal snakebites in Venezuela, 2003–2007

L. Parra<sup>1</sup>, J. Peña<sup>1</sup>, A. Rísquez Parra<sup>2</sup>, L. Echezuria<sup>2</sup>, A. Rodriguez-Morales<sup>2</sup>

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**Background:** There have been few studies evaluating snakebite mortality in Venezuela and South America. In this study we evaluate trends in fatal snakebites occurring in Venezuela, 2003–2007.

**Methods:** Epidemiological data for this study were retrieved from the records of the Ministry of Health of Venezuela (ICD-10 codes to search for deaths due to snakebites). We analyzed the impact of these envenomations in Venezuela during the study period.

**Results:** During the study period, there were 176 reports of death due to snakebite (0.63 deaths/100,000pop., ranging from 0.08 to 0.17), showing a slight decrease in the mortality rates from 0.13 deaths/100,000pop. in 2003 to 0.08 in 2007 ( $r^2=0.3942, b=-0.014000, P=0.2634$ ); 72.7% were males, 27.3% were females ( $P<0.05$ ). Annual mean deaths numbered 35 per year. Of total deaths, 30.1% occurred in victims 55–70 y-old (age adjusted rate of 1.4 deaths/100,000pop.). Deaths in young children (<5 y-old) accounted for 4.5% of the total (age adjusted rate of 0.28 deaths/100,000pop.). Mortality by age showed an age-dependent pattern, with higher rates in older ages ( $r^2=0.259, b=0.392998, P=0.0156$ ). Regarding the place of envenomations occurrence in 15.3% were at home, 12.5% at roads and 5.1% at farms ( $P<0.05$ ).

**Conclusion:** These figures are similar to a previous report (*Wilderness and Environmental Medicine* 2007;18:209-213), however previously was reported a slight increase in the mortality in the last eight years, herein we showed a decrease. Additionally also an increase in the female deaths has been observed. Unfortunately morbidity data at the national level is not optimal (underreporting) to perform further analyses beyond the primary analysis of the trends in snakebite mortality. Conversely, all cases of deaths are specifically and obligatorily reportable, and mortality information is more available and accurate. Snake envenomations are an important cause of injury and deaths in Venezuela as in many American countries. Surveillance of envenomations is essential for establishing guidelines, planning therapeutic supplies, and training medical staff on snakebite treatment, as well as assessing risk zones for travelers.

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**Type: Poster Presentation**

Epidemiology of drowning deaths in Venezuela, 1996-2007

**C. Madrid<sup>1</sup>**, M. H. Maldonado<sup>1</sup>, A. Rísquez Parra<sup>2</sup>, A. Rodríguez-Morales<sup>2</sup>

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**Background:** Over the past four decades, medical community has learned considerably more about the pathophysiology and treatment of drowning. This, coupled with increased emphasis in improvement in water safety and resuscitation, has produced a threefold decrease in the number of deaths, indexed to population, from drowning in countries such as United States and Australia yearly. However in many countries these trends are not the same.

**Methods:** Epidemiological data for this study were retrieved from the records of the Ministry of Health of Venezuela, using ICD-10 codes to search for all deaths due to drownings during the study period (1996-2007). Using these data, we analyzed the impact of these accidents in Venezuela, a significant beaches touristic destination for travelers.

**Results:** During the study period, there were 7,071 reports of death due to drownings (rates ranging from 1.88 deaths/100,000pop. to 2.87), showing a significant decrease in the mortality rates from 2.87 deaths/100,000pop. in 1996 to 1.88 in 2007 ( $r^2=0.8971, b=-0.947, P<0.001$ ); 81.97% were males (rates 3.11-4.55 deaths/100,000pop), and 18.03% were females (rates 0.70-1.16 deaths/100,000pop) ( $P<0.01$ ). Annual mean deaths numbered 589 per year ( $\pm 32$ ). Highest rates occurred in victims aged less than 5 y-old (3.71 deaths/100,000pop.), however when adjusted by sex highest rate was observed in males 15-24 y-old (4.86 deaths/100,000pop.). Female rates were highest at less than 4 y-old (3.03 deaths/100,000pop.). Regarding the place of drowning occurrence in 52% was at natural waters (beaches, rivers, lakes) (1.04 deaths/100,000pop.), 4% at swimming pools (0.08 deaths/100,000pop.) and 1% at bathtub (0.02 deaths/100,000pop.).

**Conclusion:** Drownings in male adolescents and young adults (and female children less than 5 years) continue to be a great challenge for water safety organizations, legislators and parents. Drownings in the indigenous community and among tourists requires more detailed study and action. Prevention especially during holidays or vacations requires a multidisciplinary approach, including travel medicine practitioners, in order to keep the decrease of these fatalities.

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**Type: Poster Presentation**

Travellers' Hantavirus Pulmonary Syndrome in Andean Patagonia. Argentina

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**Background:** Andean Patagonia is an important tourist destination in Argentina. It offers beautiful wild landscapes -mountains, lakes and forests-. Andes virus (ANDV) is the etiologic agent of hantavirus pulmonary syndrome (HPS) in this region and Chile. Transmission to humans occurs through inhalation of rodent excreta and person-to-person transmission has also been demonstrated for this genotype. The incubation period is between 8 and 45 days. HPS has low incidence but high mortality rate (40%).

**Objective:** To identify ANDV infection cases associated with travelling in the region.

**Methods:** Epidemiological charts of 80 HPS cases were reviewed.

**Results:** Cases were grouped according to different "travel circumstances":

A) International travellers (n= 0).

B) Argentine travellers far (>1000 km) from home (n=7). a) One tourist started symptoms 21 days after cleaning a rodent infested cabin; b) one tourist guide, exposed in a National Park; c) five cases belonging to an outbreak in 1996 that started in El Bolsón (with one index-case followed by 15 secondary patient-cases). These five patients lived and started symptoms in Buenos Aires (1400 km from El Bolsón). Travelling people and interhuman transmission resulted in this phenomena.

C) Local travellers (n= 4). Two children from different cities shared common rodent exposures during a week's holiday in a wild area; symptoms started 22 and 24 days after (cluster for coexposition). Other 2 patients who lived in urban centers were exposed during holidays (fishing and trekking).

The situation of workers exposed when moving from their urban residence to wild areas for deforestation, building and ecologic activities, was considered: 7 men became ill. Two of the workers' wives were also infected by interhuman transmission (family clusters).

**Conclusion:** Precautions to avoid exposure to rodents should be strongly recommended. In patients with febrile respiratory distress syndrome, HPS should be considered: travel and possible exposure antecedents, and estimation of incubation period will help to identify imported cases. If no epidemiologic risk is found, data of previous contact with a febrile patient exposed in ANDV endemic area may suggest interhuman transmission. Isolation precautions must be applied to prevent person-to-person transmission. Molecular characterization is useful to identify the infecting genotype.

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**Type: Poster Presentation**

Characterization of pre-travel consults at a travel medicine clinic in Buenos Aires in 2008:  
Experience with 1,439 Travelers

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**Background:** Travel industry grows steadily even in the middle of economic crisis, high-impact outbreaks or tragic events. The vast majority of travelers, however, do not seek pre-travel advice. To date our center has a large experience in Latin America, since the launch of the Travel Medicine Division in 1993. We have counseled 54,100 travelers. The objective of this study is to describe some aspects of our experience during 2008.

**Methods:** This is a retrospective observational study. A randomized sample of pre-travel consults stratified by month was obtained. Medical records were reviewed. Data collected included age, sex, destinations, trip purpose, length of stay, time to consult before departure and evaluation of malaria chemoprophylaxis.

**Results:** Among the 1,439 travelers included for analysis, 562 (42.4%) were female and 763 (57.5%) male, mean age was 35.3 years (+/-13.8).

Main reasons for travel were tourism, 849 (58.3%) and business, 517 (35.4%).

Conventional tourism accounted for 433 consults (51%); adventure tourism for 236 (28%) and ecotourism for 180 (21%).

Main destinations were: South America, 510 (31,6%); Africa 267 (16,4%); Central America and Caribbean, 176 (11%); Argentina, 145 (9%), Indian Subcontinent, 117 (7,2%).

Median length of stay was 16 days.

High-risk travel was the most prevalent category 683 (54,2%).

The number of vulnerable travelers (HIV, cancer, pregnancy, diabetes) was very low 37(<0.5%). The median time from consult to departure was 20 days.

Only 141 (24%) high-risk travelers consulted with enough anticipation.

Malaria chemoprophylaxis was prescribed to 398 (31,6%) travelers, being mefloquine the most frequent drug (62,3%).

Mistakes in the selection of chemoprophylaxis for chloroquine resistant areas were not found.

Chemoprophylaxis for short stays in the Indian Subcontinent was prescribed in 31% of cases.

**Conclusion:** Tropical and subtropical regions were the most common destinations chosen in our series.

Despite a majority of high-risk travelers, only 24% consulted with enough anticipation.

Thus, we should place further emphasis on travelers education in order to optimize pre-travel consult time, particularly for high-risk destinations, and to detect vulnerable population for counseling.

The low rate of malaria chemoprophylaxis for travelers to the Indian Subcontinent agrees with recent changes in prevention recommendations for that region.

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**Type: Poster Presentation**

Risk assessment of potential anthroozoonotic pathogen transmission from ecotourists to wildlife populations in Borneo

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**Background:** Over half of all human infections are zoonotic in origin. Nonhuman animal populations are also susceptible to human pathogens. Expanding travel and ecotourism are increasing the likelihood of contact between populations of immunologically-naïve animals and potentially infectious travelers. While the benefits of ecotourism appear clear, anthroozoonotic pathogens transmitted from ecotourists could negatively impact wildlife populations.

**Methods:** To better understand potential infection transmission associated with ecotourism travel, we employed the largest survey to date of self-perceived health and vaccination status in ecotourists. Anonymous surveys were randomly obtained from 633 visitors at the Sepilok Orangutan Rehabilitation Centre (Sabah, Malaysia), Asia's most frequented wildlife tourism destination. The questionnaire recorded demographic information, history of recent travel, recalled recent contact with livestock, domestic and wild animals, recent diagnoses and symptoms of various infections, and recalled current vaccination status for several infectious diseases.

**Results:** Over half of the sample reported being currently vaccinated against tuberculosis, hepatitis A, hepatitis B, polio, and measles. 15% of the sample self-reported at least one of the following current symptoms: cough, sore throat, congestion, fever, diarrhea, and vomiting. Participants with recent animal contact were more likely to report current respiratory symptoms compared to individuals with no such animal contact (aOR 2.4). Likewise, participants with a medical-related occupation were more likely to report current respiratory symptoms compared to participants with non-medical occupations (aOR 2.2). 67.1% of those with medical-related occupations reported not being currently vaccinated for influenza.

**Conclusion:** Ecotourists represent a potentially significant source of anthroozoonotic infections. Like other international travelers, ecotourists are not adequately protected against vaccine-preventable illnesses. We demonstrate that potentially infectious tourists were visiting a wildlife sanctuary, despite significant animal contact prior to arrival (which may have exposed them to other sources of infection), and despite having at least some basic knowledge about infection transmission (i.e., medical-related occupation). We conclude that a significant proportion of ecotourists are uninformed of the risks they pose to nonhuman animal health. It is the combined responsibility of the medical and tourism communities to accurately communicate the risks of zoonotic and anthroozoonotic infections in ways that best support the needs of humans and wildlife alike.

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**Type: Poster Presentation**

Malaria in a community hospital

**O. Mascaro**, C. Socolich, A. Pedragosa, E. Reynaga, G. Lucchetti, X. Gimeno, J. Bruges  
Consorti Hospitalari de Vic, Vic, Spain

**Background:** To identify all the cases of malaria in our region during the last decade, clinical features, diagnosis, severity, prophylaxis and treatment.

**Methods:** Descriptive retrospective study of malaria cases in Osona (Barcelona) admitted to the Department of Internal Medicine in a community hospital since January 2000 to November 2009. We reviewed all cases with the microbiological diagnosis of malaria. We analyzed the epidemiological and clinical data. The inclusion criteria were: older than 15 years, and admission in hospital > 24h.

**Results:** We had 48 cases of malaria (40 with the inclusion criteria). The age average was 33.78 years (21-71): 31 men (75.03%) and 9 women (24.97%). We classified the cases according to their nationality: Ghana 19 (47.5%), Nigeria 10 (25%), Iberian Peninsula 7 (17.5%), Mali 1 (2.5%), Senegal 3 (7.5%). 85% (34 cases) were black, and 6 caucasians (15%). The average stay in Spain before the episode was 54.28 months (12-96). 17.5% (7 cases) had previous episode of malaria. The classification according to the visited area was: Ghana 19 (47.5%), Nigeria 10 (25%), Senegal 5 (12.5%), Honduras 2 (5%), Guinea Bissau 1 (2.5%), Cameroon 1 (2.5%), Mali 1 (2.5%), Uganda 1 (2.5). The average stay in hospital was 4.02 (1-13 days). 25% of cases (10) took any prophylaxis, 3 cases (7.5%) were successful. The most frequent type of Plasmodium was *falciparum* 37 (92.5%), only one case due *Plasmodium ovale* (2.5%), and two were undetermined for low parasitemia (5%). 10% had one or more severity criteria of WHO. 92.5% had thrombocytopenia (<150000). All cases were treated with quinine sulfate and doxycycline, except 2 cases treated with chloroquine and one case with the unknown treatment. We found one case of recurrence.

**Conclusion:** Most reported cases were people from endemic areas (most of Ghana) and were black people. The most frequent type of Plasmodium was *falciparum*. 75% of patients did not take any prophylaxis. The thrombocytopenia was the most frequent laboratory finding, without any relation with severity criteria. Complications were rare, probably related to the epidemiological characteristics of patients (originating from endemic areas, black race...).

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**Type: Poster Presentation**

Meningococcal disease in travelers: A problem more than a 100 years old

L. De Tora, **L. Da Silva**

Novartis Vaccines and Diagnostics, Cambridge, MA, USA

**Background:** The risk of meningococcal disease to travelers has been of increased interest in the past few decades due to the well-known risk in Hajj pilgrims following outbreaks of invasive disease caused by serogroup W-135. In addition, cases have been associated with air travel. Currently, preventive vaccination against appropriate serogroups is recommended for travelers to regions with hyperendemic or epidemic disease such as the "meningitis belt" in Africa.

**Methods:** As part of an ongoing project on the history of meningococcal disease in Novartis Vaccines and Diagnostics, we investigated the occurrence of invasive disease in travelers in historical documents and recent historical reviews gleaned from various sources, including Medline, Google, and Web of Science. Disease entities such as sinking typhus, cerebral typhus, spotted fever, cerebrospinal fever, epidemic cerebro-spinal meningitis, which have been strongly associated with meningococcal disease, were investigated.

**Results:** Several case histories and historical events place meningococcal disease as a recognized risk of travel in the late nineteenth century, shortly after the first isolation of the bacterium. Dissemination across large distances was well-recognized by the beginning of the twentieth century, particularly among the military, in which small outbreaks were observed periodically. The first clear mention of the disease as associated with travel dates from 1898, when several authors describe outbreaks in ships carrying Indian laborers from Calcutta to East Africa and the West Indies (specifically Jamaica and the then British Guyana). Following the increased development of institutions of public health and epidemiology in the United States, an epidemic of meningococcal disease in Asia in 1928-1929 was tied to cases in the West Coast, carried by steerage passengers from the Philippines. In addition to new rules for steerage, the National Origins Formula of 1929 drastically restricted immigration and travel from Asia to the US.

**Conclusion:** Literature about travel and meningococcal disease shows clear evidence of risks to travelers for at least 110 years. The long-standing nature of invasive meningococcal disease as a human-specific pathogen causing potentially fatal illness is of interest when investigating new options for prevention and control.

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**Type: Poster Presentation**

Free living amoebae encephalitis infection in a child who travelled to Peru  
**C. A. Mora**, N. Orellana, A. Schteinschnaider, N. Arakaki, M. Del Castillo  
FLENI, Buenos Aires, Argentina

**Background:** An 8 year-old Hispanic boy who was living in Argentina, travelled to Perú in December 2008 to visit some relatives. He had chicken pox when he was 4 years old, and the family medical history was positive for tuberculosis in the patient's father.

**Methods:** One week before coming back to Argentina he experienced cough and low grade fever for which he was treated: Ibuprofen and amoxicillin. Nine days after he was back from Peru, he experienced headaches, vomiting. His parents noticed mild right ptosis, he developed acute ataxia. MRI findings: two ring-enhancing lesions, one in the left occipital area and other one in the brain stem. Spinal tap: CSF: cell/mm<sup>3</sup>, Glucose level: 58 mg/dl, Protein level: 0.38 mg/dl. PCR assays for HVS-VZV and cultures for bacterial, mycobacterial and fungal were negative.

**Results:** Serologic studies: HIV(-), ELISA *Cysticercus*(-), IgM *Mycoplasma* (-), IgG *Mycoplasma* (+), ID *Histoplasma* (-). PPD 2 UT (-)

Preliminary diagnosis was Acute Disseminated Encephalomyelitis which was treated with parenteral steroids. He showed no improvement, he started treatment with intravenous immunoglobulin. The patient showed deterioration: MRI showed that the lesions had progressed in size. Excisional biopsy of the occipital lesion was performed. In the tissue sections there was no evidence of granulomas with caseification, toxoplasmosis, cysticercosis, fungi and desmyelination.

The presence of structures with spheroid nucleus and clear cytoplasm induced to search for amoebas. The Trichromic modified stain Gomori Wheatley showed images similar to the ones of the amoebic trophozoites.

He received treatment with pentamidine, rifampicine, liposomal, amphotericin, sulfamethoxazole trimethoprim, clarithromycin and fluconazol for a period of 60 days.

He remained clinically stable throughout that period but experienced gross neurological sequelae. Serial MRI studies showed gradual resolution of the lesions with a decreased in size. After 6 months of finishing his treatment, at this day he still remains alive.

**Conclusion:** Even though the confirmation of the diagnosis of free-living amoebae encephalitis was not confirmed by the indirect immunofluorescence assay, the clinical course of the illness, the imaging studies, the microscopic findings and the fact that he didn't get worse induces us to believe that Granulomatous Amebic Encephalitis is a possible diagnosis.

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**Type: Poster Presentation**

New world cutaneous Leishmaniasis in travelers (1994-2008) experience In Argentina

**T. Orduna**<sup>1</sup>, S. Lloveras<sup>1</sup>, G. D. Gonzalez<sup>1</sup>, C. C. Falcone<sup>1</sup>, S. L. Garro<sup>2</sup>, S. E. Echazarreta<sup>3</sup>

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**Background:** Leishmaniasis is a common cause of dermatosis in returning travelers. The New World cutaneous leishmaniasis (NWCL) is caused by multiple species including complexes *L (V) braziliensis* and *L (L) mexicana*.

**Materials and methods:** A retrospective, cross-sectional and descriptive analysis was performed based on medical reports of travellers with clinical and epidemiological diagnosis of NWCL, assisted from 1994 through 2008.

**Results:** 39 cases of NWCL were recorded (29 males, 10 females, with age ranged from 17 to 72 years). Twenty-nine (74%) patients were residents of Argentina, 10 (26%) were foreigners in transit. The reason for travel was tourism in 21 (54%), work 14 (36%) and friends and relatives visit (VFR) 4 (10%).

26 patients (67%) acquired the disease outside Argentina; 13 (33%) in Argentina, who were domestic travelers to endemic areas.

At query time 16 (41%) patients had lesions 30 to 60 days of development, 19 (49%) 75 to 120 days.

Estimated average time of exposure in risk area was 20 days.

Thirty patients (77%) had multiple lesions and 9 (23%) had single lesion.

84.61% of the lesions were ulcers; 67% of the lesions were localized in the extremities, 23% of the face and 10% in trunk.

The diagnosis was made by direct microscopic examination in 29 (74%), and 10 (26%) by biopsy, 7 were cultured (5 were positive). No species identification was made in either case.

36 patients received as first treatment schedule meglumine antimoniate intramuscular (20mg/kg/day for 21 days), 2 patients amphotericin B deoxycholate 0.5 mg/kg/day up to 1,5 grams total and 1 patient who travelled around Panama received fluconazole 200mg/day for 6 weeks. 4 patients treated with meglumine antimoniate had adverse effects.

85% of patients cured with first therapeutic regimen.

3 patients treated with antimonials reported relapses, one patient had therapeutic failure. All healed without subsequent relapse.

**Conclusion:** Cutaneous leishmaniasis is a risk for travelers to tropical areas of America and is necessary to include prevention guidelines in pre travel advisory. It is important that physician be trained in the recognition of this condition and consider the possibility of mucosal involvement in patients infected with *L braziliensis*.

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**Type: Poster Presentation**

Imported malaria in travelers assisted in Buenos Aires

**S. Lloveras**<sup>1</sup>, S. E. Echazarreta<sup>2</sup>, S. L. Garro<sup>3</sup>, C. C. Falcone<sup>4</sup>, G. D. Gonzalez<sup>4</sup>, T. Orduna<sup>1</sup>  
<sup>1</sup>Corunna, Argentina, <sup>2</sup>Hospital F.J.Muñiz, Buenos Aires, Argentina, <sup>3</sup>Hospital F.J. Muñiz, Buenos Aires, Argentina, <sup>4</sup>Hospital F. J. Muñiz, Buenos Aires, Argentina

**Background:** Malaria is the most important parasitic infection that produces human disease. It is caused by protozoa of the genus *Plasmodium* and transmitted by the bite of the female *Anopheles* mosquito. It's endemic in over 90 countries and is the most common specific etiologic diagnosis in febrile travelers.

**Methods:** A retrospective, cross-sectional and descriptive analysis was performed based on medical reports of travellers with diagnosis of imported malaria, assisted from 1981 through 2008.

**Results:** Of 1010 returned travelers (domestic and international) seen at our clinic, 337 (36.36%) patients cited fever as a chief reason for seeking care and 143 (42.43%) of them had malaria.

There were 135 (94.40%) cases of imported malaria, 100 (74.07%) males and 35 (25.92%) females, from 3 to 73 years. 127 (94.07%) travelers were residents.

The species involved was *P. vivax* in 61 cases (46.18%), *P. falciparum* in 59 (43.70%); *P. ovale* in 1 (0.74%), mixed infections of *P. falciparum* and *vivax* malaria in 3 (2.22%) and 11 cases (8.14%) without identification.

74 (54.81%) travelers acquired malaria in Africa, 48 (35.55%) in South America, and 7 (5.18%) in Central America.

The reasons for travel were: 56 (41.48%) work/business; 47 (34.81%) tourism; 18 (13.33%) visiting friends and relatives; 13 (9.62%) missionary/volunteer; and 1 (0.74%) per education.

In travelers to Africa the species most frequently involved was *P. falciparum* (52/74, 70.27%) and *P. vivax* in South America (38/48, 79.16%).

Only 40 (2.96%) travelers received medical advice before the trip, of them 32 (80%) received chemoprophylaxis for malaria, 3 (9.37%) of which were inappropriate according to the area visited. None of the chemoprophylaxis included primaquine. All patients improved with treatment. 5 / 59 (8.47%) travelers had *P. falciparum* severe malaria.

**Conclusion:** In febrile returned traveler, we must always consider the diagnosis of malaria regardless of the time elapsed since leaving the malaria area. *Plasmodium falciparum* malaria is a medical emergency. The treatment depends on the knowledge of the geographical distribution of parasite resistance against antimalarial drugs, especially when no parasite species identification is possible.

It should be emphasized prevention with personal protection measures and adequate chemoprophylaxis.

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**Type: Poster Presentation**

Travel medicine working group

**A. Macchi**<sup>1</sup>, A. Lepetic<sup>2</sup>, C. Biscayart<sup>1</sup>, P. Elmassian<sup>1</sup>, V. Verdaguer<sup>1</sup>, M. P. Della Latta<sup>1</sup>, C. Torroija<sup>1</sup>, E. Sturba<sup>3</sup>, M. Arrestia<sup>1</sup>, D. Stamboulia<sup>4</sup>

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**Background:** Travel Medicine Working Group (TMWG), established in 1992, is the first program for prevention of travel medicine related infectious diseases in Buenos Aires. TMWG aims to improve travelers health and to raise awareness of its importance to the Argentinean public. The objective of this presentation is to describe the scope of a multidisciplinary TMWG.

**Methods:** TMWG comprises infectious diseases specialists, high-tech, up-to-date laboratory facilities and vaccination centers. We designed a program aimed to assist travellers before departure, en route, after return and to optimize vaccination in adult population. Activities focus on four basic areas:

Community-oriented education: travel-oriented brochures and handouts, travel medicine newsletters and travel warning.

Health care professional education: Seminars and symposia, medical rounds, periodic work meetings and annual courses.

Research: Characterization of medical consults.

Medical assistance: pre-travel consult, specific medical record, tailored counseling, telephone and electronic real-time support and referral to local reference medical centers. Post travel medical evaluation of asymptomatic long-term travellers, prompt diagnosis and treatment of symptomatic travellers and epidemiological surveillance activities.

**Results:** Between 1992 and 2008 the TMWG has counseled 54,100 travelers. The average annual consult increase was 25% in last four years. In 1998 only 11.8% of high-risk travelers consulted with enough anticipation; in 2008, 24%. 0.4% of a sample of travelers came to our center referred by travel agencies and 0.5% by embassies. Routine and special vaccine shots increased progressively each year. In a group of 10-50 years old travelers, 8.3% were susceptible to chickenpox, 11.4% to mumps, 10.2% to rubella and 8.9% to measles. Hepatitis A seroprevalence was 40%.

**Conclusion:** Our experience shows this new medical specialty is increasingly demanded. We must deepen our work in an interdisciplinary manner to obtain traveler referrals from embassies and travel agencies, because of individual and community travel associated health risks. The development of TMWG has created awareness of the need for pre travel advice in the medical and general community and provides opportunities to update routine vaccinations in adults.

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Profile of imported malaria in travelers from the north of Portugal

**A. Silva**<sup>1</sup>, A. R. Silva<sup>1</sup>, T. Teixeira<sup>1</sup>, H. Coelho<sup>1</sup>, R. Sarmiento e Castro<sup>2</sup>  
<sup>1</sup>Hospital Joaquim Urbano, Porto, Portugal, <sup>2</sup>Porto, Portugal

**Background:** Malaria was eradicated from Portugal since the 50's, but every year, hundreds of Portuguese travelers arriving from Malaria Endemic Countries are diagnosed *plasmodium* positive.

The objective of this study is to investigate the clinical and epidemiological imported malaria between January 2004 and October 2009 in the Travel Medicine Department of Hospital Joaquim Urbano in Porto - Portugal.

**Methods:** Review of the records and data of 160 malaria diagnosed positive cases, confirmed by microscopy, in the last 6 years. Variables analyzed: age, gender, country visited, pre-travel consultation, chemoprophylaxis, plasmodium species, reason of travel and symptoms.

**Results:** The average age of the 160 patients was 42,18 years old. From this group 108 patients (67,5%) were male.

The main visited countries were Angola with 92 cases (57,5%), followed by Mozambique with 23 (14,4%), São Tomé with 7 (4,8%), and the rest distributed over fifteen other countries. The most common agent of plasmodium identified by laboratorial exam was *P.falciparum* with 31 cases (19,38%), followed by *P.vivax* with 26 (16,25%). Although, in 91 cases (56,88%) was not possible to detect the plasmodium species.

The malaria chemoprophylaxis was not made by 85 patients (53,12%). Those who did it, 38 (84,44%) took mefloquine.

The inpatient ratio was 43 (26,88%) of the 160, with a fatal case (0,63%).

**Conclusion:** The geographic areas of acquisition were the former Portuguese Colonies in Africa, with 78,75 % of imported malaria.

The *P. falciparum* was the most frequent species.

Another problem was the diagnosis made by microscopy with lower parasitemia, that did not identify the plasmodium species. By this reason, more sensitive and accurate methods must be used.

To reduce risk of imported malaria, all travelers should have a pre-travel counselling, so major investment is advised to be made in this important emerging field of Travel Medicine in Portugal.

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**Type: Poster Presentation**

Demographics and travel patterns of travelers to Central America, South America and the Caribbean seen in the Boston Area Travel Medicine Network (BATMN)

**J. B. Trivedi**<sup>1</sup>, N. S. Hochberg<sup>2</sup>, W. B. Macleod<sup>3</sup>, M. Pfaff<sup>3</sup>, M. M. Sosa<sup>4</sup>, C. Benoit<sup>5</sup>, L. H. Chen<sup>6</sup>, M. E. Wilson<sup>6</sup>, L. Kogelman<sup>7</sup>, W. W. Ooi<sup>8</sup>, A. W. Karchmer<sup>4</sup>, E. D. Barnett<sup>1</sup>, D. H. Hamer<sup>3</sup>

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**Background:** Travelers to Central America (CAm), South America (SAm) and the Caribbean may face regional travel-related health risks. Our objective is to describe demographics, trip characteristics and differences in pre-travel antimalarial prescriptions for travelers to CAm, SAm and the Caribbean.

**Methods:** Demographics, health, and trip information was collected for travelers seen in the 5 clinics of the Boston Area Travel Medicine Network (BATMN) from March 1, 2008 to September 30, 2009. For analysis, Mexico was included in the CAm category.

**Results:** Of 9203 travelers seen in participating clinics, 2834 (30.8%) planned to visit CAm, SAm and the Caribbean including 1411 (49.8%) to SAm. Travelers to CAm and SAm were predominantly white (>80%) and less often black (2.6%) compared with 58.3% white and 20.8% black travelers to the Caribbean. Most trips (71.6%, 53.8%, and 72.8% for CAm, SAm and the Caribbean respectively) were <2 weeks duration. Most traveled for tourism (53.7%, 56.6% and 30.3% of visitors to CAm, SAm and the Caribbean) while visiting friends/relatives (VFR) accounted for 7.3%, 13.5% and 23.7% of visits to these regions, respectively. Caribbean and CAm travelers also went for missionary/volunteer work (21.9% and 13.3%). Travelers to the Caribbean were more likely to stay at a local residence (49.8%) than those traveling to CAm or SAm (27.9%/31.9%); however, CAm and SAm travelers were more likely to stay at a hotel/hostel (70%) than Caribbean travelers (40%). More travelers to CAm (65.6%) and the Caribbean (68.8%) visited a malaria risk country than those traveling to SAm (43.6%). Of the Caribbean travelers who were prescribed antimalarials, the vast majority (96%) were visiting Haiti and the Dominican Republic. Caribbean and CAm travelers were predominantly prescribed chloroquine (82.3% and 74.5%) or Malarone (18.6% and 23.7%); SAm travelers appropriately received Malarone (85.4%) or doxycycline (8.2%).

**Conclusion:** Caribbean travelers were more likely to travel as VFRs or volunteers and stay in local residences than those traveling to CAm or SAm. South American travelers visited for longer periods of time and often to non-malarious areas. All travelers received antimalarials appropriate for their destinations.

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Hookworm-related cutaneous Larva Migrans: An annoying souvenir of some trips

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<sup>1</sup>Corunna, Argentina, <sup>2</sup>Hospital F.J.Muñiz, Buenos Aires, Argentina

**Background:** Hookworm-related Cutaneous Larva Migrans (HrCLM) in travellers is a common but neglected parasitic skin disease that results from a zoonotic nematode infection and shows a characteristic creeping eruption due to penetration and migration of the larva within the epidermis.

**Methods:** We performed a retrospective survey of patients with this illness assisted at our Unit from 1999 through 2008.

**Results:** A total of 64 individuals received a diagnosis of HrCLM, and among them there were 55 (85.93 %) who had acquired it in the Brazilian beaches, but also in Costa Rica, Paraguay, Peru, Senegal, Thailand and Venezuela.

Three patients were domestic travelers and they had acquired the creeping eruption in Argentina. Fifty percent of the 64 affected patients were young adults between 20–39 years. Lesions were mainly unique (90.6%) and affected feet (82.75%).

The symptoms appeared to 70.35% (n=45) of patients, between 3 and 7 days after returning from the trip. Eleven (17.18%) patients had secondary bacterial infection. Out of 64 patients assisted, 62 (96.87%) were cured by a single course of treatment: 48 patients with ivermectin, 11 with oral thiabendazole plus topics of 10% thiabendazole cream; one received oral albendazole, 400 mg a day for 3 days and one patient's treatment was not recorded. Two patients (3.12%) required a second course of therapy.

**Conclusion:** As travel to the tropics increases, many travelers may be returning to their countries with this infection, which is often misdiagnosed or incorrectly treated.

Although there are various treatments available, it is necessary to have prospective and randomized controlled trial to compare their efficacy.

Among the preventive measures, that can be suggested to travelers, are to avoid walking barefoot has proved to decrease the disease impact and also reduce the contact of skin areas with contaminated soil or beaches, by lying on a canvas for sunbathing or to rest.

Furthermore, Public Health measures must be put into practice to avoid the presence of loose animals and to promote deworming of the same.

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**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Immunocompromised travellers in the pre-travel appointment: A report from Portugal

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**Background:** As both international travel and the number of immunocompromised travellers increase, concerns related to the efficacy of immunizations and malaria prophylaxis, drug interactions and worse of the basal medical condition in this population are a challenge for practitioners.

**Methods:** The charts from pre-traveller appointments of consecutive travellers were reviewed selecting our target population: those with HIV infection, malignant diseases in treatment, solid organ or stem cell receptors, under immunosuppressive therapy and splenectomized. Demographic data, destination, duration and reasons of travel, malaria quimioprophylaxis and immunizations were considered.

**Results:** From the 2101 travelers 23 (1,1%) meet criteria for immunosuppression. Eleven (48%) had HIV infection (all with CD4+ counts between 200-500/ul, 5 AIDS, 8 under HAART, 3 HCV co-infected), 10 (43%) were under immunosuppressive drugs (7 had autoimmune diseases, 2 solid organ transplant receptors, 1 under systemic corticotherapy for severe asthma), 2 (9%) were splenectomized. Their ages ranged from 21-56 years, mean 41; 17 (74%) were man. Mean time between the consultation and the date of travel was 23 days. Africa was the destination of 16 (70%) travelers (13 for Angola), tropical South America in 5 (22%), Indian subcontinent and Central America in 1 each. Excluding 2 travelers (one emigrant and one who lived in the country of destination) the duration of travel ranged from 3-180 days, mean 29days. The reason for travelling was work in 12 (52%), tourism in 9 (39%), humanitarian mission in 1 and 1 was resident. Malaria quimioprophylaxis was indicated in 12 (52%) and in 3 drug interactions changed the the first choice. Yellow fever vaccination was required for 12 travelers, in 6 a medical excuse was done, 4 were vaccinated (2 HIV not severe immunosuppressed, 1 was splenectomized, 1 before immunosuppressive drugs ) and 2 had actualized vaccination.

**Conclusion:** Immunosuppressed represent 1% of our travellers ; HIV infected travellers are common, followed by patients under immunosuppressive drugs for autoimmune diseases. More frequently the traveler is a young male, travelling for a month to Africa for work. The excuse for yellow fever vaccination and the possibility of drug to drug interaction in malaria prophylaxis makes the medical prevention less than optimal in this vulnerable population.

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**Type: Poster Presentation**

Trichinellosis in domestic travelers in Argentina: Which is the advice for international travelers?  
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**Background:** Trichinellosis is a parasitic zoonosis caused by tissue nematodes of the genus *Trichinella* spp. *T. spiralis* is the most frequently involved worldwide. The disease spreads by eating raw or undercooked meat containing cysts of the parasite. Outbreaks occur worldwide, more rarely presented as sporadic cases or travel related disease.

**Methods:** A retrospective, cross-sectional and descriptive analysis was performed based on medical reports of travelers with diagnosis of trichinellosis assisted from 1981 through 2008.

**Results:** Of 1010 returned travelers (domestic and international travelers) seen at our Unit, 296 (29.30%) were domestic travelers and 71 (24 %) of them had trichinellosis, 55 (77.46%) males, the age range from 12 to 67 years old.

65 travellers (91.54%) acquired the disease by eating food in the Province of Buenos Aires; 53 cases (74.64%) during an outbreak in Saladillo county, in Easter 2005.

The implicated food was pork, 95.77% (n = 68) as food processing sausage-shaped, 2.81% (n=2) as fresh meat and 1.40% (n = 1) have eaten both kinds. All were due to consumption of food purchased commercially produced by small commerce engaged in the slaughter of animals without adequate sanitary control.

The mean time from ingestion until the presentation of symptoms was 15 days (range 4 to 30 days).

All the travelers had fever. The most common symptoms were myalgia (68 cases, 95.77%), periorbital edema (54 cases, 76.05%), headache (52 cases, 73.23%) and conjunctival injection (39 cases, 54.94%).

The outcome was favorable. One patient developed a severe form with central nervous system involvement.

65% of patients, who got the result of specific serology, had at least one positive sample.

**Conclusion:** In our country there are reported cases of trichinellosis annually, often as disease outbreaks involving local people and travelers.

Argentina has more than 900 agricultural and livestock establishments for rural tourism and the tourist-recreational mode allows knowing, sharing and learning customs and traditions of our country, but travelers are exposed to diseases acquired by consumption of regional foods. It is very important that international travelers to Argentina consume cooked pork and they should not eat food from street sellers.

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**Type: Poster Presentation**

Health surveillance of Brazilian military peacekeepers deployed in the United Nations Stabilization Mission in Haiti (MINUSTAH), 2007 - 2009

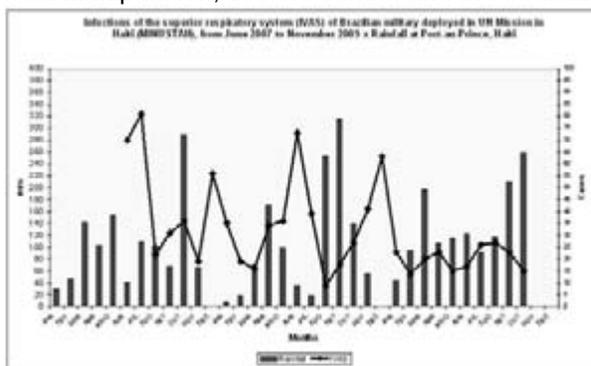
**J. R. P. De Andrade-Lima<sup>1</sup>, R. B. Batista<sup>2</sup>**

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**Background:** Military are deployed for peacekeeping missions in different regions of the world, being exposed to some endemic diseases in these operational environments. The surveillance of military health is the tool to the precocious detection of outbreaks, the adoption of preventive strategies for the more serious or frequents diseases, as well as the appropriate planning of health assistance.

**Methods:** In the second semester of 2009, a System of Health Surveillance (VigSau) was adopted in the Medical Unit Level 1 (MU 1) of the Brazilian Peacekeeping Battalion (BRABATT), analyzing the data of the medical attendance of about 5,000 Brazilian military deployed in the United Nations Stabilization Mission in Haiti (MINUSTAH), from June 2007 to November 2009. Around 60% of the Brazilian military in Haiti were included in VigSau. A computerized system was adopted in MU 1, having all the new medical visits been registered in 58 categories of attendance. The retrospective survey of the data registered in books of MU 1, since June of 2007, has also been made. The monthly and weekly incidence rates were determined and trends were analyzed.

**Results:** The total incidence rates of patients varied from 11,2% to 39.3% of the total of supported military/month. Amongst the diagnostic categories, the four categories that had greater load of diseases and temporary incapacity of the Brazilian military in Haiti were: infections of the superior respiratory system (22%), dermatological diseases (15%), musculoskeletal injuries (14%) and gastroenteritis (11%). The sazonalidade of some diseases was evidenced. The first results of the surveillance guided environmental interventions in the sanitation of drinking water, food manipulation, vectors control and the education in health.



**Conclusion:** Despite the logistic difficulties and deficiencies, the VigSau proved to be a practical and effective tool for the protection of the health of Brazilian troop in Haiti. The surveillance to the health of the military is a modern strategy to guarantee the troop performance, having to be enclosed into the doctrine of military health, in times of peace or war, to cooperate in the reduction of the diseases rate during the multinational peacekeeping operations.

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**Type: Poster Presentation**

Immunizations in travelers attending a private center for infectious diseases and travel medicine in Buenos Aires, Argentina, 2005-2008

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**Background:** According to Argentina's official estimations for 2006, 1.5 million people traveled abroad. Immunizations constitute an important part of the pre-travel consult. As such, and with the aim of describing some trends, we present a four-year experience at Centros Médicos Dr. Stamboulian, an ambulatory center for Infectious Diseases and Travel Medicine with vaccination facilities in Buenos Aires with travelers that sought pre-travel advice.

**Methods:** This is a retrospective, descriptive analysis. Immunization records were reviewed. Hepatitis A and B data was excluded (they are part of Argentina's immunization program). We focused on locally available recommended immunizations: typhoid fever, rabies, inactivated polio vaccine, and meningococcal A + C. Yellow fever was the only required vaccine in Argentina, since A, C, Y, W 135 meningococcal vaccine is not available.

**Results:** Between January 2005 and December 2008, a total of 2,342; 2,775, 3,501 and 4,710 travelers attended the center per year, respectively. Most common destinations were South America, Central America and the Caribbean, Asia and Africa. Recommended vaccine shots taken as a group increased 141% percent during the studied period (typhoid fever shots increased the most: 101%). Yellow fever vaccination increased dramatically in 2008, due to the ongoing outbreak in Misiones, Paraguay and Brazil. Polysaccharide typhoid fever vaccine was almost invariably used, except for a brief period of unavailability, when it was replaced for oral live vaccine.

Vaccine	Number of vaccines per year			
	2005	2006	2007	2008
Meningococcal A + C	664	784	667	537
Polio Salk	90	924	926	948
Rabies	224	238	267	177
Yellow fever	261	194	234	2495
Typhoid fever (Polisaccharide)	1571	1978	2600	2901
Oral Typhoid fever	37	304	0	0
Total	3240	4512	5255	7807

Of note, rabies vaccine shots include only records of corporate travelers, since we are not able to discriminate between shots given to travelers and people locally bitten by animals.

**Conclusion:** The tendency in pre-travel consults and immunizations at our center could reflect that, even taking into account Argentina's unsteady economic situation, people are willing to travel and to protect themselves. When events such as the yellow fever outbreak in 2008 occur, there is an extra demand of preventive measures.

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**Session: Tuberculosis: Epidemiology, Prevention & Control**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Clinical manifestations of tuberculosis among pediatric household contacts with active culture confirmed disease

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**Background:** Previous research on tuberculosis in children has been largely limited to cross sectional studies in settings where MTB culture was not performed. To further characterize the clinical presentation of children with TB, we performed a retrospective cohort study of pediatric household contacts (HHC) with culture confirmed disease in Uganda.

**Methods:** We reviewed clinical, radiologic, and epidemiologic characteristics of 79 pediatric subjects with active culture-confirmed TB. The cohort was derived from a longitudinal HHC study of adult infectious cases in Kampala, Uganda. Analysis included stratification by age group (young children (YC)= ages 0-2, older children (OC)= ages 3-14).

**Results:** Median age was 2.7 years, 42 were young children, and 45 were female. Cough  $\geq 3$  weeks was the most common symptom (80%) and was the only symptom present in the majority of subjects. Disease presentation varied significantly by age group. The frequency of abnormal findings was significantly higher amongst young children (YC) vs older children (OC), including fever (YC 19/42 vs OC 7/37;  $p=0.01$ ), weight loss (15/42 vs 7/37;  $p=0.004$ ), sick general appearance (17/42 vs 4/37;  $p=0.02$ ), and abnormal respiratory exam (20/42 vs 9/37;  $p=0.03$ ).

**Conclusion:** Clinical assessment of TB in child household contacts of infectious cases is challenging given the relative paucity of findings on clinical, microbiologic, and radiographic examination. In our study, cough was the only symptom present in the majority of cases, CXR was positive in 55% of cases, and AFB smear was only positive in 22% of cases. Clinical manifestations of disease varied significantly with age. Young children were more symptomatic and more likely to have multiple abnormal findings on physical exam than older children who had few signs or symptoms. In fact, more than one third of older children had no signs at all on physical exam. The reasons for these differences in presentation are unknown; it is possible that the immature immune response of children  $< 2$  years is dysregulated and over-produces pro-inflammatory cytokines resulting in systemic symptoms. Whereas in older children, the mature host cellular immune response may be sufficient to contain the organism in granulomas with minimal systemic signs or symptoms. Further research is needed to understand the effect of age and immune response to *M. tuberculosis*.

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**Type: Poster Presentation**

A validated clinical practice guideline for community health nurses working in tuberculosis Out-patient Clinics

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**Background:** Tuberculosis is a major contributor to disease burden in developing countries; it is considered the second fatal disease all over the world and the third most important public health problem in Egypt. The direct causes of increasing the burden of tuberculosis are the inconsistent and fragmented health services. The nursing interventions of tuberculosis in community settings require system of recommendation that ensures the consistency of care. The present study aimed at provide a valid clinical guideline that assist nurses to intervene consistently to the newly diagnosed pulmonary tuberculosis patient.

**Methods:** The needs analysis of community health nurses working in out patient chest clinics in addition to the expectation of newly diagnosed pulmonary tuberculosis patient regarding nursing interventions. The guideline development process established according to the criteria of experts of guideline development organizations. The Scottish Intercollegiate Guidelines Network (SIGN) research appraisal tools were used for the critical appraisal phase of the obtained evidence. AGREE instrument was used for assessing the internal validity of the guideline. The guideline and appraised for internal validity by academic nursing and medical staff, nursing, and medical practitioners.

**Results:** The scores of all appraisers in relation to scope and Purpose, stakeholder involvement, rigour of development ranged from (62.9- 77.7%, 53.5- 77.7%, and 66.6- 76.2%), respectively. While the scores for the clarity and presentation were 50- 76.4, applicability were 61.9- 68.5, and editorial independence were 88- 93. The four groups of revision strongly recommended the application of the guideline.

**Conclusion:** In conclusion, the developed guideline based on the needs of the intended users of nurses and end points beneficiaries, i.e., patients. It was strongly recommended by the reviewers and stockholders to be used in the out-patients chest clinics. It is recommended that the guideline should disseminate to the authoritative level to be applied in the chest out patient clinics and evaluated for applicability and achieved outcomes.

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**Session: Tuberculosis: Epidemiology, Prevention & Control**

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**Type: Poster Presentation**

Genetic diversity of *Mycobacterium tuberculosis* population in Bulgaria

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**Background:** Molecular typing of *Mycobacterium tuberculosis isolates* is a useful tool for epidemiological studies at different levels. Tuberculosis remains an important public health issue for Bulgaria, a Balkan country located in the world region with contrasting epidemiological situation for tuberculosis. Here we presents the insight into the population structure and drug resistance of *M. tuberculosis* strains currently circulating in Bulgaria, as a necessary step towards an implementation and better understanding of molecular epidemiology of TB here. We further looked at our data at a global scale through comparison with the international SITVIT2 database.

**Methods:** Study sample included 133 *M. tuberculosis* clinical isolates originating from different regions of Bulgaria and analyzed by various DNA fingerprinting methods We additionally investigated molecular basis of drug resistance of the studied strains.

**Results:** Comparison with SITVIT2 database (Institut Pasteur de Guadeloupe) revealed a heterogeneous population structure of *M. tuberculosis* in Bulgaria. The Beijing genotype strains were not found in spite of close links with Russia in the recent and historical past. Novel 15/24-loci MIRU-VNTR format achieved the highest discrimination. We additionally investigated molecular basis of drug resistance of the studied strains. Three types of the *rpoB* mutations were found in 20 of 27 RIF-resistant isolates; *rpoB* S531L was the most frequent. Eleven (48%) of 23 INH-resistant isolates had *katG* S315T mutation. *inhA* -15C>T mutation was detected in one INH-resistant isolate and three INH-susceptible isolates. A mutation in *embB306* was found in 7 of 11 EMB-resistant isolates.

**Conclusion:** This study gave a first molecular snapshot of *M. tuberculosis* strains circulating in Bulgaria. It demonstrated a heterogeneity of the Bulgarian *M. tuberculosis* population that appears to be dominated by several worldwide distributed and Balkan specific spoligotypes. Comparison with genotyping data did not reveal a statistical difference in the prevalence of drug resistance between clustered and non-clustered isolates. Emergence and spread of drug-resistant and MDR-TB in Bulgaria is not associated with any particular spoligotype or MIRU-VNTR cluster.

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**Type: Poster Presentation**

Transmission of drug resistant tuberculosis and its implication for TB control in rural China

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**Background:** The transmission pattern of drug resistant TB might vary due to the difference in the geographic feature, socio-economic development and TB epidemic from specific areas and populations. This might also matter how effectively TB control program would work. The present study attempted to describe the transmission of drug resistant TB in two comparable rural Chinese areas albeit with different duration of direct observed treatment, short-course(DOTS) implementation, as well as to discuss about its possible implication for TB control policies.

**Methods:** Setting in two rural counties: ten years' DOTS covered Deqing and one year's DOTS covered Guanyun, this study included the pulmonary drug resistant TB patients registered in local TB dispensaries as the subject. Proportion method and DNA sequencing determined drug resistant pattern and genetic mutation of *M. TB* isolates. IS6110-RFLP identified clustered patients and their epidemiological link was confirmed using concentric circle method.

**Results:** Totally 223 of 351 isolates(63.5%) were resistant to at least one anti-TB drug, including 53(15.1%) simultaneously resistant to isoniazid and rifampicin or multidrug resistant(MDR-TB, 18 from Deqing and 35 from Guanyun). Of the 168 patterns identified by IS6110-RFLP, 20 were shared only between drug resistant isolates; 12 were shared between the drug resistant isolates and the pan-drug sensitive isolates. Deqing and Guanyun had significantly different cluster proportion of drug resistant TB(32/101.vs.55/122, $p=0.041$ ) but similar cluster proportion of MDR-TB isolates(11/19.vs.22/34, $p=0.624$ ). The significant high cluster proportion was observed in the previous treated patients both in two counties but in the sputum smear positive patients with cavitaries alone in Guanyun, so does happen to those infected with the strain from Beijing genotype and resistant to isonazid and/or rifampicin. Of 87 clustered drug resistant strain, 67.8% was clustered in same or neighboring village ; The remained 71.4% were scatted along the traffic arteries.

**Conclusion:** Transmission pattern of drug resistant TB was consistent with attenuated properties of pathogen as well as social activity of rural populations. Meanwhile, DOTS itself might not work enough on the recent transmission of MDR-TB. These observations might suggest the need of supplementing the additional strategies, including active case finding within village and effective treatment on patients previously treated or with cavitaries.

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**Type: Poster Presentation**

Evaluation of cost and methods for detecting latent tuberculosis infection among target individual groups in Trinidad & Tobago

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**Background:** A huge amount of valuable time and resources is expended in the country using tuberculin skin test (TST) assay to screen for TB contacts and other subjects in the country. This study was carried out to compare using TST and QuantiFERON®-TB gold (QFT-G) assays in screening and diagnosis of latent tuberculosis infection (LTBI) in a cross section of high risk individuals for LTBI in Trinidad and Tobago.

**Methods:** During a 9-months period, over 550 subjects including contacts of TB patients (n=200), HIV positive patients (n= 70), health care workers (45), prison inmates (65) and TB patients (180) used as controls were recruited for the study. Blood specimen was drawn from the subjects and processed in the laboratory using QuantiFERON®-TB Gold (QFT-G) kit and tuberculin solution was administered on the forearm. Data were analyzed with Epi Info 3.5.1 software (CDC, Atlanta, GA, USA). The chi-squared test and Fisher's exact test were used as appropriate to compare data from the different groups. Data were descriptive and were reported as comparisons of frequency distributions. A *P* value <0.05 was considered significant.

**Results:** The QFT-G assay detected LTBI in 41.1% of the subjects with the highest (65.6%) positive results occurring among control group. The TST test detected LTBI in 19.9% of the subjects, a lower result than the QFT-G method, (*p*=0.001) Overall, QFT-G method detected more LTBI in all the different groups than the TST except among prison inmates where the detection was almost similar. The QFT-G gave a high rate of indeterminate and non reactive results among HIV positive subjects. Despite using less time completing QFT-G assay (23.3 hours) against TST method (70.2 hours, *p* < 0.0001), the cost was less performing the TST in each subject (\$3.70) when compared to QFT-G that was \$18.60, and the difference was statistically significant, *p*=0.0008.

**Conclusion:** The QFT-G gave a faster turn around time result, effective and more expensive than TST method. However, because the QFT-G gave indeterminate and non reactive response for immunocompromised subjects such as HIV positive patients, care must be taken when screening or making a diagnosis of LTBI based on QFT-G results in a poor resource and high HIV prevalence setting like Trinidad & Tobago.

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**Type: Poster Presentation**

HIV prevalence and MDR TB among DOTS attendees in a rural area of Haryana, India

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**Background:** MDR-TB was a potential threat to tuberculosis control in India. We wanted to estimate the prevalence of HIV infection and MDR TB among DOTS attendees in Ballabgarh, Haryana, India.

**Methods:** The study was carried out in two DOTS centres of Ballabgarh Tehsil of district Faridabad, Haryana. It was a health facility based cross sectional survey. Study subjects were all TB patients (Pulmonary and Extra Pulmonary) who were registered for DOTS. Data was collected from January 2007 to June 2008. We enrolled 413 eligible patients who were informed about the study objectives and written consent was obtained. All patients were offered free Liver Function Test. Unlinked anonymous testing on aliquot of LFT blood samples was performed after removing all identifiers. HIV testing was done using three E/R/S. Sputum was collected from patients belonging to Categories I and II, under Revised National TB Programme. Anti TB sensitivity testing was done on randomly selected patients of Category I (50 % of patients) and all of the Category II patients.

**Results:** Four hundred and thirteen TB patients [CAT I-220(53.3%), II-101(24.5%), and III-92 (22.2%)] were interviewed and blood samples could be obtained from 368 patients (89.1%). Four blood samples got contaminated and labels of ten samples was lost during the transportation. Finally, of the 354 samples tested two were found HIV sero reactive. Prevalence of HIV among TB patients was 0.56% (95 % CI 0.068-2.02).

Sputum collection was attempted from 211 patients. Thirty eight patients (18%) had difficulty in producing sputum. Of the 173 sputum samples collected, eighty were culture positive for Mycobacterium tuberculosis. Among them nine samples were found to be multi drug resistant by RLBA. The prevalence of MDR among TB patients was 11.25 % (95 % CI 5.27 – 20.28).

**Conclusion:** HIV prevalence was low and similar to the rate observed among pregnant women (0.13%) who could be considered as proxy to general low risk population. Multi drug resistance was high among TB patients. It was reassuring that HIV – TB co infection was low and none of the MDR TB patients were HIV positive.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

The social representation of DOT-related tuberculosis by patients of Guarulhos at the metropolitan area of Sao Paulo, Brazil

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**Background:** Setting: Public healthcare service (HCS) of Guarulhos municipality, in the metropolitan area of Sao Paulo, Brazil.

**Objective:** Analyzing the individual perception of patients concerned to supervised treatment (DOT)-related tuberculosis (TB) offered by HCS.

**Methods:** Qualitative approach using interviews with patients. The Collective Discourse (CD) allows capturing a pool of social representations, gathering answers from different individuals with discourse content of similar sense to build collective statements.

**Results:** Answers of 65 patients (interviewers can give more than one central idea), in three questions (Q) about DOT – (Q1) ‘Someone tells you about DOT. What is DOT for you?’, (Q2) ‘Tell us about DOT on a daily basis?’, and (Q3) ‘DOT takes six months and after one month patients do not have more symptoms. Why do you think this happen?’. According to Table 1, the central ideas presented were: Q1, (A) It's a guidance regarding where treatment will take place; (B) B. It's a guidance regarding time and frequency of TB drugs; and (C) It's an explanation about TB. Q2, (A) Patients report to go to HCS to take TB drugs; (B) Patients report the side-effects to TB drugs and to the aggressive treatment; (C) Patients report that treatment is incorporated on routine and do not affect the routine; and (D) Patients report improvement of health condition after the beginning of treatment. Q3, (A) Because symptoms decrease or disappear and patients feel good and think they're cured; (B) Due to lack of consciousness and responsibility and lack of love to life, health and to the next one; (C) Due to side effects and lack of information regarding the treatment of TB.

**Table 1.** The social representation of DOT-related tuberculosis by patients of Guarulhos at the metropolitan area of Sao Paulo, Brazil

Questions	Results				
	Central Ideas presented**	N	N1	N2 %	
1 Someone tells you about DOT. What is DOT for you?	A. It's a guidance regarding where treatment will take place	119		45	69.2
	B. It's a guidance regarding time and frequency of TB drugs			30	46.2
	C. It's an explanation about TB			17	26.2
	D. Others (7 categories)			27	-
2 Tell us about DOT on a daily basis?	A. Patients report to go to HCS to take TB drugs	65	82	24	36.9
	B. Patients report the side-effects to TB drugs and to the aggressive treatment			17	26.2
	C. Patients report that treatment is incorporated on routine and do not affect the routine			14	21.5
	D. Patients report improvement of health condition after the beginning of treatment			12	18.5
	D. Others (5 categories)			15	-
3 DOT takes six months and after one month patients do not have more symptoms. Why do you think this happen?	A. Because symptoms decrease or disappear and patients feel good and think they're cured	90		39	60.0
	B. Due to lack of consciousness and responsibility and lack of love to life, health and to the next one			17	26.2
	C. Due to side effects and lack of information regarding the treatment of TB			14	21.5
	D. Others (8 categories)			20	-

\*\* Interviewers can give more than one central idea

N: Number of TB patients interviewed in the study

N1: Number of answers presented in the question

N2: Number of ideas in the category presented (unifying of central ideas)

**Conclusion:** TB patients have correct knowledge about the disease, understanding the necessity of DOT and also they report their ways on the process of TB diagnosis which can compromise their treatments. So, it is necessary that HCS pay attention on TB diagnosis with commitment and training of professionals on DOTS Strategy. They don't perceive DOT as a barrier, but as a strengthening for a very difficult treatment.

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Prevalence of bacteriologically confirmed pulmonary tuberculosis in the Bhutanese refugees in Nepal. Results of active case finding

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**Background:** Approximately 100,000 Bhutanese refugees live in camps in southeastern Nepal. Since December 2007 the International Organization for Migration (IOM) in Nepal has conducted medical screening of Bhutanese refugees prior to resettlement in USA, Canada, Australia, New Zealand, Denmark and Norway. Screening for TB included both sputum smears and cultures. The estimated prevalence of all forms of TB was 243 per 100,000 in Nepal (WHO, 2006), and 217 per 100,000 in Bhutanese refugee camps prior to resettlement (UNHCR, 2007).

**Methods:** Depending on the age group and specific instructions of the resettlement countries, suspected TB cases were identified with the combination of the medical history, physical examination, CXR and TST. Suspected cases were referred for microbiological examination of three sputum samples by both acid-fast bacilli staining and liquid culture for TB. Drug susceptibility testing (DST) was performed on all new positive cultures. If smears or cultures were positive, or if clinical and radiological findings were consistent with active TB, directly observed treatment was performed.

**Results:** From December 13, 2007 to July 31, 2009, IOM Nepal conducted medical examinations of 23,459 refugees, of which 2,391 (10.2%) were suspected TB cases. Prevalence of bacteriologically confirmed (positive sputum smears or/and cultures) was 644 per 100,000. Prevalence of smear-positive cases was 230 per 100,000. Compared with culture, sensitivity of sputum smears was only 32%. DST yielded 2% MDR TB among culture confirmed cases; 5% with resistance to more than one drug, but not MDR TB; 3% INH mono-resistant TB; 3% PZA mono-resistant TB.

**Conclusion:** Results of this cross-sectional study show high prevalence of infectious pulmonary TB among the Bhutanese refugees in Nepal. These results highlight the increase in case detection when sputum culture is performed, which can detect TB cases with low bacillary load. We identified prevalence of pulmonary TB at least 2.7 times greater than previously reported prevalence of all forms. This points out the benefit for wider use of sputum culture to detect infectious TB in high burden countries. A significant proportion (13%) of cases with drug resistance highlights the need for DST to direct TB therapy.

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**Type: Poster Presentation**

Newly diagnosed tuberculosis patients and tobacco use in North Malaysia: The prevalence of tobacco use, knowledge, and attitudes

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**Background:** Sufficient evidence concludes that tobacco smoking is strongly linked to tuberculosis (TB). It was suggested that a considerable proportion of the global burden of TB may be attributable to smoking. This study aimed to document the prevalence of smoking among newly diagnosed TB patients and to learn about their tobacco use knowledge and attitudes.

**Methods:** Data on smoking prevalence were obtained based on reviews of routinely collated data from January through December 2008 in the state of Penang. The study setting comprised of five chest clinics located within Penang and Wilayah Persakutuan Kuala Lumpur health districts, Malaysia. A validated 58-item questionnaire was used to assess the tobacco use knowledge and attitudes of those TB patients who were smokers.

**Results:** Smoking status was determinant in 817 of 943 new cases of TB during study period. Of this, it was estimated that the prevalence rates of current and ex-smoking among TB patients was 40.27% (329/817) and 13.95% (114/817), respectively. Of 86 cigarette smokers who were eligible for a larger project, 93% responded to the survey. The mean ( $\pm$ SD) total score of tobacco use knowledge items was 4.23 $\pm$ 2.66 (maximum possible score=11). More than half of the participants (51.3%) were moderately dependent on nicotine and less than half (47.5%) had knowledge about the body system on which cigarette smoking has the greatest negative effect. The majority wrongly believed that smokeless tobacco can increase athletic performance (60%) and that it is a safe and harmless product (46.2%). However, an overwhelming proportion (>80%) of the patients believed that: tobacco use is dangerous to health and that smokers are more likely to die from heart disease when compared with non-smokers. The use of smokeless tobacco was moderately prevalent among the participants with 28.8% reporting ever snuffed, but the use of cigar and pipe was uncommon.

**Conclusion:** Smoking prevalence rate was high among patients with TB in Malaysia. These patients generally had deficiencies in knowledge of tobacco use and its health dangers, but had positive attitudes against it. Efforts should be geared towards reducing tobacco use among this population due to its negative impact on TB treatment outcomes.

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**Type: Poster Presentation**

Frequency of tuberculosis in the biggest island of the Persian Gulf

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**Background:** Despite availability antituberculosis drugs for almost 50 years, tuberculosis (TB) continues to exert an enormous toll on world health. The incidence of TB is increasing all over the world. Qeshm represents a region in south of Iran that is the biggest island in the Persian gulf with 23 thousands inhabitants with a long tradition in TB control, including a centralization of the bacteriological diagnostic facility. The present study was intended to analyze the transmission of *Mycobacterium tuberculosis* by a combination of conventional epidemiological approaches.

**Methods:** *Mycobacterium tuberculosis* analyzed in this study were collected at the Health Care Center in Qeshm, Iran. A total of 81 new, bacteriologically verified TB cases were registered in Qeshm Island between 2003 and 2008. All the isolates were examined for their susceptibility to ethambutol, isoniazid, streptomycin, rifampin, and pyrazinamide by using a radiometric culture system (BACTEC). The data obtained from the cultures analyses were interpreted by using demographic data, such as age, sex, ethnicity, and residence, for the patients. The risk factors among the patients for being part of an active chain of transmission, as opposed to demonstrating reactivation of a previously acquired latent infection, were estimated by statistical analyses (SPSS).

**Results:** A total of 81 clinical isolates belonging to patients having pulmonary and extra pulmonary tuberculosis were collected during Jan 2003 to Nov 2008. The incidence of tuberculosis in female was 25.9% and in male was 74.1%. This survey observed 47.1% of immigrated Afghans and 39.1% of Pakistanis were infected with tuberculosis. Regarding the literacy 57% were unlettered. 91.7% of people referring to health center were new patients. 68.8% people were infected with pulmonary tuberculosis. The peoples over 60 year were highest group infected to pulmonary tuberculosis (30.4%) and age groups 30-44 were highest the cases infection external pulmonary tuberculosis. The major chains of recent transmission were localized to distinct geographical regions in the area.

**Conclusion:** TB is frequent among immigrants, especially from Afghanistan and Pakistan, but it is apparently readily suspected, diagnosed, and treated by the health care system. Indigenous patients with pulmonary symptoms are not primarily suspected to have TB and, therefore, play an important role in recent TB transmission in Qeshm.

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The clinical and epidemiological characteristics of *Mycobacterium tuberculosis* Beijing/W family strains in a major immigrant-receiving province of Canada

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**Background:** Tuberculosis resulting from the Beijing/W family of *Mycobacterium tuberculosis* strains is a global concern due to associations with tuberculosis outbreaks, antituberculosis drug resistance, and treatment failure and relapse. This study sought to determine if Beijing/W strains represent an emerging public health threat within Canada, a major immigrant-receiving country with low tuberculosis incidence.

**Methods:** This population-based cohort study investigated archived culture-positive *M. tuberculosis* isolates from cases diagnosed in the province of Alberta, Canada between 1990 and 2007. Isolates were Beijing/W genotyped with PCR-based region of difference analysis and DNA fingerprinted with IS6110 RFLP. In 449 isolates, Beijing/W status was validated with spoligotyping. Demographic and clinical data for each isolate was obtained from the provincial TB Registry. Statistical analyses consisted of Pearson's chi-square test, Fisher's exact test, and independent sample t-test.

**Results:** Nearly 99% (n=1,900/1,927) of isolates were available for genotyping. Beijing/W strains comprised 19% (n=372) of isolates, with foreign-born persons contributing 91% (n=337) of all Beijing/W isolates (p<0.001). The distribution of Beijing/W strains within the foreign-born population was remarkably heterogeneous; 90% (n=302) of Beijing/W isolates occurred among persons from the Western Pacific region (p<0.001). Conversely, the Canadian-born Aboriginal and non-Aboriginal subpopulations had similar distributions of Beijing/W strains (n=19 and 16, respectively; p=0.389). Cases attributed to Beijing/W and non-Beijing/W strains were comparable in terms of age at diagnosis (p=0.055), sex (p=0.488), disease phenotype (respiratory or non-respiratory disease) (p=0.947), and sputum smear positivity (p=0.121). Beijing/W and non-Beijing/W strains also had similar associations with first-line drug resistance among Canadian-born persons (p=1.000) as well as within each foreign-born subpopulation, namely persons from the Western Pacific region (p=0.902) and other regions (p=0.512). Although a comparable proportion of foreign-born Beijing/W and non-Beijing/W cases were involved in transmission clusters (p=0.329), non-Beijing/W strains accounted for a significant proportion of clustered cases within the Canadian-born population (p=0.023).

**Conclusion:** Within a major immigrant-receiving country, Beijing/W strains appear to pose no more or less of a public health threat than non-Beijing/W strains. The findings also suggest that current TB control programmes within low incidence settings can appropriately manage Beijing/W strains.

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**Type: Poster Presentation**

Evaluation of the performance of TB Control in Apac District in 2008 using Direct Observed Therapy (DOTS)

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**Background:** DOTs is a WHO Global TB monitoring, treatment and surveillance strategy based on case detection at lower treatment levels. One of its tools is detection of pulmonary TB by sputum smear microscopy. Research questions for the study were: Can we use the DOTS strategy to predict the TB burden in Apac District annually?; Did the TB control program in Apac District measure up to WHO standards recommended for areas under DOTS?; How useful is DOTS as a planning, monitoring and surveillance tool for TB control in Apac District?

**Methods:** This was a cross sectional study carried out in Apac District in Northern Uganda. The district has Kole, Maruzi and Kwania HSD. Main study subjects were persons who went for sputum smear examination and were recorded in laboratory registers and HMIS Forms 055a and 055b (HMIS 055a is the laboratory summary forms per health unit; 055b is the district summary for all laboratories reports from district health units).

**Results:** The highest prevalence (877 TB cases per 100,000 people) and incidence (280 TB cases per 100,000 people per year) were in Kole Health Sub District (HSD). District incidence was 66 cases per 100,000 persons per year. Maruzi HSD had the highest case detection (93%) while Kole HSD (27%) registered the lowest. District case detection was 49%. Kwania HSD had the highest number of sputum examinations (1,628) but with lesser sputum smear positives than Maruzi HSD.

**Conclusion:** The district case detection rate of 49% compared well with the national figure but was below the WHO target of 70%. The incidence of TB was 66 new sputum smear positives per 100,000 people in the district. However, the same must be established in the different HSD to know where the highest burden is found. DOTs tools are applicable to Apac district useful planning and can be used by the district Health Teams to monitor and evaluate progress in TB control. More intense case finding and surveys are needed in the district to establish the actual prevalence of TB. A study should be done to include treatment outcomes as required in the DOTS strategy.

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**Type: Poster Presentation**

Analysis of the decentralization of tuberculosis control program in the sanitary district of Cabula-Beirú (SDCB) Salvador-Bahia-Brazil, 2008

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**Background:** The National Program of Control of Tuberculosis (PNCT) decentralization process began in the decade of 1980 from the federal level to the state level, extending to the municipal level since 1990. **OBJECTIVE:** Describe the process of decentralization of Tuberculosis Control Program in SDCB, Salvador - Bahia-Brazil.

**Methods:** A descriptive qualitative and quantitative study has been done with views to produce information on the process of decentralization of PCT in SDCB of the municipal district of Salvador-Bahia-Brazil in 2008. Techniques for interviewing were applied in questionnaires and analysis of content of the speeches for the collection of the primary data, the secondary data were obtained consulting the Book of Registration of PCT, Report of Administration 2005-2008, Municipal Plan of Health and the database of the confirmed cases of tuberculosis registered in SINAN. The quantitative data were treated and analyzed using the programs EPI-INFO and presented under the form of tables. The qualitative analysis was accomplished starting from the depositions of the informers.

**Results:** 1,781 cases were confirmed from (2000 to 2008) of these 1,131 they presented positive bacillus-carriers. From (2005 to 2008) 1,924 cases were confirmed of these 86, 64% (1,667 cases) bacillus-carriers. It was observed that PCT of DSCB was implanted in one unit in 2004 and seven in 2008. In relation to the diagnosis in 2004 73% of the bacillus-carriers were positive in 2008 for the three studied units. 59.2% of the bacillus-carriers that tested positive, the rates of discharge for the cure were from 71.3% (2004) to 68.3% (2008) the adherence tax to the treatment was from 75.6% (2004) to 83.6% (2008) and the tax of abandonment 5.9% (2004) and 5.5 (2008). The factors that caused obstacles to the decentralization of PCT: the medical professional's absence, structures inadequate physics, disabled team and the stigma of the disease for the professionals of health.

**Conclusion:** The program was decentralized for seven units of health, with increase in the adherence taxes. That process guaranteed larger access to the diagnosis and the treatment supervised by the population, with the reduction of the taxes of abandonment for the District.

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**Type: Poster Presentation**

A community based tuberculosis control project in children in urban and rural settings: A public-private mix approach

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**Background:** The control of tuberculosis in children entails a concerted effort of both the government and private sectors thus a public-private partnership was formed and will serve as a model.

**Methods:** One urban (Sta. Rosa City) and two rural (Florida Blanca and Mabalacat) were chosen for a pilot program for the control of tuberculosis in children by the Philippine Ambulatory Pediatric Association, Inc (PAPA.) through the collaboration of the Department of Health National Tuberculosis Program (DOH-NTP), private groups and the Local Department of Education (DepEd). PAPA provided the logistics, trainings, medicines and project managers through funding from Pott's Foundation and Alliance for the Control of Tuberculosis in Children (ACTC). The chosen areas were visited by PAPA and DOH for consultation with the Local Chief Executive (LCE) and the signing of memorandum of understanding. The Local Government Unit (LGU) provided the health personnel. Trainings of the health personnel were done by PAPA. Initial screenings were fully supervised by PAPA. Screening was done twice a week and complete allocation of medicines for active disease were provided using 2HRZ and 4HR. Directly observed treatment strategy (DOTS) approach was used and the barangay health worker (BHWs) served as treatment partners. Weight monitoring and symptom monitoring were done and recorded. Mid-project assessments and end-of-project reports in each area were also done.

**Results:** The areas yielded 1923 screened patients and treated 538 (28%). The project protocol was validated and later used for National Tuberculosis Control Program for Children in the Philippines.

**Conclusion:** It was demonstrated that the control of childhood tuberculosis through private-public partnership contributed a very significant role in the detection and monitoring component thus lessening the burden of the disease and ensuring success and possible future eradication of tuberculosis in children.

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**Type: Poster Presentation**

Patterns of tuberculosis health problem in India: A gender perspective

**S. Gupta**

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**Background:** Tuberculosis is the most common cause of infectious disease-related mortality worldwide. The WHO estimates that 2 billion people have latent TB, while another 3 million people worldwide die of TB each year. Although TB rates are decreasing in the India, the disease is becoming more common in many parts of the world. Tuberculosis is an airborne contagious disease that is transmitted by coughing or sneezing. Exposure to cooking smoke can increase the risk of tuberculosis by reducing resistance to initial infection or by promoting the development of active tuberculosis in already infected persons. Air pollution is a major public health problem in developing countries including India due to these TB patients has also increased. In India, there is high differential in reporting TB patients through sex as well as state wise.

**Methods:** This paper tries to see the levels and patterns of TB patients among men and women in India. This paper also examines the relationship between TB patients with selected background characteristics using data from National Family Health Survey-1998-99 and 2005-06. Bivariate and Multivariate statistical techniques were used to test the significance impact of selected background variables on prevalence of tuberculosis among men and women aged 15-49 years living in rural and urban areas of the country.

**Results:** The analysis suggest that the prevalence of TB patients has declined from 550 to 290 patients per 100,000 population during 1998-2006 time period among women; while in case of men, the prevalence is also declining from 420 to 350 patients per 100,000 population during same time period. The results from logistic regression analysis suggest that persons (male/female) belongs to scheduled caste/scheduled tribes, poor economic status, residing in rural area and illiterate are more likely to reported TB than their respective counterparts.

**Conclusion:** Hence we conclude that men has reported higher TB patients compared to women also it is reported higher side those who have low standard of living. Further, it reveals that the variables like number of persons per room, type of house, cooking under chimney, and caste significantly influence the likelihood of tuberculosis prevalence among Indian men and women.

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**Type: Poster Presentation**

Tuberculosis in health-care workers: Results of a surveillance program in Mexico City

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**Background:** Tuberculosis is major public health problem, left untreated, each person with active TB disease will infect on average between 10 and 15 people every year. Preventive strategies are essential for the achievement of the Millennium Development Goal.

**Methods:** In 1992 a HCW's surveillance program was instituted in our hospital, which consist in an initial evaluation for every worker during their first week of working within the Institute, a questionnaire with demographic and risk variables, PPD application, and a basic risk prevention's course. PPD Booster is applied for those with an initial PPD negative test, and then annual follow-up is performed. The workers with PPD positive test are evaluated by a physician and a chest-Rx is taken. Prophylaxis is indicated in every HCW who has a PPD positive test during the follow up or risk factors for the disease.

**Results:** A total of 5513 HCW have been evaluated since 1992, 61% were women, and the median age was 27. Forty percent were physicians, 32% nurses, 10% administrative workers and the rest (18%) from other areas. Of the evaluated workers 1932 (38%) had an initial PPD positive test, all of them had a normal chest-Rx. After the Booster application we identified 286 workers with positive test. During the follow up 259 HCW have had a PPD positive test. A total of 169 prophylactic treatments have been provided, 53 workers did not finish the treatment because of mild secondary effects and personal decision. Sixteen HCWs have developed clinical tuberculosis, 9 had a previous PPD positive test and refused prophylactic treatment.

**Conclusion:** The initial PPD positive test prevalence was 43%. HCWs are in risk of this infection especially in developing countries. A specific program for them is essential in order to reduce nosocomial transmission and identify those in risk of tuberculosis.

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**Session: Zoonoses and Infectious in Animals**

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**Type: Poster Presentation**

Prevalence and associated risk factors of mastitis in lactating dairy cows

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**Background:** Mastitis has been known to cause a great deal of loss or reduction of productivity, to influence the quality and quantity of milk yield, and to cause culling of animals at an unacceptable age. Apart of its economic importance it also carries public health significance. Although sub clinical mastitis (SCM) is more prevalent than clinical mastitis in developing countries including India, as sub clinical disease is not manifested as visible changes in the mammary glands or in the milk, it is therefore not easily recognized by farmers. .

**Methods:** A study was conducted to determine the prevalence of mastitis and associated risk factors. The screening for mastitis was done by Sodium lauryl sulphate test (SLST) as per the method of Pandit and Mehta (1969) and Somatic cell count (SCC) (Schalm *et al.* 1971). Clinical mastitis was confirmed on the basis of physical examination of udder and visual examination of milk for presence of flakes, clots, pus, odor and discoloration apart the tests used for SCM. Quarter was defined as mastitis positive if it have a SLST score of >1+ or SCC  $5 \times 10^5$  per ml of milk and a animal was define as mastitis positive when it has atleast one of quarters with a SLST score 1+ or  $>5 \times 10^5$  per ml of milk. For SCC, a quarter will be considered to have mastitis when the SCC  $>5 \times 10^5$  per ml of milk.

**Results:** In the present study, a total of 46 (18.04%) cows and 65 (6.97%) quarters had clinical mastitis. A total number of cows affected with sub clinical mastitis were 196 (76.86%) out of 255 by SLST and 172 (67.45%) out of 255 cows by SCC and 8.63% teats were blind. Among risk factors, the higher prevalence was in 2nd and 8th parities and late stage of lactation. Age wise 3 to 6 years old cows had high prevalence (55.10%) followed by >6 years age and <3 years old age cows.

**Conclusion:** Sub clinical form of mastitis in dairy animals is highly prevalent than clinical. Prevalence is high in 3-6 years old cows. Therefore, need special attention towards its controll measures by awaring the farmers and updating the veterinary practitioners regarding this deadly disease.

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**Type: Poster Presentation**

The clinico-pathological features associated with experimental concurrent PPR virus and *Mannheimia hemolytica* A: 2 infections in the West African dwarf goats

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**Background:** Small ruminants represent an important aspect of the livestock economy in developing countries in the humid tropics as more than 80 % of rural families keep sheep and goats. The most common viral and bacterial respiratory disease of Nigerian goats are Peste des petit Ruminants and Mannheimiosis. The endemic nature of PPR infection especially in goats and the usual association with *Mannheimia hemolytica*, in the pneumonia of small ruminants on the field, makes it necessary to study the clinicopathological changes associated with experimental co infection of PPRV and MH with a view of understanding the role of this bacterium in the pathology of PPRV infection in West African dwarf goat.

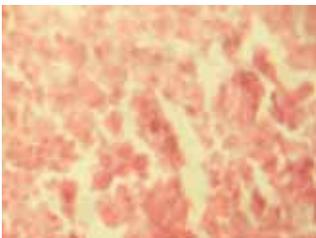
**Methods:** Twenty apparently normal West Africa Dwarf (WAD) goats six months of age were used for the experiment. 15 goats were infected by intratracheal inoculation of 1ml of pure cultured 106.5 TCID<sub>50</sub> PPR virus grown in Baby hamster kidney cell lines and a week later 1ml of pure culture (109 CFU) of MH A2 to study its clinicopathological features and five goats served as controls. The clinical signs were observed and two goats were euthanized at predetermined intervals for gross examinations, bacteriological, virological and histopathological investigations on tissues collected using standard techniques.

**Results:** The clinical signs were severe and the order of manifestation was anorexia, pyrexia, dyspnea, oculo-nasal discharge, recumbency and death. The lesions observed were severe pulmonary consolidation and pleural adhesions of the apical lobes and pleurisy with marked erosive stomatitis and acute enteritis. The average percentage lung consolidation for the infection was 7.01% and the right lung was more affected ( $p < 0.05$ ) while the overall mortality was 33.3% at 15 dpi. Histologically, the lung lesions were typical of fibrinous bronchointerstitial pneumonia with thickened alveolar septa, edema and neutrophilic infiltrations of the interstitium with giant cells. *Mannheimia hemolytica* A:2 was re-isolated bacteriologically from the lungs, at necropsy.



**The lung of a WAD goat infected with PPRV and MH showing marked hyperemia, consolidation and fibrinous deposits on the right cranial, middle and anterior portion of the caudal lobe**

**Conclusion:** The association of the two agents results into very fulminating disease with severe pulmonary and enteric lesions. This study further showed that the survivability of animals affected by PPRV and MH depends on the early commencement of antibiotics treatment to curtail the bacterial involvement and the electrolyte loss associated with PPR.



**Photomicrograph of the lungs of a WAD goat infected with PPRV and MH showing giant cells H&E X 400**

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**Type: Poster Presentation**

*Ancylostoma spp.* in sand of beaches of Navolato, Sinaloa, México  
**M. C. Rubio Robles**, S. M. Gaxiola C., N. Castro C.  
Universidad Autonoma de Sinaloa, Culiacan, Sinaloa, Mexico

**Background:** Eggs of *Ancylostoma spp.* disseminated for defecate of dogs and cats in public areas, are extremely resistant to adverse environmental conditions, capable of surviving in soil for many months; this represent risk for health humans and pets that have contacted with contaminated sand of beaches can act as reservoir or vector of this zoonotic parasite, that potentially can infect the host by eggs and larvae penetrating the skin of bare feet or hands and produce Larva migrans, also known as creeping eruption or sandworm eruption, characterized by tortuous migratory lesions of the skin. The objective of this work was to determine the presence of *Ancylostoma spp.* in moist sand of beaches of Navolato, Sinaloa, México.

**Methods:** The composite samples of sand of seven beaches were determined for representative samples described by the technique of Thrusfield (2005) was used:  $n = [t \cdot SD / L]^2$ . Where  $n$ =sample size,  $t$ =value of the normal distribution (Student  $t$ ) for a 95% confidence level ( $t = 1.96$ ),  $L$ =accepted error or precision (5%), and  $SD$ =weighted disease prevalence (%); the total of composite sample of sand determined by random samplings was 254, took surface moist sand scraping of 100 grams of sand for each sample and deposited it in plastic bags; transferred to the laboratory of parasitology of the FMVZ-UAS to be analyzed by the sedimentation technique.

**Results:** Of the 254 composite samples of sand of the seven beaches, 96 (37.7 %), were positive to *Ancylostoma spp.*

**Conclusion:** The contamination with *Ancylostoma spp.* represent high risk for the pets and public health, yet residents as visitors ignore about parasitic diseases that dogs can transmit them it is necessary implement control strategies and education for the prevention of the infections.

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**Type: Poster Presentation**

*Arcanobacterium pyogenes* associated with pulmonary and submandibular lymph node abscessation in white tailed deer (*Odocoileus virginianus*)

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<sup>1</sup>Afifi Department of Wildlife & Zoo Medicine, Faculty of Veterinary Medicine, Suez Canal University, Ismlia, Egypt, <sup>2</sup>Sleeman, USGS National Wildlife Health Center is located at 6006 Schroeder Road, Madison, Wisconsin 53711, <sup>3</sup>Saunders, & Kaur, Department of Biomedical Sciences & Pathobiology, Virginia–Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA

**Background:** Thin, lactating and uncoordinated female white-tailed deer was submitted for necropsy as part of a surveillance program for chronic wasting disease (CWD). Laboratory tests for CWD and rabies were negative. Post-mortem examination revealed pulmonary and submandibular lymph node abscesses associated with *Arcanobacterium pyogenes* and *Pasteurella*. The overall presentation suggests that the infections may have been associated with chronic stress.

**Methods:** Brain tissues were removed aseptically and transferred to Virginia's Department of Game and Inland Fisheries for analysis for CWD and rabies virus, and Brain culture swabs were sent to the Virginia-Maryland Regional College of Veterinary Medicine for aerobic and anaerobic bacterial cultures, including *Listeria monocytogenes* culture. Lung, lymph node, brain, intestine and heart samples were preserved in 10% neutral buffered formalin for histopathologic examination. Lung and lymph node samples were taken aseptically for aerobic culture, including culture for *Mycoplasma* and *Salmonella*. Lung and lymph node were plated onto Blood Agar, MacConkey Agar and Columbia CAN Agar. Culture swabs of lung tissue were plated onto Chocolate agar, TSA and CAN agar. Chocolate Agar plates were incubated in 5% CO<sub>2</sub> incubated at 37 °C with no CO<sub>2</sub> analysis.

**Results:** *Arcanobacterium pyogenes* was isolated from lung and submandibular lymph node, and identified using bioMérieux API Coryne strips. *Pasteurella* spp. was isolated from the same lymph node, and identified using bioMérieux API 20 NE strip. Laboratory tests for rabies, CWD, *Listeria*, *Mycoplasma*, *Mycobacterium* and *Salmonella* were all negative. Histopathologic examination was performed on lung, brain, spleen, lymph node, intestine, heart and liver. The lung had multiple discrete nodules of coagulative necrosis containing neutrophils and macrophages (figure 1). A rim of neutrophilic inflammation surrounded the necrosis; peripheral to this was a layer of fibroplasia and fibrosis.

**Conclusion:** In conclusion, even though it was not isolated from the lung, *Pasteurella* was the primary cause of infection in the lung and from there it spread to the lymph node. *A. pyogenes* was considered to be a secondary infection in the lungs where pneumonia was already present. The final diagnosis was pulmonary and lymph node abscesses due to *A. pyogenes* and fibrinopurulent and necrotizing bronchopneumonia due to a mixed *Pasteurella* and *A. pyogenes*.

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**Type: Poster Presentation**

Phylogenetic analysis of sporadic hepatitis E virus in Eastern China

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<sup>1</sup>School of Public Health, Fudan University, Shanghai, China, <sup>2</sup>Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China, <sup>3</sup>Deqing County Center for Disease Control and Prevention, Deqing County, Zhejiang, China

**Background:** Recently, evidence for the existence of hepatitis E virus (HEV) was reported all over the mainland of China. The number of acute sporadic hepatitis E cases increased annually and small outbreaks happened more frequently, probably due to food-borne transmission. The phylogenetic characteristics of the circulating HEV worth further understanding.

**Methods:** A total of 413 serum samples were collected from acute sporadic hepatitis E patients in 14 hospitals in Eastern China from 2005 to 2008 under informed consent. All the samples were detected with a nested RT-PCR assay for HEV RNA, and a 150-nt fragment within HEV ORF2 region was sequenced for phylogenetic analysis using Neighbor-Joining method with reference HEV sequences from the GenBank.

**Results:** The ratio of male to female was 1.75: 1 among all the patients. The majority (61.5%) of them was 40-69 years old, with an average age of 50 ± 16 years old. 140 out of 413 (34.0%) sera were positive in HEV RNA, and all the isolates were sequenced subsequently. Phylogenetic analysis revealed that all these isolates belonged to genotype-IV with much high similarities, sharing 77.9%-88.3%, 80.8%-90.6%, 73.4%-85.2% and 91.0%-95.4% nucleotide identities with prototype I (D10330, Burma; D11092, China; X98292, India; AY230202, Morocco; AY204877, Chad), II (M74506, Mexico), III (AB089824, AB189070, Japan; AY115488, Canada) and IV (AB097812, AY594199, AB108537, China) HEV strains, respectively. Those isolates could be further divided into six clusters within genotype IV, but no obvious geographical difference was observed among the clusters.

**Conclusion:** It is evident that genotype-IV HEV had been the principle causative agent of acute sporadic HEV infection for human in Eastern China.

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**Type: Poster Presentation**

Molecular characterization of VP1-3 and NSP1-3 genes of porcine group A rotavirus G12 strain RU172: Evidence for porcine origin of human G12 strains

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**Background:** Group A rotavirus is an important cause of infantile diarrhea in humans, and classified into 23 G and 31 P genotypes. Among them, G12 has been regarded as an important emerging genotype of human strains, worldwide. On the other hand, the only G12 strain reported from animals was a G12P[7] strain, RU172, detected in a diarrheic piglet in Eastern India. The VP4, VP6, NSP4 and NSP5 genes of porcine G12 strain RU172 exhibited genetic relatedness to human Wa-like G12 strains. Although the origin of human G12 genotype remains obscure, some recent studies on human G12 rotaviruses suggested that the G12 genotype might be of porcine origin. To determine the true origin of G12 rotaviruses and decipher the exact genetic relatedness between human and porcine G12 strains, we characterized genetically the remaining six genes (VP1-3 and NSP1-3) of porcine G12 strain RU172.

**Methods:** The VP1-3 and NSP1-3 gene sequences of porcine G12 strain RU172 were obtained by RT-PCR and direct sequencing using end primers and several internal primers, designed from conserved stretches of several published sequences.

**Results:** The VP1-3 and NSP1-3 genes of porcine G12 strain RU172 exhibited high sequence identities to Wa-like porcine and human strains, including human G12 strains, and by phylogenetic analyses, clustered within the Wa genogroup along with human Wa-like G12 strains.

**Conclusion:** Wa-like human and porcine group A rotaviruses are believed to be genetically related and have a common origin. Therefore, based on full genome analyses of porcine G12 strain RU172 and human Wa-like G12 strains, we propose that the Wa-like human G12 strains might have resulted from reassortment events involving Wa-like human non-G12 and porcine G12 strains, or more favorably, both the porcine and human Wa-like G12 strains might have evolved from a common progenitor, maybe of porcine origin. The AU-1-like and DS-1-like G12 strains might be the result of reassortment events involving non-G12 strains of these genogroups and human Wa-like G12 strains. Therefore, the present study deciphers the probable origin of human G12 genotype, and provides evidence for porcine-human transmission of rotaviruses.

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**Type: Poster Presentation**

Veterinary practitioners and the spread of infectious diseases

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**Background:** There is an increasing overlapping among livestock, pets and human beings, highlighting the need for a well defined biosecurity plan to reduce the opportunity for infectious agents to gain access to and spread within a veterinary premises or any other animal housing unit. And the recent outbreaks of infectious diseases around the world have clearly shown the threats to human and animal health arising from emerging and re-emerging infectious disease, a fact which has huge economic and public health implications.

**Methods:** In order to provide the best veterinary care possible, veterinarians have to redefine their underlying responsibility to minimize the risk of additional; harm that might unintentionally befall a patient because of their interventions.

**Results:** This Paper focused on an investigation to determine the possible roles of veterinary practitioners in the spread of infectious diseases.

**Conclusion:** This paper focused on an investigation to determine the possible roles of veterinary practitioners in the spread of infectious diseases, discusses the need for biosecurity programs in veterinary practices, and relates a practical approach for developing biosecurity practices that are tailored to individual facilities, to help ensure that veterinary practitioners retain their role in the control rather than the spread of infections.

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Trypanosome infections in dogs from Chagas disease endemic regions in Panama, Central America

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**Background:** Chagas Disease remains a major parasitic zoonosis in Latin America affecting 9.8 to 11 million people. The infection is caused by *Trypanosoma cruzi*, a protozoan naturally transmitted to mammals, including humans, by triatomines. In endemic areas, humans and animals become mainly infected through contact with parasite-infected excreta from triatomines. The sylvatic triatomine, *Rhodnius pallescens* is considered the main vector of *T. cruzi* and *T. rangeli* in Panama. In many countries, such as Panama, non-domiciliated vectors remain responsible for a significant transmission risk and their control poses a challenge for disease control. Dogs are important reservoirs of the disease in the domestic transmission, and due to the close proximity with humans they may represent a high risk to humans. However, the role of dogs as reservoirs and as risk factor for human transmission in the peridomestic and/or sylvatic habitats has only been partially explored. Consequently we evaluate the prevalence of canine trypanosomiasis rural endemic communities where the non-domiciliated *R. pallescens* is responsible for *T. cruzi* transmission to humans.

**Methods:** During 2007, a cross-sectional study was designed to evaluate the presence of anti-*T. cruzi* antibodies and blood trypanosomes in dogs from the rural communities of Las Pavas and Lagartera Grande in Central Panama. A questionnaire was applied to the dog owners to assess epidemiological data and risk factors associated with the disease.

**Results:** Of the 94 dogs analyzed, 51 were male and 43 females. The mean age for both males and females was 3.6 years (range 4 months – 15 years). Serological and parasitological tests revealed that 12 dogs (12.8%) were trypanosome infected (Table 1). Nine dogs (9.6%) had antibodies against *T. cruzi*. Trypanosomes were isolated in three (5.3%) hemoculture samples. Molecular analysis showed that isolated trypanosomes were *T. rangeli*. None of these *T. rangeli* positive dogs had detectable antibodies against *T. cruzi*. Four infected dogs belong to people with Chagas disease diagnosis.

**Conclusion:** In conclusion our data demonstrate that dogs are frequently infected with Trypanosomes in this area of Panama with a prevalence similar to the one observed in the human population. This study improves our understanding of the epidemiology and control of Chagas disease in rural areas of central Panama.

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**Type: Poster Presentation**

Molecular evidence of genetic diversity of *Borrelia burgdorferi* sensu lato detected in *Ixodes granulatus* ticks removed from rodents in Taiwan

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**Background:** Genetic diversity of *Borrelia* spirochetes in *Ixodes granulatus* ticks of Taiwan remains unknown and needs further identified.

**Methods:** A general survey was conducted to collect *I. granulatus* ticks removed from trapped rodents in Taiwan. Total genomic DNA was extracted from individual tick specimen by using DNeasy Blood & Tissue Kit (Qiagen). Genetic identities of *Borrelia* spirochetes detected in *I. granulatus* ticks were determined by analyzing the gene sequences amplified by a nested polymerase chain reaction (PCR) assay based on the 5S-23S intergenic spacer amplicon gene of *B. burgdorferi* sensu lato. Phylogenetic relationships of these detected spirochetes were further analyzed by neighbour-joining (NJ) compared with maximum parsimony (MP) methods.

**Results:** A total of 261 *I. granulatus* ticks (156 adults and 105 nymphs) were tested by nested-PCR assay and *Borrelia* spirochetes were detected in 80 adults and 52 nymphs with an infection rate of 51.3% and 49.5%, respectively. Phylogenetic analysis reveals that all these detected spirochetes constitute two major separate clades distinct from other *Borrelia* genospecies in both NJ and MP methods. Within the clades, 10 strains of *Borrelia* spirochetes detected in *I. granulatus* ticks were closely related to the genospecies of *B. burgdorferi* sensu stricto and 15 strains of detected spirochetes were closely related to *B. valaisiana*.

**Conclusion:** Our results demonstrate the genetic diversity of *B. burgdorferi* sensu lato spirochetes detected in *I. granulatus* ticks collected in Taiwan. The genetic identities of these detected spirochetes were clarified by analyzing sequence homology of 5S-23S intergenic spacer amplicon gene. Further investigations on *Borrelia* spirochetes detected in variant tick species and reservoir hosts would be beneficial to the better understanding of genetic heterogeneity of *Borrelia* spirochetes in Taiwan.

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**Type: Poster Presentation**

Occurrence of *Ureaplasma diversum* in cows with various reproductive disorders

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**Background:** *Ureaplasma diversum*, a bovine species was first isolated by Taylor Robinson and co-workers in 1967 from cattle. The genital ureaplasmosis in cows occurs in various clinical forms viz. urethritis, endometritis, salpingitis, granular vulvovaginitis, abortion and neonatal calf mortality leading to temporary or permanent infertility.

**Methods:** During present study, a mycoplasmological examination of cervico-vaginal swabs/vaginal discharges from 136 cows including 86 with various reproductive disorders (22 anoestrus, 25 repeat breeder, 6 cervicitis, 17 metritis, 16 abortion/still-birth) and 50 apparently healthy cows was conducted. The U-9B liquid medium was used for isolation of *Ureaplasmas*. *In-vitro* antibiotic sensitivity of ureaplasma isolates against ten selected antibiotics was performed at first stage of their cultivation in U-9B colour test liquid medium.

**Results:** The mycoplasmological examination of cervico-vaginal swabs/vaginal discharges from 136 cows including 86 with various reproductive disorders and 50 apparently healthy cows resulted in isolation of 14 *Ureaplasma* species along with 11 *Mycoplasma* and 8 *Acholeplasma*. The incidence of mollicutes was found higher in genitally diseased cows (92.907%) as compared to apparently healthy cows (16%). The prevalence of *Ureaplasma diversum* was more in repeat breed cow (20%) than anoestrus (9.8%), cervicitis and metritis (4.9%). However, no *Ureaplasma* strain was isolated from abortion cases. The concentration of nine strains of *Ureaplasma* isolated from cows with various reproductive disorders and 5 from apparently healthy ranged between 5x10<sup>2</sup> to 5x10<sup>4</sup>ccu/ml.

All the test strains of *Ureaplasma*, were found sensitive for lincospectin and resistant to ampicillin. However, variable resistance was shown by 6 isolates to tetracycline, 4 isolates to enrofloxacin, spiramycin and chloramphenicol, 3 isolates to tylosin and erythromycin and one isolate to tiamutin and sparfloxacin.

**Conclusion:** All the fourteen strains of *Ureaplasma* isolated from cows with various reproductive disorders show multiple drug resistance against tested antibiotics.

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**Type: Poster Presentation**

One health: Collaboration, recent research and developments in the global effort to eliminate Rabies

**R. DEDMON**<sup>1</sup>, D. Briggs<sup>2</sup>, T. Lembo<sup>3</sup>, S. Cleaveland<sup>3</sup>

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**Background:** Rabies causes 55,000 deaths annually, half of which are in children less than 15 years of age, and has the highest mortality rate of any infectious disease. It is both preventable and controllable. Economic impact of rabies in developing countries in Asia and Africa, where over 98% of all human rabies deaths occur from exposure to canine rabies, rises each year. Estimated annual human rabies prevention costs are about \$800 million U.S. Even so, continuing vaccine and RIG shortages in countries with the highest incidence of rabies continue unabated, leaving the most vulnerable at greatest risk. New lyssavirus genotypes continue to be discovered in bats in Asia and Africa-needing continuing widespread surveillance globally. Burden of disease and suffering cannot be over-stated. Rabies victims in developing countries are often sent home to die, due to lack of treatment facilities and fear of the disease. Additionally, lack of surveillance and adequate control measures have enabled rabies spread to previously rabies-free areas (e.g recent outbreak in Bali leading to human rabies deaths).

**Methods:** Literature review-Mesh rabies, monoclonals, vaccine, epidemiology, surveillance Meeting abstracts 2008-09

**Results:** Recent advances in rabies prevention and control strategies: 1. New vaccine both for humans and animals. Molecular biology has brought new understanding to lyssavirus evolution, to possible new reduced vaccine schedules, and to an effective substitute for equine or human rabies immunoglobulin through the development of promising monoclonal antibodies now in clinical trials. Oral animal vaccines have been developed using human adenovirus as the rabies G-Protein carrier. 2. Diagnosis of rabies in the field has been made possible via the dRIT light microscope immunohistochemical test. 3. GIS systems and computer modeling have improved rabies surveillance. 4. Partners for Rabies Control has developed an action blueprint for controlling rabies in developing countries. 5. Substantial funding from Bill and Melinda Gates Foundation is targeted to studies of dog rabies elimination in Tanzania, KwaZulu-Natal, and the Philippines. 6. A registered charity in the U.K. and U.S., the Alliance for Rabies Control, has become a beacon for rabies education and prevention.

**Conclusion:** Rabies is both preventable and controllable-many challenges, but brighter future!

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**Session: Zoonoses and Infectious in Animals**  
**Date: Wednesday, March 10, 2010**  
**Time: 12:30-13:30**  
**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Epizootiology of foot and mouth disease in Nepal

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**Background:** Foot and Mouth Disease (FMD) is endemic in Nepal. The details of epizootiology of FMD in Nepal were set out in this work. We studied epizootiology of the disease for the prevention and control strategies of FMD for the veterinary service, taking into account the peculiarities of the country.

**Methods:** We studied the spread of FMD in animals of different species by seasons of the year, regions and ecozones, types, and virus serotypes. The work was carried out using monthly epidemiological reports on the disease from 75 districts to Veterinary Epidemiology Centre, Directorate of Animal Health, Kathmandu, Nepal from 2000 to 2007. The results were processed and analyzed with the use of the computer program Microsoft Excel Programme.

**Results:** FMD was ranked first in terms of the number of outbreaks, the number of affected and dead animals in the structure of the major infectious and invasive diseases in Nepal. The predominant serotypes responsible for epidemic outbreaks of FMD in Nepal are *O*, *Asia 1* and *A*, which are identical to other countries in South Asia. Cattle and buffaloes are the most susceptible animals to FMD in Nepal, whereas goats and sheep are relatively less susceptible. Hill and Terai (Plain) ecozones of Nepal are the most stressful areas and persistent disadvantage for the disease. The most vulnerable to the disease are the regions of the Far-west and Central. Although the outbreak of FMD is reported all the year round, high incidence of FMD is noticed twice a year: in April-June and December (the movement of animals in previous religious activities).

**Conclusion:** On the basis of our study, we recommend to launch 100% vaccination of susceptible animals at first in Far-western development region adding other regions in the next years using trivalent vaccine, containing virus serotypes *O*, *A* and *Asia-1* to acquire the herd immunity for successful FMD planning. Expeditionary activities, pre-vaccination campaign, increase monitoring of veterinary regulations, disinfection, immediate isolation and vaccination of animals, quarantine, and creation of normative-legal provision for the implementation of activities, seminars and training of veterinary professionals are important in Nepal to implement prevention and control strategies of FMD.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

The micro-adenomatous lesions associated with *Lawsonia intracellularis* in the pig intestine

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**Background:** *Lawsonia intracellularis* orally infects and causes marked hyperplasia of enterocytes in pigs. The infected intestinal wall makes thickening remarkable. This disease is called porcine proliferative enteropathy (PPE) or porcine intestinal adenomatosis(PIA). *L. intracellularis* spreads all over the world and that the microbes were infected with pigs of a lot of farms have been reported. The characteristic pathological lesion of PIA is well known, but the pathogenesis mechanism is not clear. In this study, the localization of *L. intracellularis* and the mucosal lesions were investigated on the intestine with onset or healthy pigs.

**Methods:** The histopathological examination of the intestines of the 25 poor-growth piglets and 67 healthy pigs at the meat inspection station were examined by Hematoxylin-Eosin staining, Warthin-Starry(WS) staining and immunohistochemical(IHC) method used the anti-*L. intracellularis* antibody. In addition, it was examined detection of a specific gene of *L. intracellularis* by PCR method about an intestinal frozen-, a formalin- and a paraffin-specimen.

**Results:** In necropsy, no thickened intestinal wall was found in 25 poor-growth piglets, however, in two of them, the typical PIA histological lesions were found in from the jejunum to the rectum. These cases were diagnosed as atypical PIA. In addition, in the other two of them, an island-shaped micro-PIA lesion was distributed in the intestinal mucosa. The comma-shaped small bacteria were observed by WS staining, and the antigens of *L. intracellularis* were detected with IHC methods in the enterocytes of the micro-adenomatous lesions. The antigen of *L. intracellularis* was also detected in the intact superficial enterocytes. In 67 healthy pigs, the thickened intestinal wall was not found macroscopically. However, the focal adenomatous lesions with clear boundaries were observed to three pigs in them. A specific gene of *L. intracellularis* was detected by PCR method in the intestinal frozen-, the formalin- and the paraffin-specimen.

**Conclusion:** In this study, it was confirmed that there was atypical PIA, and the island-shaped or the focal adenomatous lesions were also distributed in a normal intestine macroscopically. The microlesions was suggested on the stage of an early infection or the subclinical infection with *L. intracellularis*.

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**Type: Poster Presentation**

Emerging and zoonotic disease risk mitigation: Rabies prevention as a template for best practices

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**Background:** Like many zoonotic and emerging diseases, rabies prevention requires the cooperation of animal control, law enforcement, natural resource personnel, veterinarians, diagnosticians, public health professionals, physicians, and other professionals. Despite the forced extinction of dog-to-dog types of rabies viruses in most of Europe and the Americas, the recent translocation of dogs from Puerto Rico, Thailand, India, and Iraq, which developed rabies from their places of origin upon movement into the United States, demonstrates the risk of human travel and movement of animals, some of which can be mitigated through carefully crafted requirements. While the methods for measuring immunity to rabies and for diagnosis are powerful, they include some limitations innate to biological assays.

**Methods:** For example, we report the results of an inter-laboratory comparison of rabies serology by Kansas State University (KSU) and the New York State Department of Health (NYSDOH). Of 90 samples presented blinded, 37 were from rabies vaccinated individuals and 53 were from patients sero-positive for other pathogens.

**Results:** Among sera from vaccinated persons, 87% yielded concordant results of  $\geq 0.50$  IU/ml. A discordant result occurred in 5 samples at or near 0.50 IU/ml. Among 53 samples from non-rabies-vaccinated humans, a low level of neutralizing activity was reported by NYSDOH in 21 samples and by KSU in 9 samples, most likely indicative of nonspecific antiviral activity or cross-reactive antibodies.

**Conclusion:** There remains a need for proficiency testing and advancement of quality control practices to optimize human and animal rabies diagnostic and serological practices. Although rabies excites the imagination, current vulnerabilities include the potential for re-introduction of dog-to-dog transmitted rabies, a decline in diagnostic expertise and capacity, commercial enterprises answering a perceived need for diagnosis and serology but with limitations in test accuracy and specificity, and a lack of basic research, especially to understand recent advances towards treatment of clinical rabies. As a global community with rapid and high volume exchange of animate beings and inanimate products, diligent attention and dedicated effort will be required to maintain, and indeed, even advance, emerging and zoonotic disease control, with rabies as a tangible "best-practices" template, beyond major advances of the last half-century.

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**Date: Wednesday, March 10, 2010**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Identification and characterization of Shiga toxin-producing *Escherichia coli* (STEC) isolated from cattle, sheep and meat samples in Tehran Province, Iran

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**Background:** Shiga toxin producing *Escherichia coli* (STEC) has been associated with hemolytic uremic syndrome, outbreaks of diarrhea and hemorrhagic colitis in human. Infection is mainly acquired by ingestion of contaminated food. In Iran, STEC strains have been frequently isolated from cattle and humans. This study aimed at the assessment of the distribution, virulence gene profile and phenotypes of STEC strains isolated from dairy cows and sheep feces and from raw meat samples in Tehran province.

**Methods:** A total of 326 samples, including 120 from cattle feces sampled in three farms, 102 sheep feces and 104 meat samples from slaughtered cattle in Tehran's abattoir, were assayed for *stx1*, *stx2*, *eae* and *α-hly* genes by PCR. STEC isolates were further characterized for their O and H serotypes by slide agglutination assay.

**Results:** STEC strains were isolated from 67.5% (81 out of 120) of the cattle stool samples, 83.3% (85 out of 102) of the sheep feces and 49.1% (51 out of 104) of the meat samples. 52.5% STEC strains isolated from sheep and 36.9% of the cattle isolates possessed the *stx*-coding and *eae* genes. *α-hly*-coding gene was observed in 13.5% of STEC isolates. *E. coli* O157:H7 was detected in nine (4.1%) of the bovine samples (including both faecal and meat samples). The most commonly isolated STEC serogroups were O146, O112a and O44 in meat samples and O127a, O142 and O1 in bovine feces. No *E. coli* strains belonging to the classical pathogenic STEC serotypes, such as O145, O111, O103 and O26 were identified.

**Conclusion:** The present study confirms the potential of sheep and cattle to serve as a reservoir for potentially virulent STEC strains in Iran. The observation that the most of the STEC strains isolated in study harboring the *stx* and *eae* genes belonged to unusual serogroups may reflect differences in the epidemiology of STEC infection in Iran. This finding, should it be confirmed by clinical investigations aiming at defining their role as causative agent of diarrhoea and/or HUS, may influence the strategies to be adopted for the diagnosis and control of STEC infections in Iran.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Brucellosis – Emerging zoonosis in Bosnia and Herzegovina

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**Background:** Brucellosis is the most widespread zoonosis, that affects more than a half million people per year, all over the world. Causative agent is Brucella species, domestic and wild animals are reservoirs, and it affects mostly humans. The disease appeared in Bosnia and Herzegovina right after the war 1992-1995, and ever since then it has been constantly spreading among animals and humans, with no signs of reduction in number of infected and affected. The aim of this work is to analyze cases of human brucellosis hospitalised in Bosnian hospitals for the period 2000-2009. Another aim is to encourage better cooperation between human, animal medicine and local authorities, in order to utilize all resources to combat and control of brucellosis in the most efficient way.

**Methods:** We did a retrospective study of brucellosis files from three clinics and four departments for infectious diseases from both Bosnian entities (Federation of B&H and Republic of Srpska). Diagnosis was confirmed either by ELISA and Rose-Bengal test or by isolation of Brucella species in blood.

**Results:** In period 2000-2006 brucellosis was spreading through all the country slowly becoming continuing epidemic. There were 305 cases registered in Federation of B&H, and 5 cases in Republic of Srpska. There were registered 1741 cases for the period 2006-2009 in Bosnia and Herzegovina, and 283 of them were registered in Republic of Srpska.

**Conclusion:** Brucellosis has become an emerging zoonosis in Bosnia and Herzegovina. First cases were registered after the war in Bosnia (1992-1995). It has become a continuous infection. For the past ten years it has become an endemic disease. Brucellosis cannot be controlled due to administrative borders and lack of political will. Scientific teams must be founded in order to make a national program for treatment and control of the disease.

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**Session: Zoonoses and Infectious in Animals**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Prevalence ectoparasites in dogs of beaches and fields fishing of Navolato, Sinaloa, Mexico

**M. C. Rubio Robles**, S. M. Gaxiola Camacho, N. Castro del C., J. Gaxiola M.

Universidad Autonoma de Sinaloa, Culiacan, Sinaloa, Mexico

**Background:** Fleas are a nuisance to humans and their pets, can cause medical problems including flea allergy dermatitis, secondary skin irritations, in extreme cases, anemia. Although bites are rarely felt, it is the resulting irritation caused by the flea salivary secretions that varies among individuals. Some may witness a severe reaction (general rash or inflammation) resulting in secondary infections caused by scratching the irritated skin area. Also, may transmit Tapeworms (common infection is *Dipylidium caninum*) normally infest dogs and cats but may appear in children if parts of infested fleas are accidentally consumed. The ticks are painful and sometimes very dangerous. These blood sucking insects feed on all vertebrates but are particularly skin to dogs and cats as they tend to enjoy the same places that pets. They can be found infesting lawn and garden, and can transmit diseases how Lyme disease, Rocky Mountain Spotted Fever, Typhus, Rickettsial Pox, Tularemia, Babesia and Anaplasma. Generally, these different diseases are unique to different ticks which carry causal organisms of these such diseases and can be confined to certain regional areas.. The objective of this work was to determine the prevalence of ticks and fleas in dogs of beaches and fields fishing of Navolato, Sinaloa, Mexico

**Methods:** The samples were determined for a representative sample with both sexes and cradle described by the technique of Thrusfield (2005) was used:  $n = [t * SD / L]^2$ . Where n = sample size, t = value of the normal distribution (Student t) for a 95% confidence level (t = 1.96), L = accepted error or precision (5%), and SD = weighted disease prevalence (%). With This technique, the number of animals determined for random sampling was 242; collected into identified plastic bags; and observed at microscope

**Results:** 194 (80.16 %) positive to ticks; and 211 (79.92 %) positive to fleas *Ctenocephalides spp.*

**Conclusion:** Is considerable number of positives animals and to continue in the present conditions, this problem can take importance in the society, because frequently these dogs are on different points from the town and can pass the infection of other healthy animals, visitors and even the same family

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**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Phenotypical and genotypical traits of *Listeria monocytogenes* strains isolated from tonsils of wild boars hunted in Switzerland

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<sup>1</sup>Institute of Food Hygiene and Technology, Veterinary Faculty, Oberschleißheim, Germany,

<sup>2</sup>Vetsuisse Faculty, Institute for Food Safety and Hygiene, Zurich, Switzerland, <sup>3</sup>Veterinary Affairs Service (SCAV), Geneva, Switzerland

**Background:** During the last decades, wild boar populations have increased in Europe and spread over the entire continent. The population densities of wild boars in Switzerland are among the highest reported in Western Europe. High wild boar densities and increasing popularity of outdoor ranging of fattening pigs may intensify the risk of contacts between wild boars and domestic pigs and, therefore, the transmission of microorganisms and parasites. With this background and with the background of increasing per capita consumption of wild boar meat and the high per capita consumption of pork, knowledge of the situation of the food-borne pathogens circulating in the wildlife population is an important public health issue.

**Methods:** In this study, tonsils of 153 wild boars hunted in the Western part of Switzerland were enriched overnight in TSB and screened on the presence of *L. monocytogenes* using VIDAS®. Positive samples were cultured on two selective agar media. Presumptive positive colonies were biochemically identified. *L. monocytogenes* strains were further characterized by serotyping and genotyping methods.

**Results:** *L. monocytogenes* are food-borne pathogens that are distributed in a wide variety of environments. Human infection may lead to a serious and potentially life threatening illness known as listeriosis. Reports from the USA show that *L. monocytogenes* infections are responsible for the highest hospitalization rates (91%) amongst known food-borne pathogens. *L. monocytogenes* were isolated from tonsils of twenty-six (17%) animals. Of the 26 isolates, 45% were of serotype 4b, 38% were of serotype 1/2a and 17% were of serotype 1/2b. Epidemiological data from different countries shows that the majority of human infection outbreaks are primarily associated with three *L. monocytogenes* serotypes (1/2a, 1/2b and 4b), despite the fact that there are 13 serotypes potentially capable of infecting humans. Further genotyping results of the isolated strains will be presented.

**Conclusion:** This study shows, that tonsils of wild boars are not only a reservoir for *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, but also for *Listeria monocytogenes*. This has mainly to be considered by the hunters, who may be handling carcasses under minimal hygiene conditions. Moreover, outside rearing of domestic pigs and close contact to wild boars may increase the risk of transmission.

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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

The "One Health" Initiative: Using open source data for disease surveillance

**M. Greene**

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**Background:** We have created a self-organizing ontology that allows us to organize and filter external data by relating email exchanges through their references into "reference-connected sets". These sets of messages can be generated dynamically and constructed in real-time to identify subject categories (e.g. disease outbreaks) as they evolve. This allows the filtering of less important information into sets of messages that uniquely identify events so the user is not overwhelmed with irrelevant information. .

**Methods:** This paper describes the approach as it is applied to ProMEDmail, an Internet-based system dedicated to rapid global dissemination of information on infectious diseases. This official program of the International Society of Infectious Diseases has the largest reporting base of any health organization and can be used as a model for a bio-threat surveillance system that takes advantage of decentralized, Internet-based social networks.

**Results:** The presenter will demonstrate how the technique was used in an analysis of the evolution of pandemic influenza messages. The presenter will also discuss the benefits of expanding the approach through new science and technology solutions to increase global health security.

**Conclusion:** A medical informatics solution to surveillance of outbreaks of zoonotic diseases can be achieved through user-friendly graphic interfaces; automated extraction and formatting of data; expert systems for epidemiologic analysis; powerful algorithms for data and information fusion; interconnection with other networks (public health agencies, hospitals, animal/zoonotic disease surveillance); standard forms through an internet.

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**Type: Poster Presentation**

Assessment of *Brucella melitensis* disease burden in lactating goats in Mizque, Bolivia

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**Background:** Brucellosis is a zoonotic disease that can be transmitted from goats to people by direct contact or through ingestion of unpasteurized dairy products. In Latin America, where goats are a significant part of agriculture, human cases of brucellosis are reported in Mexico, Argentina, and Peru. In Bolivia, human cases of brucellosis have been described, but there is minimal epidemiologic knowledge. The objective of this study was to assess *Brucella melitensis* disease burden in lactating goats in Mizque, Bolivia.

**Methods:** Milk and blood samples were collected from 229 lactating goats on 26 farms in Mizque, Bolivia, an agricultural town 100km from Cochabamba, where human cases of brucellosis have been described. Herds, and goats within herds, were selected via convenience sample. Efforts were made to minimize selection bias. In herds with 16 or fewer lactating goats, all goats were sampled, otherwise, one-third of the lactating goats were sampled. Information from each herd and animal was collected by survey. Milk was analyzed via culture. Serum was analyzed for antibodies using the Rose Bengal plate test and the Lateral Flow Assay.

**Results:** Median herd size was 33 adult goats (range: 10-150). The average reported age of the animals sampled was 3.4 years (SD:  $\pm 1.5$ ). None of the animals sampled had a reported history of vaccination against *Brucella melitensis*. 20 (8.7%) goats sampled from 13 (50%) farms had a reported history of abortion. Of the 229 animals sampled, 0 had positive milk culture and serology results (95% CI = 0 – 1.6%).

**Conclusion:** This region of Bolivia may be free from disease, or may have disease prevalence too low to be detected by the sample size. Given the tendency for disease to cluster within herds, the high disease prevalence in bordering countries, and lack of disease monitoring within Bolivia, the presence of *Brucella melitensis* in the Bolivian goat population cannot be ruled out. Larger studies conducted in multiple geographic regions of the country are warranted. For example, assuming 100% sensitivity of the tests, a population of 2500 animals, and desiring 95% certainty, the maximum possible prevalence is 1.3% even after finding 0 of 229 positive in this sample.

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**Type: Poster Presentation**

Analysis of circulation influenza virus A on the Chany Lake (Novosibirsk region, Russia) in 2008 year

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**Background:** The natural host species of type A Influenza viruses are wild-waterfowl and shorebirds. The virus easily transfers to the domestic birds, which congestion promotes spread of the infection. Birds migrations assist spread of various variants of virus on the huge territories. Monitoring and studying genetic, antigenic and pathogenic properties reveal to estimate zoonotic danger of such variants. Biological material was collected from captured free-flying birds on the Chany Lake in 2008 year. This is a territory of birds population from Africa, Average and Southern Asia and Europe during nested periods and migration flights.

**Methods:** Viruses were obtained from cloacal swab material. Avian influenza was isolated by virus isolation methods in embryonating chicken eggs. chorioallantonic fluid from the infected embryos was used as the virus source. RNA was extracted with SV Total RNA Isolation System () in accordance with manufactures instructions and transcribed into cDNA for a subset of samples. In RT-PCR subtype-specific primers were used. Sequence analyses of HA and NA genes were obtained. For each data set, sequence alignments were created using CLUSTAL. Phylogenetic trees were constructed using MEGA 4.1

**Results:** The virus was detected in 12 probes out of 255. Subtypes of isolates were identified. After making phylogenetic analysis homology of isolates from the Chany Lake with strains circulating in Mongolia, Sweden, Germany and Hokkaido was shown.

**Conclusion:** Our findings indicate of facility influenza A virus's transmission on the long distance by wild birds of passage.

This work was supported by Russian Government and Bio Industry Initiative (BII), USA (ISTC#3436) and was done in collaboration with the Novosibirsk State University.

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**Session: Zoonoses and Infectious in Animals**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Epidemiological study on infectious bovine rhinotracheitis in cattle

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**Background:** The study was conducted on 5 farms belong to the General organisation of Cattle (GOC) in Syria for epidemiological & Sero investigation of infectious bovine rhinotracheitis (IBR) .

**Methods:** Data was collected from individual farm included animal characteristics and farm specifications.

Random blood samples were collected from individual animal from the farms study.

ELISA test was used to test all samples to detect positive antibodies LPEC model Program was used to estimate the economic losses of the disease

**Results:** The sero prevalence of the disease was ranged between 10-20 percent in the study farms. It was carried out an quantitative epidemiological and economic study to evaluation the epidemiology of the disease and economic losses caused by the disease . It was confirmed that there were strength significant association between the occurrence of the disease and advance ages of animals and increased the size of herd in individual farms. The economic losses resulted of the occurrence of the disease were estimated as individual level for a cow and generally on the farm level. The sero prevalence of the disease was ranged between 10-20 percent in the study farms. It was carried out an quantitative epidemiological and economic study to evaluation the epidemiology of the disease and economic losses caused by the disease . It was confirmed that there were strength significant association between the occurrence of the disease and advance ages of animals and increased the size of herd in individual farms. The economic losses resulted of the occurrence of the disease were estimated as individual level for a cow and generally on the farm level.

**Conclusion:** The study was concluded that there was an increase in the seroprevalence in advance ages in all regions study, this was because there was no vaccination program in all regions study. Biosecurity procedures should be taken in consideration in order to decrease the disease occurrence. The economic losses resulted from the disease reached to 4000 US\$ at the farm level and about 100,000 US\$ at the GOC level.

Control strategies should be carried out in order to prevent the high prevalence level of the disease

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Clinical manifestations of leptospirosis

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**Background:** Leptospirosis is an acute generalized infectious disease which belongs to the zoonoses group. Leptospirosis mainly causes interstitial nephritis in animals, but people accidentally get infected in a direct or indirect contact with the urine of the infected animal. Our goal was to display the clinical manifestations in 150 hospitalized patients, treated at the Clinic for infectious diseases in Novi Sad during the past ten years.

**Methods:** A retrospective study of the clinical manifestations in patients diagnosed with leptospirosis, hospitalized on the Clinic for infectious diseases from the year 1999 to year 2000.

**Results:** Non icterous form of leptospirosis was found in 21,33% (32/150) patients; high body temperature with a generalized infectious syndrome was present in 43,75%; 8,33% of the patients had a high body temperature followed with severe hemolitical anemia; aseptic meningitis was present in 28,1%; gastroenteritis in 15,6%; bronchopneumonia in 6,25%; nephritis in 6,25% and pneumonia, aseptic meningitis and gastrointestinal bleeding joined simultaneously in 8,36% of the patients. The most severe form of leptospirosis, Weil's syndrome, was present in 78.6% (118/150) patients. 100% of the patients with Weil's syndrome had renal failure; 50% hemorrhagic syndrome; 45,8% neurological disorders (38,7% meningitis, 5.0% meningoencephalitis, 1,6% convulsions, 0,8% poliradiculitis); 43,3% atypical pneumonia; 21,2% gastrointestinal symptoms, 15,2% rash; 8,4% cardiovascular disorders; 6,7% sight impairment and 3,3% pancreatitis. In patients with a non icterous form of leptospirosis, no lethal outcome has been noted. Among the patients with Weil's syndrome the mortality was 16,1% (19) and 65% among the elderly patients over 60 years.

**Conclusion:** Clinical manifestations vary among the patients, from easy forms to severe multi organ forms of leptospirosis. The highest impact on the prognosis of the disease has the age of the patients, co morbidity ( alcoholism ), early corticosteroid therapy in patients with pulmonary symptomatology and early haemodialysis in patients with renal failure.

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**Type: Poster Presentation**

Epidemiology of avian influenza viruses in wild birds in Mongolia

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**Background:** A combination of geographical features makes Mongolia an ideal location for understanding the epidemiology of avian influenza viruses in wild birds. There are four main migration routes (*East-Asia/Australasia, Central-Asia/India, West-Asia/Africa and Mediterranean/Black-sea*) passing through Mongolia. Around 391 species of migratory birds arrive in Mongolia. Mongolia also represents an important site for molting of Anseriformes.

**Methods:** Since 2006, we have collected 3950 feces samples and 150 tissue samples from wild birds during the active surveillance in Mongolia.

Viruses isolated by the egg inoculation method, and RT-PCR, qRT-PCR and Neuraminidase inhibition test were used for subtyping.

The confirmation of the isolated highly pathogenic avian influenza virus (HPAIV), and intravenous pathogenicity index (IVPI), phylogenetic analysis were performed by the OIE Reference Laboratory for HPAI at Hokkaido University, Japan.

The confirmation of the low pathogenic avian influenza virus (LPAIV) isolates and phylogenetic analysis were carried out in the National Veterinary Research and Quarantine Service of Korea.

**Results:** In 2005 and 2006, 2 HPAIV (H5N1) were isolated from wild birds (*whooper-swan, bar-headed-goose and common-goldeneye*) at Erkhel and Khunt Lakes. These viruses were classified into the clade 2.2 so-called Qinghai Lake-type.

In May and August, 2009, 2 HPAIV (H5N1) were isolated from wild birds (*whooper-swan and bar-headed goose*) at Doitiin tsagaan and Duruu Lakes. These viruses were classified into the clade 2.3.2. The IVPI was high /2.97-3.00/.

Also we isolated 21 LPAIVs in 2007 and 2008. The subtypes were H3N8(11), H4N6(4), H7N7, H7N9, H3N1, H3N2, H4N2 and H10N6. The viruses were related with the Euro-Asia lineage.

In 2009, 7 LPAIVs were isolated from wild birds (*whooper-swan, ruddy-shelduck, Mongolian-gull, mallard and gadwall*) in Central and Eastern Provinces. The subtypes were H3N8(3), H10N6(3) and H4N6.

**Conclusion:** We isolated 4 HPAIVs and 28 LPAIVs from wild birds in Mongolia genetically related to the Euro-Asian AIVs. All HPAI outbreaks were restricted to the wild birds in north-central Mongolia.

The phylogenetic differences of the H5N1 isolates from 2005, 2006 and 2009 indicate that the role of the migratory birds in Mongolia in the AIV mutation should be clarified.

Therefore, it is necessary to continue the research on avian influenza in Mongolia.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Virological studies on migratory penguins captured in Brazilian southeast coast

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**Background:** The migratory birds represent the main vector of Orthomyxovirus and Paramyxovirus in nature, spread among birds in temperate regions. They have been reported circulating among Antarctic penguins through serological surveys, especially in Adelie penguins (*Pygoscelis adeliae*) of eastern Antarctica. The Magellanic penguins (*Spheniscus magellanicus*) are distributed on the southern shores of South America. Their breeding colonies were distributed from the coast of Chile to the Peninsula Valdez in Argentina, on the southern tip of South America. This penguin performs migration to the north, and such shift is an annual event considered characteristic of the species. The birds breed in colonies north of its distribution, as in the southern coast of Brazil, where they stay in the winter. Some individuals have demonstrated irregular movements that may occur with or in large numbers, causing errant individuals. It is believed that for this reason this species have reached the Northeast of Brazil in 2008 and made an unusual situation.

**Methods:** We analyzed the presence of Orthomyxovirus and Paramyxoviruses in penguins captured on the coast of the Espírito Santo state, Brazil, due to the increasing number of species in the region in 2008. Were inoculated into the allantoic cavity embryonated fowl eggs suspensions of 73 cloacal swabs taken from live migratory penguins at Brazilian Southeast Coast, between September - October 2008. The allantoic fluids were tested for haemagglutination activity (HA). In samples with positive HA, we performed hemagglutination inhibition (HI) test against antibodies to H1N1, H2N2, H3N8, H7N7, and Newcastle disease virus (NDV). Neuraminidase activity (NA) assay was performed to characterized the isolates. Were performed a RT-PCR to protein M of Influenzavirus and L protein of broadly range of Paramyxovirus.

Sample	FMP (ua)	FMI (ua)	T1
NDV (+)	18394,46	444,89	41,34
56	23659,12	1273,81	18,57
60	25118,95	1893,23	13,26
64	25248,39	1343,51	18,79
66	25018,11	610,98	40,94
87	26148,81	2500	10,45
119	23635,86	1494,16	15,81
129	26903,40	1890	14,23
439	25262,89	818,95	30,84

**NA activity and inhibition by DANA of penguins isolates.**

**Results:** Our results shown that 9 samples (12%), were positive by haemagglutination test, but no inhibition by influenza sera observed. Partial inhibition by NDV serum was observed in all samples. All samples presents NA activity. All samples amplified L protein gene of Paramyxovirus (Avulavirus), demonstrating a strong band of 500 bp on agarose gel.

**Conclusion:** Our results shown that Avulavirus is present on these birds and may cause diseases on this species, contributing to clinical deterioration of the animals.

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**Session: Zoonoses and Infectious in Animals**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Poultry farmers' response to AI outbreak and its control in Indonesia

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**Background:** AI outbreak in Indonesia had started since August 2003 up to now. GOI continuously try to control it by introducing 9 strategies on bio-security policies, for instance, vaccination, depopulation, transportation and trading of poultry products. Poultry industry development provides employment for villagers and it is very important for low income villagers, in particular poultry rearers in sector IV.

**Methods:** In order to observe socio economic impacts of AI outbreak in sector IV, survey had been implemented in three provinces in Indonesia in early 2008.

**Results:** Results indicated that in general farmers did not know the 13 symptoms as overall AI symptoms. Overall only 2.6 percent of the respondents knew all of the AI symptoms. This knowledge was very low and, therefore, they could not deal with AI outbreak properly. Overall, only 25.1 percent of the respondents knew all of the seven items relating with control measures as (i) stamping out, (ii) vaccination, (iii) spraying disinfectant (iv) isolation, (v) ) burning, (vi) compensation and (vii) provide antibiotic, while the rests knew only some of the items. The higher the disease attack the smaller the percentage of the respondents knew all items of AI outbreak prevention. Most farmers conducted vaccination (65.3 percent). Only few smallholders in West Java applied vaccination (27.1 percent). Almost all farmers said that all of the methods of controlling AI outbreak did not give economic benefit. Survey also revealed that location of poultry enterprise is always sticking to and extend according to the pattern of residential areas.

**Conclusion:** In densely populated Java, it is almost impossible to differentiate between areas to rear poultry and residential areas. In one hand, such situation hardly can be excused considering environmental aspects for instance, but on the other hand, poultry in sector IV also need to be developed to provide employment for villagers.

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**Session: Zoonoses and Infectious in Animals**

**Date: Wednesday, March 10, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Cluster of MRSA in cats and staff of a veterinary clinic: Follow-up and possible implications for control

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**Background:** At approximately 1% the prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in the Netherlands is among the lowest in Europe.

Voluntary notification of a veterinarian of 4 successive but unrelated cats with postoperative wound infections with an identical, human MRSA strain prompted this investigation. The prevalence rate of human MRSA carriage in all veterinary staffmembers was measured, and subsequently we wanted to prevent MRSA infections in cats and MRSA carriage in this clinic.

**Methods:** After informed consent all 44 veterinary staffmembers were questioned for MRSA risk factors. Cat case histories were reviewed (Result A). Hygienic procedures were updated (Result B). Staffmembers were screened for MRSA, and positives were treated. Posttreatment cultures were all sampled every 2 weeks during 8 weeks (Result C). A selective broth was used for 24 hours, after which the IDI test was performed. Each positive sample was subcultured on blood agar and an antibiogram was made using the Vitek-2 system (BioMerieux, Lyon, France) or E tests when appropriate. Each detected strain was sent to the national reference laboratory (RIVM) for pulse field gel electrophoresis (PFGE) typing. MRSA carriage was treated using local and systemic antibiotics.

**Results:** Result A: Professionals worked in individual (operation) rooms and had no cattle contact. No one had MRSA risk factors or MRSA infections. One positive professional had controlled skin eczema. The owner of the first cat and his family were MRSA negative. Result B: the updated hygienic protocol included improved hand hygiene, surface cleansing and more extensive use of gloves and masks. Result C: 7 persons (16%) were MRSA positive (nose, throat) with MRSA PFGE type 113 (national typing). Two of 7 professionals spontaneously turned negative. Four were treated successfully, but the last one surprisingly had a positive culture in the second round. She stayed negative thereafter. On 5 cultures additional spa and MLST typing of the strain was performed, matching the results of the four cats (spa type 739, ST 45 with clonal cluster 45).

**Conclusion:** A specific human MRSA cluster in humans and cats was found and successfully treated in humans. This may decrease future new infections in cats. Veterinary clinics should implement guidelines for dealing with MRSA, and be aware of increased risks for contracting MRSA.

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**Session: Zoonoses and Infectious in Animals**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

An exploration of the knowledge, attitudes and perceptions of the local, adult, non-medically trained Grenadian population about certain zoonotic diseases

**S. Bidaisee**

St. George's University, Grenada, St. George's, Grenada

**Background:** Zoonotic diseases represent a leading cause of illness and death from infectious diseases in humans. In the Caribbean generally and in Grenada specifically, to the best of our knowledge, no reports on examining people's knowledge, attitudes and perceptions towards zoonotic diseases have been published. The objective of this research study was to explore the knowledge, attitudes and perceptions of the local, adult, non-medically trained Grenadian population about certain zoonotic diseases.

**Methods:** The study consisted of a quasi-experimental design consisting of 450 participants, selected using a convenience sampling in the Grand Anse and the Carenage areas of St. George's, Grenada. A questionnaire was employed to collect data on the knowledge, attitudes and perceptions towards five zoonotic diseases (Ringworm, Leptospirosis, Creeping Eruptions, Rabies and Salmonellosis).

**Results:** The overall level of distribution of Knowledge of zoonotic diseases was 38.6%. Knowledge of Ringworm (81.0%) was predominant among participants while Leptospirosis and Creeping Eruption demonstrated the greatest deficiency in participants' knowledge. Knowledge of zoonotic diseases was found to have an effect on the attitudes and perceptions of persons towards the diseases. Education ( $p=0.0000$ ) and income ( $p=0.0000$ ) were found to be determinants of zoonotic disease knowledge while age ( $p=0.56$ ) and gender ( $p=0.97$ ) had negligible influence on the measure of knowledge, attitudes and perceptions.

**Conclusion:** The overall level of distribution for correct knowledge towards zoonotic diseases was found to be less than 50% (38.59%) among the study participants. Education and income assumed the role as confounders which together act to determine participants' level of zoonotic disease knowledge. Age and gender was found to have no effect on either participants' attitudes towards pet care or their perceptions of zoonoses.

Decisions on zoonotic safety involve consideration of a wide range of concerns to effectively address the public health concerns of such diseases. Scientific advice is relevant to inform effective and efficient interventions that are environmentally specific and culturally sensitive.

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**Session: Zoonoses and Infectious in Animals**  
**Date: Wednesday, March 10, 2010**  
**Time: 12:30-13:30**  
**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Leishmaniasis in Albania

**S. BINO**

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**Background:** Different aspects of Leishmaniasis in Albania has been described in different research papers. Leishmaniasis is mandatory notified near public health services. From 1960-2001 an average of 80 cases per year has been described in the literature. Also other studies of vectors as well as studies of seroprevalence in dogs has been performed recently.

Data from ecological survey, reservoirs and vectors were analysed in different geographical areas.

**Methods:** A retrospective analysis of cases admitted in district and tertiary care hospitals from 1997 -2008

Also such analysis has included confirmed Visceral Leishmaniasis (VL) cases notified to the the national surveillance center

VL cases were reported from 35 out 36 districts, characterised by different levels of morbidity.

Mortality and co infection data were also analysed.

ELISA and IFAT were used for diagnosis.

**Results:** A total of 1439 cases of Visceral Leishmaniasis cases were analysed. The incidence rate ranked from 3.4 - 4.3 cases/10000 population.

About 89% percent of the districts are infected with VL. The most affected areas are: Shkodra 1.4, Lezha 1.6 , Berat, 1.1, Elbasan 0.7, Tirana 0.3 and Vlora 0.4cases/10.000population.

A high proportion of cases occurred among infantile population: especially children below 5 years, and 79.6% below 10 years of age.

*P. neglectus* and *P. papatasi* are the common while *P. tobi* is concentrated only in one geographic area.

The seroprevalence in 340 dogs collected from 7 regions show a rate of 5.8%.

**Conclusion:** VL in Albania represents a serious health problem.

Paediatric cases are exceeding 80%. It is present all over the country A control program needs to be established

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Prevalence of HPAI in live-bird markets in the Jabodatabek region of west Java, Indonesia in 2009

**C. Lockhart**<sup>1</sup>, E. Wuryaninggsih<sup>2</sup>, E. Brum<sup>1</sup>, P. R. Barrios<sup>1</sup>

<sup>1</sup>Avian Influenza Control Programme, Indonesia, Jakarta, Indonesia, <sup>2</sup>Campaign Management Unit, Jakarta, Indonesia

**Background:** Outbreaks of highly pathogenic avian influenza (HPAI) due to H5N1 began in October 2003 and have affected poultry in 31 of 33 provinces in Indonesia. Very little is known about the HPAI in commercial poultry. The objectives of this study were to determine the prevalence of HPAI due to H5 avian influenza viruses in live-bird markets that trade in commercial poultry located in the greater Jakarta metropolitan area on Java Island, Indonesia. Secondary objectives were to identify geographic origin and bird type associated with the presence of HPAI within markets

**Methods:** Pooled environmental swabs were taken from a random sample of live-bird markets in 12 districts and subjected to H5 testing over a period of six months to determine the presence of HPAI. Data on type of birds as well as origin of birds were gathered in order to assess risk associated with HPAI infection in markets

**Results:** Our results show that markets were continuously infected with HPAI, with 33.3% of market samples testing positive over the study period. HPAI infection was concentrated in markets in the northeast and central regions of the study area where more than 60% of markets tested positive.

**Conclusion:** HPAI due to H5 avian influenza appears to be widespread within markets and concentrated in the northeast and central areas of the study region. Knowledge about market-level prevalence in each district provides indication of the level of HPAI circulating within the commercial poultry industry, information that is currently not available from other sources.

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**Type: Poster Presentation**

*Ancylostoma spp.* on beaches of Elota, Sinaloa, México

**M. C. Rubio Robles**, S. M. Gaxiola Camacho, N. Castro del Campo, .. PROFAPI 2009/160  
Universidad Autonoma de Sinaloa, Culiacan, Sinaloa, Mexico

**Background:** Eggs and larvae of *Ancylostoma spp.* can disseminated in the soil of public areas, and resist to adverse environment conditions, capable of surviving for many months. Humans and pets can infect that have contact with contaminated soil of parks and sand of beaches.

Ancylostomiasis can be a zoonotic infection with hookworm species that do not use humans as a definitive host, the most common being *A. braziliense* and *A. caninum*. The normal definitive hosts for these species are dogs and cats; humans may also become infected when filariform larvae penetrate the skin. With most species, the larvae cannot mature further in the human host, and migrate aimlessly within the epidermis, causing cutaneous larva migrans (also known as creeping eruption), sometimes as much as several centimeters a day. Some larvae may persist in deeper tissue after finishing their skin migration. Occasionally *A. caninum* larvae may migrate to the human intestine, causing eosinophilic enteritis. *Ancylostoma caninum* larvae have also been implicated as a cause of diffuse unilateral subacute neuroretinitis. The objective was determine the presence of *Ancylostoma spp.* in sand of beaches of Elota municipality of Sinaloa, México.

**Methods:** The composite samples of sand of three beaches, were determined for representative samples described by the technique of Thrusfield (2005) was used:  $n = [t \cdot SD / L]^2$ . Where  $n$ =sample size,  $t$ =value of the normal distribution (Student t) for a 95% confidence level ( $t = 1.96$ ),  $L$ =accepted error or precision (5%), and  $SD$ =weighted disease prevalence (%); the total of composite sample of sand determined by the double W samplings was 225, took surface moist sand scraping of 100 grams of sand for each sample and deposited it in plastic bags; transferred to the laboratory of parasitology of the FMVZ-UAS to be analyzed by the sedimentation technique.

**Results:** 45 (20 %) of the 225 composite samples of sand and in two of the three beaches were positive to *Ancylostoma spp.*

**Conclusion:** The contamination with *Ancylostoma spp.* represent risk for the pets and public health, yet residents as visitors ignore about parasitic diseases that dogs can transmit them it is necessary implement control strategies and education for the prevention of the infections.

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**Type: Poster Presentation**

Characterization of Venezuelan field strains of EEEV by RT-PCR and SSCP

**V. M. Bermudez G.**, M. C. Gonzalez, J. M. Ruíz , J. Rivero, F. Herrera

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**Background:** Genetic characterization of three field strains of VEEE, by RT-PCR and SSCP shows difference in virulence, antigenic variation and immune response. Two genetic regions of the virus: )No Transducible RNAm 26S and a region of the gen nonstructural protein P4 (nsP4 '3 might relate to heterogeneity and behavior of the strains.

**Methods:** EEEV field virulent strains were isolated from epizootic outbreaks in equines and named: Strain 1 or PAO from Cojedes State in 2002. Strain 2 or MERCEDES Guárico State in 1996. Strain 3 or TUCACAS from Falcón State in 1984. Primers from known EEEV were used to copy and to amplify a fragment of 542 pb of the region No Transducible RNAm 26S and '3 another 532 pb fragment of the gen nsP4. Once amplified a RT-PCR was run and DNAc of both fragments from each of the three strains were verified and compared by SSCP. The double chains of DNAc obtained were denatured and turned into single chains by quick heat and cold stress to promote in chain reassortments or accessory chains. The end products were separated by low amperage PAGE.

**Results:** SSCP of the three strains of EEEV studied showed a wide range of band patterns with marked difference in the migratory pattern of the reassorted singlets (RSS) revealing and showed difference in the migratory patterns of the denatured singlet (DSS). This results uncovered that insertions or deletions of nucleotides took place in the gen sequence of these strains of EEEV.

**Conclusion:** The three field strains of EEEV showed genetic polymorphisms in the genome regions studied which characterize EEEV variants of South America.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Discovery of reverse zoonotic transmission of pandemic H1N1 influenza virus infection in cats following the initiation of a real time sero-molecular epidemiological study

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**Background:** Influenza viruses remain the most significant infectious disease One Health concern entering into 2010. The highly infectious pH1N1 virus is a human host adapted virus with unknown host susceptibility that remains problematic for pandemic influenza preparedness plans. Atypical hosts provide an opportunity for endemic influenza evolution, a new reservoir host, or a site for future mutational events. Following the discovery of the index case for feline pH1N1 infection, we are employing a sero-molecular epidemiological study to monitor infection rates and evolution of this virus at the human-animal contact interface to advance the understanding of influenza virus evolution in populations and predict, prevent or curtail emergent events.

**Methods:** This study utilizes molecular testing; real time RT-PCR differential pH1N1 detection assays; virus isolation, gene sequence analysis for virus detection, typing and viral genomics. Host immune responses are assessed via differential H1N1 ELISA, and specific hemagglutinin inhibition assays and virus neutralization assays.

**Results:** Our epidemiological study discovered the first two feline cases of pH1N1 infections following apparent reverse zoonosis from humans. Both cases include cats older than 10 yrs, clinical signs include respiratory disease, lethargy and inappetence, 3-5 days following influenza like illness in humans. One cat was moderately febrile. Both cats developed lower respiratory disease characterized by a bilateral dorsal alveolar pattern on radiography and had lymphopenia. Bronchoalveolar lavage or possibly deep oropharyngeal swabs were sufficient for PCR based viral detection. The power of serology in diagnosis will be emphasized. As the study continues, additional cases will be presented.

**Conclusion:** Here we report on the discovery and a diagnostic approach of the first pH1N1 virus infection in the atypical domestic cat which was associated with significant lower respiratory disease which is similar to more recent human cases possibly suggesting the discovery of a relevant animal model for future experimental trials with pH1N1 viruses. Moreover these atypical index cases demonstrate the possible development of new endemic infections of influenza virus in close household or community human/animal contacts complicating preparedness plans and reinforcing the need for expanding professionally trained comparative clinical scientists via the One Health initiative.

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Antimycobacterial activity of pyrimido[4,5-*b*]diazepine derivatives

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**Background:** Human tuberculosis is a contagious-infectious disease mainly caused by *Mycobacterium tuberculosis*, which is an aerobic pathogenic bacterium that establishes its infection usually in the lungs. About one third of the world's population is currently infected with *M. tuberculosis*; 10% of those infected will develop clinical disease, particularly those who also have the human immunodeficiency virus (HIV) infection. This problem is further compounded by a dramatic increase in multidrug-resistant strains of *M. tuberculosis* which demands the search for alternative antimycobacterial drugs. In this effort, diazepine derivatives, which showed a broad spectrum of biological activity especially antitumoral is presented as a promising source of new compounds-leaders. Using the existing capacity that posses the Colombian National Institute of Health (NIH) for to develop reproducible *in vitro* tests against mycobacteria species and the research trajectory of Grupo de Investigación de Compuestos Heterocíclicos, the aim of this study was to determine the activity against 15 *Mycobacterium* spp strains and clinical isolates of 12 diazepine derivatives.

**Methods:** The synthesis of pyrimidodiazepines by microwave irradiation (200 °C, 300 W) during 2-8 minutes of equimolar mixture of 2-R-4,5,6-triamino-pyrimidines and chalcones in DMF (0.5 mL) and catalytic amount of BF<sub>3</sub>.OEt<sub>2</sub> (0.5 mL). The minimum inhibitory concentration (MIC) was determined by colorimetric broth microdilution method. The activity was evaluated against five *M. tuberculosis* ATCC strains, 5 isolates of Beijing genotype and 5 clinical isolates of nontuberculous mycobacteria. Isoniazid and rifampicin were used as control drugs.

**Results:** In total six diazepine derivatives showed moderate activity at concentrations in a range between 16-32 µg/mL (Good activity CMI <10 µg/ mL), three were active against 13 microorganisms; the rest just inhibited the growth of *M. tuberculosis* strains.

**Conclusion:** Diazepine derivatives scaffolds over the years have gained an ongoing interest for biological activities as anticancer, anti-bacterial, psychotropics, anticonvulsant, anti-viral, and herbicidal. This is the first report of antimycobacterial activity of diazepine compounds developed entirely in Colombia, in addition, also includes multidrug-resistant isolates classified in the national resistance studies conducted in the Colombian National Institute of Health (NIH).

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Five-year trend of antimicrobial susceptibility rates and daptomycin activity among *Staphylococcus aureus* isolates collected in Latin American medical centers (2005-2009)  
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**Background:** Daptomycin is lipopeptide with a unique mechanism of action and rapid bactericidal activity against Gram-positive cocci. Daptomycin is approved for the treatment of Gram-positive pathogen associated complicated skin and skin structure infections (cSSSI) and *S. aureus* (SA)-associated bacteremia and endocarditis and in the United States, Europe and some Latin American (LA) countries. The in vitro activity of daptomycin and comparator agents was evaluated against clinical isolates of SA collected in four LA countries over five years.

**Methods:** 6,031 SA isolates were collected in 10 medical centers located in Argentina (1,075), Brazil (2,637), Chile (1,345) and Mexico (974). Isolates were mainly from bloodstream (42.1%) and cSSSI (28.6%). Susceptibility (S) was determined by the CLSI broth microdilution method in cation-adjusted Mueller-Hinton broth supplemented to 50 mg/L of calcium for daptomycin tests.

**Results:** The overall oxacillin resistance (MRSA) rate in LA was 41.1% and ranged from 32.1-39.0% in Brazil and Mexico to 51.7-51.9% in Argentina and Chile. MRSA rates increased continuously from 34.7 (2005) to 48.4% (2009). Daptomycin was highly active against SA in all LA countries with MIC<sub>50</sub> and MIC<sub>90</sub> values of 0.25 and 0.5 mg/ml, respectively. Overall, daptomycin was highly active against MRSA (100.0% S, see table) as was vancomycin (MIC<sub>50/90</sub>, 1/1 mg/ml; 100% S) and linezolid (MIC<sub>50/90</sub>, 1/2 mg/ml; >99.9% S). Erythromycin resistance was higher in Chile and Mexico (46.5-54.5%) than in Argentina and Brazil (38.2-39.4%). Overall constitutive clindamycin resistance was 78.8% in LA. TMP/SMX and tetracycline resistance was much higher in Brazil (20.6-23.6%) compared to other countries (1.5-6.2%). Levofloxacin and gentamicin resistance was highest in Chile at 52.1% and 42.8%, respectively.

Nation (no. of SA strains)	Cumulative % inhibited at daptomycin MIC ( $\mu$ g/ml) of:					
	$\leq 0.08$	0.12	0.25	0.5	1	2
Argentina (1,075)	0.0	2.3	62.1	98.5	100.0	-
Brazil (2,637)	0.1	2.1	61.1	98.4	100.0	-
Chile (1,345)	0.2	2.8	57.3	97.9	100.0	-
Mexico (974)	0.5	3.9	74.6	99.2	99.9	100.0
All regions						
Methicillin-S (3,552)	0.2	3.3	77.3	99.1	>99.9	100.0
MRSA (2,479)	0.1	1.5	41.6	97.5	100.0	-

a. Bolded percentages indicate susceptibility rates.

**Conclusion:** Significant resistance variations among SA and several classes of antimicrobial agents were observed in LA countries. Daptomycin showed consistent potency against recent clinical isolates of SA collected in LA medical centers, including MDR strains. Resistance to other compounds did not adversely influence daptomycin potency and with S rates at over 99.9% during the past five years suggests that daptomycin has maintained in vitro activity in LA countries.

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In vitro activity of new compound YF-13-1 against gram-positive and gram-negative bacteria strains of clinical isolates in China

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**Background:** The Present study is undertaken to evaluate and compare the activity of the new compound YF-13-1 alone and with eight antibiotics against gram-positive and gram-negative bacterial strains. The majority of bacterial strains evaluated in this study were recent clinical isolates obtained from several hospital sources in China.

**Methods:** Determination of antibacterial activity in vitro: Minimum inhibitory concentrations (MICs) were determined according to CLSI (formerly NCCLS) guidelines using cation-adjusted Mueller-Hinton agar by the agar dilution method with a Multiple inoculator replicator (steers-folty replicator). Serial two-fold dilutions of the compounds will be prepared 10-fold in agar, ranging from 128 to 0.008 µg/ml (for extremely sensitive organisms, further dilutions). The lowest concentration which allowed the growth of no more than three colonies is considered to be the MICs.

**Results:** The results showed that YF-13-1 was a broad-spectrum antibiotic. MIC<sub>50</sub>, MIC<sub>90</sub> values for YF-13-1 were each 0.25 mg/L and 1 mg/L against ciprofloxacin-susceptible and levofloxacin-susceptible *S. aureus* strains. It inhibited 90% tested ciprofloxacin and levofloxacin-resistant *S. aureus* strains at less than 16 mg/L. MIC<sub>90</sub> values against *S. aureus* MRSA, MSSA were 4 mg/L, 0.25 mg/L respectively. YF-13-1 was more active against coagulase-negative staphylococci and *S. epidermidis* MRSE, MSSE, MIC<sub>90</sub> were 1 mg/L, 8 mg/L; 2 mg/L and 0.25 mg/L respectively. MIC<sub>90</sub> was ≤4 mg/L against *S. pyogenes*, *S. pneumoniae* and *Enterococcus*. MIC<sub>90</sub> 4, 8, 0.5 mg/L against *Branhemella* bacteria, influenzae and Gonococcal respectively. Whereas, the YF-13-1 MIC range for *E. coli*, *K. pneumoniae*, *Proteus*, *Serratia marcescens*, *E. aerogenes* and *cloacae* were 0.008- 32 mg/L.

MIC<sub>50</sub>, MIC<sub>90</sub> was 2 mg/L and 8 mg/L against *P. aerogenes* respectively. But, it is less active against *Acinetobacter baumannii*, MIC<sub>50</sub>, MIC<sub>90</sub> was 8 mg/L, 128 mg/L respectively.

**Conclusion:** The results showed that YF-13-1 was a broad-spectrum antibiotic.

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10-hydroxy-2-decenoic acid induce dispersion of *Streptococcus mutans* biofilms

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**Background:** Hydroxy decenoic acid is a bioactive component of royal jelly that occupies its 10% of the total weight. Biofilms progress through multiple developmental stages, beginning with reversible attachment to a surface, followed by irreversible attachment and development of microcolonies, when dispersion occurs, releasing cells into the bulk liquid. Colonization and Biofilm formation of *Streptococcus mutans* is the causative agent of dental plaques and subsequently dental caries.

**Methods:** 10-hydroxy decenoic acid was purified by means of HPLC. Glass slides were used for biofilm formation in treated media with each slide in a total area of 6 cm<sup>2</sup>. Microtiter plate dispersion bioassays were used to test various preparations for their ability to exogenously induce *Streptococcus mutans* ATCC 25175 biofilm dispersion.

**Results:** Hydroxy decenoic acid is capable of inducing the dispersion of established biofilms and of inhibiting biofilm development. When added exogenously to *Streptococcus mutans* biofilms at a native concentration of 2.5 nM, it was shown to induce the dispersion of biofilm microcolonies. These dispersion events were observed to originate at the center of microcolonies near the substratum, but only within microcolonies that had attained a minimum diameter of 40  $\mu$ m and a minimum thickness of 10  $\mu$ m.

**Conclusion:** Active at nanomolar concentrations, hydroxy-2-decenoic acid appears to be functionally and structurally related to the class of short-chain fatty acid signaling molecules such as diffusible signal factor, which act as cell-to-cell communication molecules in bacterial colonies.

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In vitro activity of biapenem against *Burkholderia pseudomallei*  
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**Background:** *Burkholderia pseudomallei*, a gram-negative bacterium, causes in humans and animals a disease called melioidosis. Biapenem has a broad spectrum of in vitro antibacterial activity against many gram negative and gram-positive aerobic and anaerobic bacteria, including those producing  $\beta$ -lactamases. The objective of the study was to determine in vitro activity of biapenem against *Burkholderia pseudomallei*

**Methods:** 110 clinical isolates of *B. pseudomallei* from different patients were selected from our collection. In vitro susceptibility of biapenem was determined by agar dilution method and Kirby-Bauer disc diffusion. Paper discs containing biapenem 10  $\mu$ g per disk (Eiken Chemical Co. Ltd., Japan) and the standard powder of biapenem (Meiji Seika Kaisha Ltd. Pharmaceutical Division, Japan) were provided by Meiji Pharmaceuticals (Thailand). The methodology used for susceptibility testing was direct colony suspension according to guidelines suggested by CLSI. The proposed breakpoints for inhibition zone diameter of biapenem recommended by the manufacture are >20 mm for susceptible, 15-19 mm for intermediate and <14 mm for resistant.

**Results:** All strains of *B. pseudomallei* had inhibition zone diameter greater than 39 mm. The MIC<sub>50</sub> and MIC<sub>90</sub> of biapenem against *B. pseudomallei* were <0.125 and 0.25  $\mu$ g/ml respectively. All strains of *B. pseudomallei* had biapenem MIC less than 0.64  $\mu$ g/ml.

**Conclusion:** Biapenem is very active against *B. pseudomallei* and biapenem could be an alternative therapy for melioidosis.

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Role of Efflux pumps in the development of quinolone-resistance in Peruvian *Escherichia coli* isolates

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**Background:** Active efflux pumps are important in the development of antibiotic-resistance. Inhibitor efflux pumps are under investigation because the possibility to be used in the future together with antimicrobial agents. Phe-Arg- $\beta$ - naphthylamide (PAN) is a broad spectrum-efflux pump inhibitor on quinolone susceptibilities in microorganisms such as *Escherichia coli*. The objective of this study is to determine the effect of PAN in the MIC of quinolones, to relate its effect with the presence of mutations in *gyrA* and *parC* genes and with the presence of transferable mechanisms of quinolone-resistance (*qnr* and *aac(6')Ib-cr* genes) in *Escherichia coli* peruvian isolates.

**Methods:** MICs for nalidixic acid (NAL) and ciprofloxacin (CIP) were determined in the presence or absence of PAN (20mg/L) by agar dilution in 67 Nalidixic Acid resistant *E.coli* isolates. Quinolone resistance mechanisms were investigated by PCR amplification of *gyrA*, *parC*, *qnrA*, *qnrB*, *qnrS* and *aac(6')Ib* genes. Amplified fragments of *gyrA* and *parC* were sequenced to detect the presence of mutations.

**Results:** The typical mutations in *gyrA* (Ser83  $\rightarrow$  Leu; Asp87  $\rightarrow$  Asn) were found in 38 and 9 isolates respectively, in the *parC* mutations (Ser80  $\rightarrow$  Ile; Glu84  $\rightarrow$  Val, Gly) were found in 15, 4 and 1 isolates respectively. Seven strains were PCR positives for QnrB and eight for *aac(6')Ib*. No QnrA or QnrS positives were found. In 30 isolates, the effect of the PAN in the MIC of NAL was higher than 4 folds, in the half of them (n=15) the effect was higher than 8 folds, while no effect in the MIC of CIP was observed. In 6 isolates the NAL resistance was associated exclusively to the effect of PAN-inhibible efflux pumps.

**Conclusion:** PAN poses a high effect in the NAL MIC, suggesting a high relevance of PAN-inhibible efflux pumps in the development of quinolone-resistance. This fact has also been supported by the presence of isolates in which only an effect of this kind of pumps was observed. Additionally presence of transferable quinolone-resistance mechanisms has been detected in the area.

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A murine gastritis model to study antibacterial effect of *Camellia sinensis* (Green Tea) against *H.pylori* infection

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**Background:** Research studies reveal that green tea contains catechins, polyphenols and related compounds possess variety anti-tumor effects and bactericidal properties. This study is meant to evaluate the antimicrobial potential of aqueous crude extract of dried leaves of *Camellia sinensis* (Green Tea) against *H.pylori* in vivo and in vitro conditions.

**Methods:** A total of 250 biopsies were collected from the patients of gastro-duodenal pathology from government sector hospitals in Karachi, Pakistan. All these biopsies were processed for detection of *H.pylori* by rapid Helicourease, culture and polymerase chain reaction (PCR) for the 16S ribosomal gene of *H.pylori*. All the isolates were screened for their susceptibility against antibiotics and aqueous extract of *Camellia sinensis* (Green Tea) by agar well diffusion as well as micro-broth dilution. In-vivo studies were carried out by developing Gastritis Models (divided into 4 groups and each has got 3 test mice and 1 control mouse) in BALB/c mice by infecting orally with sub-lethal doses of 100 µl of 10<sup>7</sup> ml of *H pylori* culture. The gastritis model was developed after inoculation *H.pylori* for about 7days, 14 days, 21 days, 28 days and 34 days with subsequent doses of 5 % aqueous extract of green tea whose MIC was 28 mg/ml.

**Results:** In this study, a total of 88, clinical *H pylori* isolates were isolated and identified by rapid and molecular methods and our isolates showed a high level resistance towards Tetracyclin (60%), Metronidazole (80%), and Amoxicillin (60%), Erythromycin (40 %), Clarithromycin (30%), Ofloxacin (8%). A significantly large zone of an average 21mm was found on most of the isolates in case of in-vitro studies. Moreover, 4 fold reductions of CfU/ g was noted in 7 days, 14 days and 21 days, but fairly moderate CFU/g was observed in control upon organ culture. The results were also found in agreement to the histopathological findings of the stomach indicating successful establishment of infection and gastritis and upon treatment by the green tea, the reduction in the bio-load

**Conclusion:** Owing to the exorbitantly high resistance like in Metronidazole that is a cause of the failure of treatment in combined therapy, the results of the current study provide useful insights to the developments of new antimicrobial agent like green tea as a therapeutic intervention tool.

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Various dosages of linezolid in treatment of difficult multidrug-resistant tuberculosis

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**Background:** Linezolid has a potential role in treatment of complicated multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB). However, more clinical experience is desirable regarding its dosing and tolerance.

**Methods:** All patients treated with linezolid plus other second-line drugs in a tertiary tuberculosis center for fluoroquinolone-resistant MDR-TB or XDR-TB from May 2004 to January 2009 were identified. Their clinical records were reviewed through October 2009.

**Results:** Twelve patients with fluoroquinolone-resistant MDR-TB and 7 patients with XDR-TB (including one probable case) were enrolled. They were given levofloxacin 600-800 mg daily together with linezolid and other second-line agents. Linezolid was administered in 600 mg twice-daily dosing in 14 patients for a median of 56 days [interquartile range (IQR) 42-56 days], and 800 mg once-daily dosing in 5 patients for a median of 84 days (IQR 42-112 days). Of these 19 patients, 8 (42%) achieved sputum culture conversion before receiving linezolid and other second-line agents and culture negativity persisted; 7 (37%) had culture converted to negative after a median of 12 weeks (IQR 8-12 weeks) of treatment; 3 (16%) had transient bacteriological conversion to negativity followed by reversion to positivity after withdrawal of linezolid; and 1 (5%) failed to achieve any sputum culture conversion.

Among the 14 patients who received 600 mg twice-daily dosing, 4 (29%) had significant gastrointestinal disturbance and 9 (64%) patients developed anemia (hemoglobin  $\leq$  10g/dL). Two (14%) tolerated treatment well without any side-effect. Among the 5 patients who received 800 mg once-daily dosing, 2 (40%) had no adverse events, 2 (40%) patients had anemia, and 2 (40%) experienced mild limb paresthesia.

A total of 6 (32%) patients completed treatment with relapse in one. Four (21%) defaulted from treatment. Two (11%) patients died, one from lung carcinoma and the other from TB. Seven (37%) patients were continuing treatment with one still on linezolid.

**Conclusion:** Complicated MDR-TB or XDR-TB may require prolonged linezolid therapy. Linezolid administered as 800 mg once daily appeared to be better tolerated than when given as 600 mg twice daily. The optimal dosage of linezolid requires further delineation through balancing efficacy and toxicity.

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Intravaginal pentamycin for the treatment of bacterial vaginosis

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**Background:** Pentamycin 3 mg vaginal tablets (FemiFect®, Lumavita AG, Basel, Switzerland) is effective in the treatment of vaginal infections caused by *Trichomonas vaginalis*, *Candida albicans* and mixed flora (Clin. Ter. 92: 137-142, 1980; Internet Journal of Gynecology and Obstetrics 11(1), 2009). While pentamycin has shown in-vitro activity against some pathogenic bacteria, it is inactive against the lactobacilli that colonize the healthy vagina, making it an ideal therapeutic candidate for the treatment of bacterial vaginoses. The objective of this study was to investigate the efficacy, safety and tolerability of intravaginal pentamycin in women with symptomatic bacterial vaginosis.

**Methods:** This open-label clinical trial included 92 women, aged 15-78 years, who attended the gynaecology clinic for symptoms of vaginitis and had a diagnosis of bacterial vaginosis on the basis of the history, complete gynaecological examination, wet mount evaluation and microbiological analysis. The causative agent was primarily *Gardnerella vaginalis* in 43 patients, while diverse bacteria were found in the vaginal secretions of the other 49 women. Twenty-five patients had previously received other antimicrobial therapies without benefit. Patients were treated with intravaginal pentamycin (6 mg daily for 5 days, n = 58; 3 mg daily for up to 10 days, n = 34), either alone (73.9% of cases) or in combination with other therapies. The gynaecological examination and microbiological analysis were repeated at the end of the treatment period, when the occurrence of systemic or local adverse events was also recorded and patient's satisfaction was assessed by using a questionnaire. Efficacy endpoints were resolution of symptoms and eradication of the causative agents.

**Results:** Resolution of symptoms and eradication of the causative agents were demonstrated in 74.4% and 69.8%, respectively, of patients with bacterial vaginoses primarily caused by *G. vaginalis* and in 87.8% and 75.5%, respectively, of patients with bacterial vaginoses caused by diverse bacteria. Irrespective of the dose of pentamycin administered, treatment acceptance was rated as "good" by 69.8% of patients with bacterial vaginoses primarily sustained by *G. vaginalis* and 79.6% of those with a disease caused by diverse bacteria. The therapeutic regimen was well-tolerated in all cases.

**Conclusion:** Intravaginal pentamycin, either alone or in combination with other therapies, is effective in the treatment of bacterial vaginoses. The drug exhibits a favourable tolerability profile and is well-accepted by patients.

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Intravenous colistin therapy for infections caused by multidrug-resistant gram-negative bacteria in critically ill patients

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**Background:** The increasing incidence of infections caused by multidrug-resistant (MDR) *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* is a worldwide problem, particularly among patients with immune deficiency, burns or neutropenia. The significant resistance of these bacteria to the commonly used antibiotics and the expected lack of new agents available in the near future lead the clinicians to test the efficacy and safety of colistin, an old antibiotic whose use decreased because of suspected toxicity and which is now used as the last therapeutic resort.

**Methods:** Thirteen (10M, 3F; mean [SD] age 45.3 [18.3] years; mean [SD] estimated body weight, 82.8 [21.9] kg;) critically ill patients with different conditions were enrolled. SAPS II and SOFA scores were used to predict mortality in the intensive care unit. All patients were treated with colistin sulphomethate sodium (CMS) intravenously (2M I.U.) every 8h. After at least three days of therapy, blood samples were collected before and at 1,2,3,4,6,8 h after the end of the 30-minute IV infusion. Colistin was measured with a specific high-performance liquid chromatography method. Pharmacokinetic parameters were determined by non-compartmental analysis using the Kinetica Innaphase™ 4.0 software.

**Results:** Patients received  $2.19 \pm 0.38$  mg/kg (range 1.58-2.49) of CMS per dose. At the steady-state, plasma colistin had a mean [ $\pm$  S.D. ] AUC (0-8h), C<sub>through</sub>, C<sub>max</sub>, t<sub>1/2</sub>, CrCL of  $11.54 \pm 6.20$   $\mu\text{g}\cdot\text{h}/\text{ml}$ ,  $1.02 \pm 0.69$   $\mu\text{g}/\text{ml}$ ,  $2.21 \pm 1.08$   $\mu\text{g}/\text{ml}$ ,  $5.87 \pm 2.56$  h and  $142.9 \pm 57.8$  mL/min, respectively. A clinical response was observed in 92% of patients; treatment failed in one patient with severe underlying conditions (cerebral cancer, pneumonia). The antibacterial effect of colistin was evaluated by the C<sub>max</sub>/MIC and AUC/MIC ratios; ratios differed according to the considered bacteria.

**Conclusion:** Based on our pharmacokinetic results, the maximum concentrations of colistin achieved were probably sufficient to ensure optimal C<sub>max</sub>/MIC ratios for all the gram-negative bacteria under study.

However, we need to redefine appropriate dosing strategies for this antibiotic in order to maximize clinical efficacy, reduce patient risk of developing bacterial resistance and minimize adverse effects.

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Pharmacodynamic profiling of intravenous antibiotics against gram-negative bacteria collected during the Tracking Resistance in the United States Today (TRUST) surveillance program  
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Hartford Hospital, 06106, CT, USA

**Background:** Antibiotic resistance in Gram-negative bacilli (GNB) is escalating globally; thus achieving optimal antimicrobial exposures has become considerably more challenging. We used data from "Tracking Resistance in the United States Today (TRUST) surveillance program" to compare the probabilities of attaining requisite antibiotic exposures against *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*.

**Methods:** Using Monte Carlo simulation, pharmacodynamic profiling was performed for standard and prolonged infusions of cefepime 1&2gq12h, 2gq8h(0.5h&3h infusions); ceftazidime 1&2gq8h(0.5&3h); ceftriaxone; 1&2gq24h(0.5h); ciprofloxacin 0.4gq8&12h(1h); doripenem 0.5, 1&2gq8h(1&4h); ertapenem 1gq24h(0.5h); imipenem 0.5gq6&8h(0.5h), 1gq8h(0.5&3h); levofloxacin 0.75gq24h(1h); meropenem 0.5gq6&8h(0.5h) then 1&2gq8h(0.5&3h); piperacillin/tazobactam 3.375&4.5gq6h(0.5h), 3.375gq8h(4h), 4.5gq6h(3h). MIC data were incorporated from the 2008 TRUST program. Pharmacodynamic targets were defined as  $fT > MIC > 40\%$  for carbapenems, 50% for penicillins and cephalosporins. A total AUC/MIC  $\geq 87$  and  $\geq 125$  was applied for levofloxacin and ciprofloxacin, respectively. The cumulative fraction of response (CFR) was determined for each regimen against each population of organisms. Optimal CFR was defined a priori as  $\geq 90\%$ .

**Results:** The fluoroquinolones displayed the lowest susceptibilities against all organisms. All cephalosporins, carbapenems and piperacillin/tazobactam had susceptibility rates  $> 90\%$  for Enterobacteriaceae. Against *P. aeruginosa*, piperacillin/tazobactam portrayed the highest percent susceptibility(90%), followed by meropenem(89.8%), cefepime(88%), and ceftazidime/doripenem(86%). All  $\beta$ -lactam regimens achieved optimal CFR against *E. coli*, and all but piperacillin/tazobactam 3.375g q6h achieved optimal CFR against *K. pneumoniae*. The fluoroquinolones achieved the lowest CFRs against all organisms. Against *P. aeruginosa*, high dose, prolonged infusion doripenem and meropenem achieved CFRs of 97.2% to 98.8%, followed by high dose, prolonged infusion ceftazidime(93.3%) and cefepime(93.2%). No regimens achieved optimal CFR against *A. baumannii*. Prolonged infusion regimens increased CFR for all  $\beta$ -lactams against the non-fermenting GNB.

**Conclusion:** Standard intravenous doses of many commonly  $\beta$ -lactam regimens have a high probability of achieving optimal exposure against Enterobacteriaceae. For non-fermenting-GNB, higher dose, prolonged infusions of cefepime, ceftazidime, doripenem, and meropenem offered the highest probabilities of achieving bactericidal exposure.

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Oral Cefixime is beneficial in acute uncomplicated *Salmonella* gastroenteritis in children with mucoid or bloody diarrhea – Do indications of antibiotics therapy need reassessment?

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**Background:** Conventional antibiotics not only provided no benefit to pediatric patients with uncomplicated *Salmonella* enterocolitis but also increased the rate of relapse and duration of fecal excretion. This study was designed to evaluate the role of cefixime, an oral cephalosporine with excellent activity against *Salmonella*, in the treatment of uncomplicated nontyphoid *Salmonella* enteritis in children.

**Methods:** In a randomized, prospective, double-blind, two-center study, children with uncomplicated *Salmonella* gastroenteritis and mucoid/bloody diarrhea were treated with oral cefixime (10 mg/kg/day divided twice daily) or placebo for 5 days. There was no significant difference between these two groups in their characteristics, severity of disease and distributions of serogroups (Table 1). The duration of fever and diarrhea were recorded by hours. Stool samples were sent for culture weekly until three consecutive negative results were obtained. Subsequent follow-up visits were at any time when diarrhea happened or at two-week intervals for the first two months, three-month interval for the following one year and two-year interval for the following six years.

TABLE 1. Comparability of Cefixime and Placebo Treatment Groups with Uncomplicated Salmonella Enteritis

Characteristics	Cefixime	Placebo	p
No. patients	37	31	
Male:female	18:19	14:17	0.815
Age (years)	5.5 (s.d. 3.7)*	5.9 (s.d. 3.4)	0.239
Age range (years)	1-16	1-16	
Total hematological and biochemical data			
White blood cell count (cells/mm <sup>3</sup> )	13402 (s.d. 4716)	13181 (s.d. 5885)	0.898
Neutrophils (%)	71.9 (s.d. 9.7)	71.8 (s.d. 9.5)	0.984
Platelet (10 <sup>9</sup> /mm <sup>3</sup> )	386.9 (s.d. 75.8)	388.2 (s.d. 77.8)	0.982
C-reactive protein (mg/dL)	86.9 (s.d. 82.2)	102.8 (s.d. 114.8)	0.912
Aspartate (U/L)	89.8 (s.d. 22.7)	112.2 (s.d. 38.2)	0.822
Alanine (U/L)	8.8 (s.d. 7.7)	14.2 (s.d. 10.7)	0.872

\*Data are shown as mean (s.d.) unless otherwise stated.

**Results:** Sixty-eight patients with acute, uncomplicated culture-proved *Salmonella* enteritis were enrolled. Serogroup B was the most commonly isolated serogroup in both oral cefixime group and placebo group (26/37, 24/31), followed by serogroup C (6/37, 3/31) and D (4/37, 4/31). The susceptibility rate of cefixime *in vitro* (100%) was equal to that of ceftriaxone (100%), and higher than those of ciprofloxacin (97.06%), ceftizoxime (97.06%), sulfamethoxazole-trimethoprim (51.47%), ampicillin (42.65%), and chloramphenicol (41.18%). The duration of diarrhea or mucoid diarrhea was significantly reduced by antibiotic treatment (mean 55.8 hours vs 79.2 hours,  $p=0.009$ ; 49.5 hours vs 73.0 hours,  $p=0.036$ ) (Table 2). Time to defervescence was significantly shortened in the group receiving cefixime (mean 15.0 hours vs 29.3 hours,  $p=0.028$ ). However, there was no significant difference with respect to the rate of clearance of *Salmonella* from stool ( $p=0.843$ ). All followed-up samples after completing therapy, remained sensitive *in vitro* to cefixime. There was no relapse of the same pathogen found.

TABLE 2. Results of Cefixime and Placebo Treatment Groups with Uncomplicated Salmonella Enteritis

Parameter	Cefixime	Placebo	p
No. patients	37	31	
Duration of diarrhea (hours)			
Pre-treatment	87.2 (s.d. 69.9)*	81.2 (s.d. 58.5)	0.884
After initiation of therapy	55.8 (s.d. 37.2)	79.2 (s.d. 58.2)	0.009
Duration of mucoid diarrhea (hours)			
Pre-treatment	79.8 (s.d. 61.2)	73.0 (s.d. 54.2)	0.227
After initiation of therapy	49.5 (s.d. 42.4)	73.0 (s.d. 48.4)	0.036
Duration of fever to defervescence (hours)			
Pre-treatment	46.3 (s.d. 48.2)	46.8 (s.d. 34.7)	0.752
After initiation of therapy	15.0 (s.d. 16.4)	29.3 (s.d. 42.6)	0.028
Duration of time to stool			
Pre-treatment	74.0 (s.d. 61.3)	71.2 (s.d. 42.3)	0.834
After initiation of therapy	12.8 (s.d. 23.4)	46.2 (s.d. 31.5)	0.028
Relapse (patients/total)	0/37	0/31	0.843

\*Data are shown as mean (s.d.) unless otherwise stated.

**Conclusion:** The clinical trial showed that cefixime could shorten the time to defervescence and the duration of diarrhea significantly. Prolonging the period of carriage or increasing the risk of relapse was not found in this study. Cefixime could be considered in acute, uncomplicated, *Salmonella* enteritis in children with mucoid/bloody diarrhea.

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Evaluation of the pharmacodynamics of Daptomycin (DAP)-Rifampin (RIF) combinations with *Staphylococcus aureus* using various in vitro methods

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**Background:** The evaluation of antibiotic combinations is often based on traditional checkerboard techniques (CT) that might or might not provide optimal interpretations. In this study, DAP plus RIF combinations were tested with *S. aureus* using CT as well as time-kill curves at constant and changing antibiotic concentrations -- static studies (SS) and dynamic studies (DS), respectively.

**Methods:** A clinical isolate *S. aureus* 866 (MIC of DAP 0.5 mg/L, MIC of RIF 0.016 mg/L) was tested using CT at DAP concentrations ranging from 0 to 2 mg/L and RIF concentrations ranging from 0 to 0.06 mg/L. In SS, the same organism was exposed to DAP (0.5, 1, 2, 4, 8 and 6 mg/L), alone and in combination with RIF (DAP/RIF 1:1.5) over a 24-hour period. In DS, *S. aureus* was exposed to daily administered DAP and DAP+RIF for 5 consecutive days; the simulated ratios of area under concentration-time curve (AUC) to the MIC were 50 and 50+75, respectively. The antimicrobial effects observed in SS and DS were expressed by areas between the bacterial concentration – time curves with and without antibiotic (ABBCs).

**Results:** Based on the CT data, DAP+RIF combinations were additive or indifferent (FIC index = 1). Based on ABBC analysis of the SS data, DAP+RIF was synergistic at relatively low AUC/MICs of DAP (24-96) or additive at higher AUC/MICs of DAP (192-770). For example, the 24-hour ABBC of DAP (AUC/MIC 48) + RIF (AUC/MIC 72) was 9-fold greater than that of DAP (AUC/MIC 48). Similar differences in the effects of DAP+RIF and DAP were established in DS: the 120-hour ABBC was 13 times greater in simulations of the combination than DAP alone.

**Conclusion:** These data suggest that (1) checkerboard technique-based evaluations of daptomycin+rifampin combinations can be misleading, and (2) in vitro pharmacodynamic data obtained in static systems are predictive of those in dynamic studies.

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The effectiveness of oral suppression in high risk prosthetic joint infections after total knee arthroplasty

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**Background:** Prosthetic joint infection (PJI) is a rare but serious complication of total knee arthroplasty (TKA) and is a frequent reason for failure to retain the prosthesis. Recent studies have reported success rates of >80 percent with debridement and retention of prosthesis between 2 to 5-year follow-up periods. Lack of consensus on definitions of PJI and risk factors between centers makes evaluation of true risk factors and outcomes a challenge. Though improved success rates have been reported for the conservative approach of debridement, intravenous (IV) antimicrobial therapy, and prosthesis retention, little data exists on combining the use of oral suppressive therapy with this treatment option.

**Methods:** Nine year retrospective study of 28 high risk PJI patients after TKA who had been treated with debridement, six weeks IV antimicrobial therapy and prosthesis retention, or the same treatment with oral suppressive therapy and had a two to five year follow-up to ascertain the effectiveness of oral suppressive therapy, excluding potential confounding factors such as chemotherapy, HIV treatment, or steroid therapy. Statistical analysis involved estimation of cumulative probability of treatment failure using Kaplan Meier method and total curve comparison was obtained by using the Mantel-Cox logrank method. Fisher's exact test and the Wilcoxon rank sum test were used for univariate analysis.

**Results:** The 2-year cumulative probability of failure for oral suppressive therapy was 7% (95% CI: 0.04 to 27.5) compared to 42% (95% CI: 17.7 to 66.07) for the control group. Oral suppressive therapy was significantly different than control ( $P= 0.033$ ).

**Conclusion:** Treating high risk patients with only six weeks IV antimicrobial therapy and debridement, especially in early postoperative infections, could lead to treatment failure sooner than when treating patients with oral suppressive therapy. The effectiveness of this treatment could reflect the importance of using rifampin/vancomycin or similar combinations during the six weeks preceding the baseline of this study.

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Population pharmacokinetics of meropenem in pediatric patients: a concurrent analysis of the plasma and urine concentration data

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**Background:** This study aimed to develop a population pharmacokinetic model for meropenem in Japanese pediatric patients, specifically focusing on the drug urinary excretion process. This study also aimed to use this model to assess the pharmacodynamics of meropenem regimens against common bacterial populations.

**Methods:** Pharmacokinetic data (229 plasma samples and 61 urine samples) were collected from 40 infected children (age, 0.2–14.8 years; body weight, 3.8–64.0 kg) in nine separate studies. The data were concurrently fitted into a multi-compartment model using the NONMEM program. The developed model was then used for a pharmacodynamic Monte Carlo simulation to estimate the probabilities of attaining the bactericidal target (40% of the time for which the free drug concentration remains above the MIC for the bacterium).

**Results:** In the final population pharmacokinetic model, body weight (BW, kg) was the most significant covariate as follows:  $CL_r \text{ (L/h)} = 0.254 \times BW$ ,  $CL_{nr} \text{ (L/h)} = 3.45$ ,  $V_c \text{ (L)} = 0.272 \times BW$ ,  $Q \text{ (L/h)} = 1.65$  and  $V_p \text{ (L)} = 0.228 \times BW$ , where  $CL_r$  and  $CL_{nr}$  are the renal and non-renal clearances,  $V_p$  and  $V_c$  are the volumes of distribution of the central and peripheral compartments, and  $Q$  is the intercompartmental (central–peripheral) clearance. The pharmacodynamic assessment based on this model showed that regimens of 10–40 mg/kg, three times a day (0.5-h infusions), achieved a target attainment probability of >80% against clinical isolates of *Escherichia coli* (MIC<sub>90</sub> = 0.03 mg/L), *Streptococcus pneumoniae* (MIC<sub>90</sub> = 0.5 mg/L), methicillin-susceptible *Staphylococcus aureus* (MIC<sub>90</sub> = 0.12 mg/L), *Haemophilus influenzae* (MIC<sub>90</sub> = 0.25 mg/L) and *Pseudomonas aeruginosa* (MIC<sub>90</sub> = 1 mg/L), in most typical patients (BW = 10, 20 and 30 kg).

**Conclusion:** These results provide a better understanding of the pharmacokinetics of meropenem in Japanese pediatric patients. They are also useful in the choice of a meropenem regimen based on the BW of the patient and the susceptibility of the causative bacteria.

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Appropriate use of fluoroquinolones in a Lebanese tertiary medical center  
**W. Kabbara**, P. Rahbany, S. Al-Natour  
Lebanese American University, Byblos, Lebanon

**Background:** Fluoroquinolones are among the most widely prescribed antibiotics especially for respiratory and urinary tract infections. However, concerns about increasing resistant micro-organisms associated with the use of these agents have emerged in both community and hospital settings. This has been particularly a rising major problem in Lebanon as the resistance to fluoroquinolones has reached 30-40 % by 2008 (for *E.coli* and *Klebsiella* species). The primary objective of this study is to assess the appropriate use of fluoroquinolones in a Lebanese tertiary medical center by evaluating the appropriate indication, dose, dosage adjustment in renal impairment, and the duration of treatment.

**Methods:** We conducted a prospective observational study at Rafic Harriri University Hospital in Beirut, Lebanon between January and June 2009. We identified 118 patients receiving broad spectrum fluoroquinolones (levofloxacin, ciprofloxacin and moxifloxacin). The majority of patients were in internal medicine floors or in the intensive care unit. A data collection form including all pertinent information was used. Patients were followed from initiation of fluoroquinolone therapy to the discharge date. The assessment for the appropriate use was based on relevant guidelines from the Infectious Disease Society of America, manufacturer package inserts and clinical judgment. Monitoring of blood glucose levels with fluoroquinolone therapy was also evaluated.

**Results:** The patient population was predominantly male (62.7%) and the mean age was 62.8 years. About one third of the patients (29.6%) had decreased renal function necessitating dosage adjustment of ciprofloxacin or levofloxacin. The main indications were community acquired pneumonia and diabetic foot infections. Cultures were taken in only 59.3% of patients. Of the positive cultures, the most common isolated micro-organisms were *E. coli* and *Pseudomonas aeruginosa*. The final percentage of fluoroquinolone appropriate indication, dose and duration of therapy was 93.2%, 74.5% and 57.6% respectively (which were also calculated for each drug separately). 57.1% of patients did not receive the appropriate dose adjustment according to the level of renal dysfunction (calculated by using Cockcroft-Gault equation). Baseline blood glucose was monitored in only 21.5% of patients.

**Conclusion:** The major clinical interventions that need improvement in our tertiary medical center are adequate renal adjustment of the dose of fluoroquinolones and the duration of therapy.

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Impact of restriction policy as part of an antimicrobial stewardship program in a university hospital of a developing country

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**Background:** In an era of growing concern about bacterial resistance and hospital costs, limiting the use of broad spectrum antibiotics is important. The purpose of this study is to evaluate the impact of restriction policy as part of an antimicrobial stewardship program.

**Methods:** The study was conducted in a 140-bed university hospital of a developing country. Tools of the program were development of guidelines of antimicrobial therapy for common community and hospital-acquired infections and education to improve antimicrobial prescription. During the first period of the program we implemented a policy of prior approval for selected antibiotics. Every day pharmacists reviewed prescriptions and the team discussed with prescribing physicians to accomplish with hospital guidelines. After one year of implementation of the program, we stopped the restriction policy and we kept on working with the other tools of the program. To assess if cessation of restriction was associated with an increase in antimicrobial consumption, we measured antibiotic consumption during the first year (period 1) and during one year after we stopped restriction (period 2). Antimicrobial consumption was measured by Defined Daily Dose (DDD) normalized by 1000 bed-days.

**Results:** During the period 2 antibiotic consumption of ceftriaxone, ceftazidime and vancomycin decreased; it remained unchanged for piperacillin-tazobactam while cefepime, imipenem and colistin increased

	Period 1	Period 2	Percentage of Increase/Decrease (%)	P value
<b>Antibiotic</b>	<b>DDD per 1000 bed-days</b>			
<b>Cefepime</b>	<b>53,73</b>	<b>80,97</b>	<b>+ 50,7</b>	<b>&lt; 0,01</b>
<b>Ceftazidime</b>	<b>17,56</b>	<b>12,57</b>	<b>- 28,4</b>	<b>&lt; 0,01</b>
<b>Ceftriaxone</b>	<b>40,00</b>	<b>32,07</b>	<b>- 19,8</b>	<b>&lt; 0,01</b>
<b>Colistin</b>	<b>9,25</b>	<b>19,50</b>	<b>+110,8</b>	<b>&lt; 0,01</b>
<b>Imipenem</b>	<b>14,11</b>	<b>21,65</b>	<b>+53,4</b>	<b>&lt; 0,01</b>
<b>Piperacillin-tazobactam</b>	<b>30,36</b>	<b>30,19</b>	<b>-0,05</b>	<b>0,9</b>
<b>Vancomycin</b>	<b>44,31</b>	<b>36,98</b>	<b>- 16,5</b>	<b>&lt; 0,01</b>

**Conclusion:** Antibiotic consumption of 4 out of 7 agents did not increased after we stopped restriction policy, while some agents suffered an increased; these agents were those mainly prescribed in intensive care unit associated to an increase in isolation of multidrug resistant *Acinetobacter sp.*

Programs aimed to improve antibiotic prescription accompanied by measurement of antibiotic consumption may help to focus the program in some particular agents and areas of the hospital, and also to reinforce other infection control measures.

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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Utilization of activated protein C (Xigris) in a large teaching hospital- Possible overuse and complications

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**Background:** The role of drotrecogin alpha (Xigris) in the treatment of septic shock remains controversial. The recent literature suggests there is little or no benefit, and definite risk, for patients whose APACHE II scores are less than 25. A 2007 Cochrane analysis stated that the benefit of Xigris in patients with higher APACHE II scores was supported only by “very weak evidence”. We reviewed our institutional experience with this agent.

**Methods:** Retrospective evaluation of the records of 73 patients receiving Xigris at an 808-bed teaching hospital from April 2003 to June 2008. APACHE II scores, demographic data, clinical outcomes, 28-day mortality, and incidence of bleeding complications were assessed.

**Results:** Forty-five of 73 (61%) patients had an APACHE II score  $\geq$  25. The overall incidence of bleeding was 18 of 73 (25%); in 8 cases, severe or life-threatening bleeding occurred. Six of 18 (33%) patients with bleeding during the infusion had APACHE II scores  $<$  25, with 4 of those 6 cases described as severe. The 28-day mortality among all patients was 25 of 73 (34%), with 19 of 25 (76%) having APACHE II scores  $\geq$  25 and 6 of 25 (24%) having APACHE II scores  $<$  25. Relative to the APACHE II scores, the 28-day mortality was 19 of 45(42%) among patients with an APACHE II score  $\geq$  25 and 6 of 28 (21%) among patients with an APACHE II score  $<$  25.

**Conclusion:** Thirty-nine percent of patients receiving Xigris had APACHE II scores less than 25; its value in these patients is unproven. The incidence of bleeding during infusion of this drug is significant and is associated with higher APACHE II scores, but severe bleeding occurred even among those with lower APACHE II values, who were less likely to derive any benefit from its use. Xigris may be significantly over utilized, and its use is not without risk.

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**Type: Poster Presentation**

Antimicrobial prophylaxis for transrectal ultrasound guided biopsy of prostate: a comparative study between single dose of Gentamicin vs. Ofloxacin

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<sup>1</sup>Ha Emek Medical Center, Afula, Israel, <sup>2</sup>Ha emek Medical Center, Afula, Israel

**Background:** Prostate cancer is the most common malignancy in men, and has been increasing in incidence in the Western world. The gold standard for diagnosing prostate cancer is a transrectal US-guided biopsy. One of the complications of this procedure is the development of urinary tract infections. It has been shown that treatment with prophylactic antibiotics prior to the procedure reduces the rate of infections. The widespread use of quinolones for this and other reasons has led to an increase in resistant bacteria.

Aims of this study : to evaluate the efficacy of single-dose gentamicin in comparison with single dose of ofloxacin in patients underwent transrectal ultrasound guided biopsy of prostate.

**Methods:** 109 patients undergoing a prostate biopsy were randomly assigned to two groups: the first group was treated with a single dose of ofloxacin (400 mg) prior to the biopsy and the second with a single dose of gentamicin (3mg/kg). Clinical signs and symptoms, and urine tests and cultures were followed 2 and 7 days after procedure.

**Results:** 57 patients received Ofloxacin and 52 Gentamicin. 3 patients in the Ofloxacin group (5.2%) and 4 in the gentamicin group (7.6%) developed urinary tract infections (N.S).

No differences were seen in the clinical outcome between the groups

**Conclusion:** The development of quinolone resistant bacteria has led us to examine alternative options for prophylactic treatment before prostate biopsies. Gentamicin, was as efficient as quinolones in the prevention of urinary tract infections. A single dose prior to the procedure is sufficient and longer treatment duration is unnecessary.

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Reducing inappropriate antibiotic usage and costs in an academic Hematology-Oncology unit via antimicrobial stewardship

**C. L. Yeo**, T. S. Wu, R. Lim, P. A. Tambyah, L. Y. Hsu  
National University Health System, Singapore, Singapore

**Background:** Rising consumption of broad spectrum antimicrobials and increasing prevalence of resistant pathogens have raised concerns about antimicrobial use in recent years. Antimicrobial stewardship programs have been reported to improve patient and institutional outcomes while potentially reducing cost and resistance rates. In the light of these findings, an antimicrobial stewardship program (ASP) was initiated in a tertiary care hospital in Singapore.

**Methods:** The program was initiated in July 2009 and piloted in the haematology-oncology department. A prospective audit and feedback mechanism was employed for review of patients on selected broad spectrum antimicrobials. Appropriateness for use was assessed based on indication of therapy, dose of antimicrobials used, and duration of therapy. Recommendations were made by infectious disease physicians to the primary team in writing. Appropriateness of use, recommendations made, patient savings and clinical outcomes were analyzed for a period of four months after initiation of the program.

**Results:** A total of 462 cases were reviewed of which 64% of them were appropriate. The most commonly prescribed antimicrobials were piperacillin/tazobactam, ceftazidime and imipenem, making up 19%, 16% and 15% of the cases audited respectively. Among the recommendations made, de-escalation and discontinuation of antimicrobials were the most common, each making up 27% of the recommendations. This was followed by recommendations for IV-to-PO switch and advice on treatment duration, which made up 11% of the recommendations each. The overall acceptance rate of the recommendations was 89%. An estimate of the potential patient savings due to the accepted recommendations came up to \$24, 590 over the four months period. There was no significant negative impact on clinical outcomes following the recommendations.

**Conclusion:** ASP has demonstrated its impact in reducing unnecessary use of broad spectrum antimicrobials and cost to patients. Extension of the program is likely to reduce selection pressure for drug-resistant pathogens in the institution and decrease antimicrobial expenditure.

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**Type: Poster Presentation**

Tigecycline usage in osteomyelitis caused by multidrug-resistant acinetobacter: A report of 10 cases from a single institution

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**Background:** Acinetobacter infectious has been identified in soldiers who participated in wars as Korean, Vietnam and Middle Orient, by now the therapeutic options are limited for this type of multidrug resistant microorganism, we know that the use is not approved for the treatment of a bone infection, so we found necessary to evaluate the efficacy of tigecycline in the treatment of this entity.

**Methods:** We identified 10 patients with chronic osteomyelitis during 2007-2008, this study of cases is prospective, descriptive analytical, we use the microbiological method of Kirby-Bauer and the criteria of inclusion were patients with clinical diagnosis, microbiology isolation, imaging and bone biopsy. Exclusion criteria: immunocompromised and pregnant woman.

**Results:** 10 patients completed the study; the 88% were males, age 19 to 72 years, mean 39 years. The more common locations were: hip, femur, tibia, sacrum and calcaneus. The risk factors identified were: open fracture, vascular compromise, prolonged use of antibiotic, long stay and re-interventions. Positive cultures for *A. baumannii* 100%. They received different regimens of antibiotics without success, at this time we began with tigecycline IV 100mg the first day and then 50 mg IV q 12hs for 6-7 weeks, all the patients included a combined surgical and medical management.

**Conclusion:** 90% showed clinical improvement, and the importance of this study is that tigecycline may be a therapeutic option for chronic osteomyelitis resistant *Acinetobacter* sp, under which treatment options are very limited.

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Tetanus immunity among intravenous drug users in Uppsala County, Sweden

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**Background:** Lately, an upsurge in cases of Clostridium tetani infections among injecting drug users (IDUs) has occurred in the USA and the United Kingdom. The majority of tetanus cases occurred among persons inadequately vaccinated or with unknown vaccination history who sustained an acute injury. This increased susceptibility may, in part, explain the disproportionate occurrence of tetanus among IDUs. The aim of our study was to evaluate the levels of antibody to tetanus toxoid among IDUs in Uppsala County, Sweden

**Methods:** Blood samples obtained from 37 IDUs were determined for the level of anti-tetanus antibodies with EIA (Enzyme Immuno Assay). Titers >IgG 0.3 IU/ml were considered protective with long lasting immunity whereas IgG 0.03-0.3 IU/ml was considered low and protective. Lower or absent levels of antibody (<0.01 IU/ml) was considered non-protective antibody activity in serum.

**Results:** Among IDUs aged 26-45 years, 88% of the tested individuals had anti-tetanus titers >IgG 0.3 IU/ml and 12% had titers between 0.03-0.3 IU/ml. Among older IDUs (>45 years), 75% had long lasting immunity (>0.3 IU/ml) whereas 25% had titers between 0.3 – 0.03 IU/ml. None of the tested IDUs lacked protective antibodies in serum.

**Conclusion:** IDUs in Uppsala County are sufficiently protected against tetanus at the moment. Because all wounds, even minor and relatively clean wounds, confer a risk for tetanus, health-care providers should review the vaccination status of all IDUs and administer indicated tetanus toxoid vaccine to keep them fully protected.

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**Type: Poster Presentation**

Three-year surveillance of community-acquired and health care-associated Methicillin-Resistant *staphylococcus aureus* infections in Uppsala County, Sweden

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**Background:** The purpose of this three-year prospective surveillance was to recognize risk factors and spread of Community-Acquired (CA) and Health Care-Associated (HCA) MRSA infections in Uppsala County and to calculate the cost by identification of patients with MRSA infections.

**Methods:** MRSA was isolated between 2004-2006 by systematic screening, regardless of symptoms, from all patients that had been treated in hospitals abroad or in the Stockholm area. During the study, 17 634 isolates were collected from 7967 patients attending two hospitals and 37 primary care clinics. A combination of molecular typing methods was used, including pulsed-field electrophoresis (PFGE) and multilocus sequence typing (MLST) to determine the prevalence of major clones. Clones were characterized by sequencing of *Staphylococcus* protein A gene (*spa* typing). The virulence factor the Panton Valentine Leukocidin (PVL) gene was determined. The majority of the MRSA isolates were grouped into resistance profiles of seven antibiotics.

**Results:** 82 cases of MRSA infections were identified, 24 HCA and 58 CA. The total cost for bacterial isolation of MRSA was 7.2 million SEK (1.14 million USD) which amounts to 86 805 SEK (13 870 USD) per identified patient.

A majority of the MRSA infections originated outside Sweden (65%).

Domestic cases showed certain risk factors as alcohol or drug abuse, immunosuppression, chronic diseases and high age.

Domestic MRSA infections decreased during the period whereas isolates originating from patients infected abroad increased.

The isolates could be assigned into 9 clonal complexes. Most frequent clonal complexes were CC8 (34%) isolates and CC80 (23%). Two clones, CC45 (6 cases) and CC1 (2 cases) were only domestic. None of the HCA isolates carried the virulence determinant PVL gene whereas 52% of CA-cases harboured the gene.

Resistance to antibiotics, other than  $\beta$ -lactams was rare in domestic MRSA-infections (3%) whereas 56 % of patients infected abroad were resistant to several antibiotics.

**Conclusion:** Sweden is a low endemic country. There was no spread of MRSA in the community or in hospital during the surveillance. Patients with family relations or travel in Middle East or Asia were highly overrepresented. To save costs selected screening of patients at risk should be advised.

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One-year epidemiology of febrile diseases on the Emergency Department of a Caribbean island: the Curaçao-experience

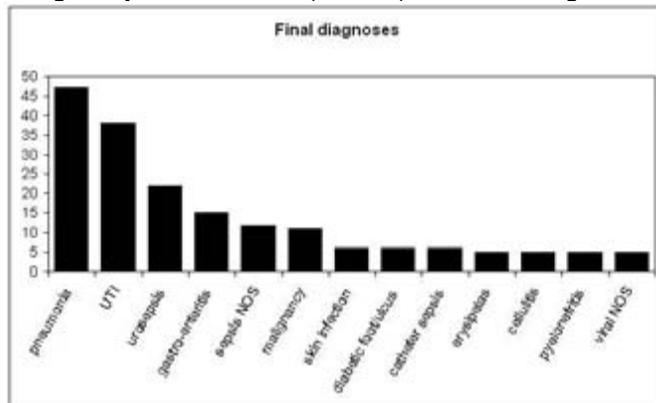
**M. Limper**<sup>1</sup>, I. Gerstenbluth<sup>2</sup>, A. Duits<sup>3</sup>, E. Van Gorp<sup>1</sup>

<sup>1</sup>Slotervaart Hospital, Amsterdam, Netherlands, <sup>2</sup>Medical & Public Health Service (GGD) of Curaçao, Curacao, Netherlands, <sup>3</sup>Red Cross Blood Bank Curacao, Curacao, Netherlands

**Background:** Fever is one of the most frequent causes of attendance at emergency departments (EDs) worldwide. The etiology of febrile diseases in tropical countries often remains poorly characterized. Curaçao is a small tropical island in the Caribbean Sea with a semi-arid climate. The purpose of this study is to describe the etiology and outcome of febrile illnesses on the ED in Curaçao, during a one-year period.

**Methods:** From April 2008 to April 2009, all febrile patients (T > 38.5 °C) between 18 and 80 years who presented at the ED of the St. Elisabeth Hospital, Curaçao, Netherlands Antilles, were included. Clinical data were recorded, routine laboratory measurements and blood cultures were taken. Supplementary diagnostics were ordered at the discretion of the attending physician. Final diagnoses were made at discharge by an independent physician and again in retrospect by the main investigator, using all available clinical data.

**Results:** Between April 2008 and April 2009, 403 patients with fever were identified (207 males, 51.4%). Mean age was 51.4 yrs (SD 21.2 yrs). 223 Patients (55.6 %) were hospitalized, 32 patients (7.9 %) died and 18 patients (4.5 %) were admitted to the Intensive Care Unit. In 129 febrile patients (32.0 %), infection was proven. 84.4 % of patients had bacterial (29.0 % urinary tract infection (UTI), 23.2 % pneumonia), 5.6 % viral and 10.0 % parasitic or fungal infection. In 34 patients (8.4 %), dengue fever was suspected; dengue was serologically proven in one patient. 21 Patients (5.2 %) were discharged with a non-infectious diagnosis, most frequently malignancy. 172 Patients (42.7 %) were discharged without a clear diagnosis.



#### **Final diagnoses of febrile patients on the emergency department, Curacao**

**Conclusion:** A high mortality rate of 7.9 % was observed in this cohort. We found a high prevalence of bacterial infections, with pneumonia and urinary tract infections as most common causes of fever. Not many typical tropical infections were seen, although many patients were diagnosed with probable dengue fever during the rain season, which could not be corroborated serologically. One in 20 patients presenting with fever did not have an infectious disease.

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Transmission risk and predictability of invasive meningococcal disease

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**Background:** In light of the media coverage concerning recent infectious disease outbreaks such as the ongoing influenza pandemic as well as previous ones involving Severe Acute Respiratory Syndrome (SARS), the public domain has become more conscience of infectious diseases as well as the effect of travel aiding their spread.

Meningococcal meningitis has been a disease which among other factors such as age, climate and life style, is facilitated by travel. The causative bacterial agent, *Neisseria meningitidis*, being spread through aerosol respiratory droplets can cause Invasive Meningococcal Disease (IMD), and is a widely distributed, complex human disease affecting all age categories.

Forecasting models exist for diseases based on vector-borne or wind related movement, as well as climate derived assessments, and recently travel oriented detection. Hence a combination of several factors ranging from meteorological to molecular level information should aid in determining likely occurrences of meningococcal meningitis epidemics in spatial and temporal means.

**Methods:** A systematic review was conducted in order to identify the different risks and models available in terms of IMD spread. Varying risk stratification was used to classify risk factors such as: travel related spread, respiratory co-infections, the effect of new meningococcal clones into a susceptible population, low humidity, high temperatures and lifestyle based aspects. These data will be used to create an early warning system generated through various technologies and transmission models in order to highlight risk areas and periods of ongoing IMD.

**Results:** As other studies have shown, travel patterns such as destination, duration and timing during the year affect risk of IMD development and spread among travelers, whilst climate, geography and factors such as virulence of circulating strain are more important in determining the severity of an outbreak in terms of local occurrence and potential epidemic developments.

**Conclusion:** Meningococcal meningitis is not only a well established threat in terms of epidemic or endemic occurrences, but also for travelers. Hence the development of more accurate and timely detection and forecasting methods are required to help in decision making processes involving prevention and early warning purposes.

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Hospitalizations due to pneumonia and case-fatality rates in Brazil between 2003 and 2007

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**Background:** In emerging countries, pneumonia is a leading cause of hospitalization and death, particularly among young children and older adults. Between 2000 and 2003, 13% of all deaths in children under 5 years of age living in Brazil were due to pneumonia. Overall, deaths in Brazil due to pneumonia averaged 4% between 1979 and 2001.

**Methods:** This was a retrospective study to quantify the rate of hospitalizations due to pneumonia (HDTP) and cases-fatality rates (CFR) in age groups ranging from <1 years old to 80 years and older. An online, interactive web-based database of individuals hospitalized during the 5-year period, 2003-2007 covered by the Integrated Health System (DATASUS) in Brazil was used to identify cases. The Instituto Brasileiro de Geografia e Estatística database served as the source of the population/denominator statistics used for the incidence rate calculations. Case-fatality rates are the percentage of HDTP cases who died.

**Results:** Overall, the observed rate of HDTP in Brazil has shown a downward trend averaging 4.5 per 1000 residents in 2003, and 3.9 in 2007. Children <1 year old had the highest reduction in rates of HDTP, from 47 per 1000 in 2003 to 36.4 in 2006. Brazilians 80+ years old had the next highest incidence rates, averaging about 23 to 27 HDTPs per 1000 residents during the study period.

CFRs related to HDTP have increased steadily over time, averaging 2.9% in 2003, and 4% in 2007. With the exception of the <1 year old age-group, CFRs increased with age, and in the 80+ age group averaged between 16% and 18%. In age groups under 5 years, CFRs have declined slightly between 2003 and 2007. In all other age-groups, particularly the ones 30 years and older, CFRs have generally risen.

**Conclusion:** As expected, the highest rates of HDTP were observed in the extremes of age, very young and old. While a slight reduction has been observed in HDTP rates, CFRs have progressively risen during this same period particularly for adults aged 80+. Pneumonia remains an important health problem in Brazil.

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Meningococcal meningitis epidemic in Arua district North West of Uganda

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**Background:** Meningitis due to *Neisseria meningitidis* has an epidemic potential and a high case fatality rate. On 3rd September 2008, a meningitis epidemic was reported in Arua district health office, in north western Uganda. The objective of the investigation was to confirm the meningitis epidemic, identify the causative agent, risk factors and assess the district response capacity to handle the epidemic.

**Methods:** Community based cross-sectional study. We purposively selected cases, their families, District health team & local leaders for interview. Cerebral spinal fluid samples were drawn from cases and sent to Central Public Health Laboratory of Ministry of Health to isolate the causative agent. Frequencies and distributions of cases with respect to age groups and affected communities with EPIINFO 2006 were done.

**Results:** A total of 44 cases were detected and 10 deaths occurred to meningitis. The Case Fatality Rate was 22.7%: highest CFR (46.2%) occurred in those above 30years. Age range was 3months to 65years. The causative agent for this meningitis epidemic was identified to be *Neisseria meningitidis* type A with latex rapid test. 70.5% of the cases were <30 years and the male to female ratio was 1:2. Characteristically, it was discovered that the epidemic occurred in the area which had missed vaccination against meningitis in the previous outbreak of meningitis. Sleeping in poorly ventilated huts and overcrowding: 3-5 huts with 7-10 people/family cluster were risk factors to this meningitis.

**Conclusion:** Highest case fatality rate occurred amongst persons who are over 30 yrs old. Overcrowding, staying in poorly ventilated huts and lack of previous vaccination were thought to be predisposing factors to this meningitis epidemic.

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The study of epidemiological data of the mumps and the effect of MMR vaccine in the Albanian children

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**Background:** Up to March 2005 in our country mumps was endemic diseases with every year epidemic and the children were unvaccinated for these diseases. From March to September 2005 was made a massive MMRII vaccination for the children under 7 years and from this year this vaccine was included in our routine national program vaccination. The incidence of this diseases in years 2003 was 72.9/100000 habitants and in 2007,2008 were respecti vely 26.9 and 2.6/100000

**The aim of the study:** was to show the epidemiological data of the mumps and his complications in Albanian children and to show the effect of the MMRII vaccine in the decrease of the incidence of this diseases in Albania.

**Methods:** This is a retrospective study .In this study were unrolled 147 children admitted in our hospital from January 2003 to December 2008.The children were the age from 18 months to 14 years old. For each children were studied the sex, the age, the season, origin, complications, length of the hospitalization and the status of the vaccination. All the patients admitted in the hospital before March 2005 were unvaccinated.

**Results:** The average age was 6.3, 96 cases or 65.3% were 5-14 years old followed by 1-4years old with 47 cases or 31.9%,predominantly male patients with 112 cases or 76.19%, 105 cases or 71,43% were admitted in the spring and summer seasons, there is not any difference for origin. We saw these complications : neurological manifestations in 61 cases or 41.5% , pancreatitis 24 cases or 16.3% , orchitis 18 cases or 12.2% manly in the patients up 12 years old , tonsillitis and maxillitis 28 cases19.1%.The spread of the patients by years was shown in the following table:

Years	2003	2004	2005	2006	2007	2008
No of cases	16 or10.89%	36 or 24.5%	61 or 41.5%	23 or 15.6%	9 or 6.12 %	2 or 1.36%

**Conclusion:** The majority of the cases was happen in the summer and spring, the male suffer more often from this diseases and the neurological manifestation were seen more often as other manifestations. In the last 2 years, the vaccine against mumps have reduce very much the incidence of this diseases in our country .

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Pertussis in Brazil: An overview from 1988 to 2009  
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**Background:** The introduction of pertussis vaccination in the past decades reduced substantially the number of cases; however, despite the high vaccination coverage rates, pertussis is still a major threat to public health all over the world including Brazil. In this study we present an overview of pertussis in Brazil in the last two decades.

**Methods:** Between 1988 and 2009, the National Reference Laboratory for Pertussis, Instituto Adolfo Lutz, São Paulo, Brazil, received a total of 985 presumptively identified *Bordetella* spp strains, isolated from sporadic cases and/or some pertussis outbreaks. The strains were forwarded by the Regional Laboratories in São Paulo State and by the Central Public Health Laboratories throughout the country. Identification of the species was done by standard methods. Detection of O1 antigen, and the serotyping were done by slide agglutination test using O1, and Fim 2 and Fim 3 antibodies, respectively. Molecular characterization was done by Pulsed Field Gel Electrophoresis (PFGE).

**Results:** All the 985 strains were confirmed as *Bordetella pertussis* and the most of them belonged to the serotype 1,3. Strains from southeastern states accounted for 67% (660/985), most of them from São Paulo State (646/985; 65.6%). The remaining 325 strains were from south region (147/985, 15%), northeastern states (87/985, 8.8%), north region (48/985, 5%) and central area (43/985, 4.4%). The great majority (81.1%, 799/985) was from children under twelve years of age, and among them 84.4% (675/799) was from infants aged less than six months. Strains from adolescents and adults accounted for 3.2% (32/985) and 8% (78/985), respectively. The age group was unknown in approximately 7.7%. Strains belonging to several PFGE patterns were identified and some were prevalent.

**Conclusion:** Different *B. pertussis* clones circulate all over the country. These data do not cover all the country, and may represent only a fraction of the actual number of pertussis cases due to the underreporting, despite pertussis is, in Brazil, a reportable disease since 2001. Data on age distribution of pertussis cases may contribute to develop the policy of booster doses of vaccine in adolescents and adults.

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Serogroup B epidemiology in the southern cone of South America. A literature review  
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**Background:** The epidemiology of meningococcal disease differs greatly by country and time period. Only passive surveillance systems for meningococcal disease exist in countries in South America. Thus, little is known about the pattern of serogroup distribution by country in this region. Existing data showed that serogroup B is prevalent worldwide, with various strains and clones causing endemic, sporadic and epidemic disease. Several of these clones are very virulent. Understanding the local and national seroepidemiology of group B meningococcal disease is critical for vaccine formulations and vaccine policies

**Methods:** We examined the current disease burden caused by serogroup B in the southern cone of South America, comprised of Brazil, Uruguay, Paraguay, Chile, and Argentina. Clinical data were retrieved following systematic review of the literature by country. The 2008 SIREVA report on invasive disease was also consulted.

**Results:** Until the late twentieth century, countries in the southern cone had similar disease epidemiology to Latin America as a whole. However, in recent years, this has changed. In Brazil, significant differences in epidemiology can be identified between areas close to other southern cone countries and areas bordering Andean or Caribbean countries. Fluctuations in the proportion of serogroup B versus serogroup C disease have been observed in southern cone countries, yet serogroup B consistently accounted for a significant disease burden throughout the early 21st century. For example, 100% of serotypable meningococcal disease reported in Chile between 1990 and 2003 was attributed to serogroup B, as was 40% to 65% of disease in Brazil. In Argentina, serogroup B consistently accounts for about 60% of invasive meningococcal disease. Even with the emergence of disease caused by Serogroups Y and W-135 in Brazil and Argentina since 2000, serogroup B has remained the cause of a consistent and ongoing public health problem.

**Conclusion:** Invasive disease caused by meningococcal serogroup B remains an important public health issue in the southern cone countries. An effective vaccine against group B meningococcal is needed to reduce the burden of disease in South America.

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Status of bloodborne pathogen education for injection drug users in Indiana hospital emergency departments

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**Background:** The problem of the study was to determine the status of bloodborne pathogen education for injection drug users in Indiana hospital emergency departments. Forty-six instruments were returned for a response rate of 43.8%.

**Methods:** The study was designed to answer the following research questions: (a) Do Indiana hospital emergency departments have written policies on bloodborne pathogen education for injection drug users? (b) To what extent do Indiana hospital emergency departments provide bloodborne pathogen education for injection drug users? and (c) What are the major barriers for Indiana hospital emergency departments in providing bloodborne pathogen education for injection drug users?

A valid instrument was developed and sent to 110 Indiana hospital emergency department nurse managers.

**Results:** The results indicated only three (7.1%) responding hospital emergency departments had written bloodborne pathogen educational policies. Ten (20.8%) emergency departments provided some form of bloodborne pathogen education for injection drug users. Major barriers indicated for not providing patient education consisted of insufficient monetary resources, injection drug users denying a drug history, and emergency department nurses being unable to identify injection drug usage.

**Conclusion:** On the basis of the findings and within the limitations of the study, the following conclusions were drawn: Indiana hospital emergency departments do not provide bloodborne pathogen education for injection drug users.

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Neighborhood urban environmental quality conditions probably drive malaria and diarrhea mortality in Accra, Ghana

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**Background:** Urbanization is an urban change process which alters the structure and function of urban environment. The alteration in the quality of urban environmental quality conditions has significant implications for urban health both in terms of change of vector ecology and infectious disease transmission.

**Methods:** Study objectives: To investigate the relationship between infectious disease (malaria and diarrhea) mortality and spatial change in neighborhood urban environmental quality in a rapidly urbanizing area in a low income economy.

Design: A time-point spatial analysis of cluster-level environmental and mortality data using Principal Component Analytic (PCA) and multiple linear regression models.

Methods: Environmental variables were extracted from the Ghana Census 2000 database and mortality data were obtained and pooled together from the Ghana Births and Deaths Registry in Accra over the period 1998-2002.

**Results:** While there was a very strong evidence of a difference in the risk of urban malaria mortality across urban environmental zones of differing neighborhood environmental quality conditions, no such evidence of a difference in urban diarrhea mortality risk was observed across these zones. Additionally, whereas bivariate analyses showed a weak to very strong evidence of association between the environmental variables and malaria mortality, not the least evidence of association was observed between urban diarrhea mortality and the environmental variables.

**Conclusion:** We conclude that environmental management initiatives intended for infectious disease control might substantially reduce and/or lower the neighborhood urban environmental quality attributable fraction of the risk of urban malaria mortality more than that for urban diarrhea mortality in rapidly urbanizing areas in a low income setting.

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Incidence of hospital- and ICU-admitted community-acquired pneumonia: A population-based study in Uruguay

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**Background:** Community-acquired Pneumonia (CAP) is still an important health problem with high mortality and intensive care unit (ICU) requirement. Basal requirement rates of hospital and ICU (intensive care unit) admission are related to age, social condition, comorbidities and clinical severity and are important background for requirements in influenza pandemic period. Objective: to estimate the incidence rates of CAP requiring hospital- and ICU-admission in the department of Rivera, Uruguay, in non influenza pandemic period.

**Methods:** a prospective cohort of all CAP patient's > 15 years admitted between 1st May 2005 and 30th April 2007 to all hospitals and their respective ICU in Rivera, Uruguay, were assessed and followed up. Rivera is a northern department of Uruguay with 78.000 inhabitants and have 3 community hospitals. Annual incidence rates were calculated for age and sex strata. Denominators were obtained from National Institute of Statistics.

**Results:** 495 (63 ± 19 years, male 53.9%) and 99 patients were admitted to hospital and ICU, respectively. Annual incidence rates are showed in table.

	Rate of hospital admitted (n)			Rate of ICU-admitted (n)			
	Males	Females	Total	Males	Females	Total	
15 - 24 y	0.82 (14)	0.89 (15)	0.89 (15)	0.85 (29)	0.06 (1)	0.12 (2)	0.09 (3)
25 - 34 y	0.68 (9)	0.83 (12)	0.83 (12)	0.76 (21)	0.30 (4)	0.35 (5)	0.33 (9)
35 - 44 y	0.99 (14)	1.35 (20)	1.35 (20)	1.17 (34)	0.14 (2)	0.20 (3)	0.17 (5)
45 - 54 y	2.37 (28)	1.83 (22)	1.83 (22)	2.10 (50)	0.59 (7)	0.17 (2)	0.38 (9)
55 - 64 y	6.31 (53)	4.37 (41)	4.37 (41)	5.29 (94)	1.07 (9)	0.96 (9)	1.01 (18)
65 - 74 y	10.15 (62)	5.24 (39)	5.24 (39)	7.45 (101)	2.95 (18)	1.21 (9)	1.99 (27)
75 - 84 y	21.37 (65)	9.81 (44)	9.81 (44)	14.48 (109)	4.60 (14)	1.56 (7)	2.79 (21)
≥85 y	30.86 (22)	22.24 (35)	22.24 (35)	24.92 (57)	5.61 (4)	1.91 (3)	3.06 (7)
Total	3.58 (267)	2.82 (228)	2.82 (228)	3.18 (495)	0.79 (59)	0.49 (40)	0.64 (99)

\* episodes/1000 inhabitants-year

**Conclusion:** Incidence of hospital and ICU-admitted CAP was 3.18 and 0.64 episodes/1000 inhabitants-year, respectively. The incidence increased with age and were higher in men.

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The Survey of leptospirosis in Mazandaran province, north of Iran by Microscopic agglutination test, 2006-2007

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**Background:** Leptospirosis, a septicemic zoonosis with multisystemic involvement, is caused by the pathogenic strains of *Leptospira interrogans*. Rural farm workers are at high risk for leptospirosis, and it can be a significant public health problem when water and food safety are not ensured. Since, the incidence of leptospirosis is significantly high in temperate regions, rice field workers and livestock animals, it will be necessary to evaluate condition of leptospirosis in Mazandaran Province (north of Iran) and recognize common serotypes for use in vaccine production.

**Methods:** In this survey, 127 serum samples of suspected human were collected from different parts of Mazandaran Province during Nov 2006- Aug 2007 and were tested by Microscopic agglutination test (MAT) with cut-off  $\geq 1:100$  in *Leptospira* Research Laboratory, Faculty of Veterinary Medicine, University of Tehran.

**Results:** Prevalence rate of leptospirosis in Mazandaran Province using MAT was 52.75%. In this survey, 67 samples were positive which 80.6% of them were belonged to male and 19.4% to female. Rice field workers showed the most frequent occurrence of infection, so among positive cases 61.21% were belonged to rice field workers and showed significant variance with other occupations ( $P \leq 0.01$ ). There was no significant variance between genus and leptospirosis ( $P > 0.05$ ). Living place (urban/rural) and contact of person to animals did show no significant variance with leptospirosis ( $P \geq 0.05$ ). The most positive cases were found in age range of 30-40 years (25.4%). Seven common serotypes which were recognized in Mazandaran Province using MAT, included, Ballum (16.77%), Sejroe (14.29%), Tarassovi (13.64%), Australis (11.69%), Pyrogenes (7.79%), Javanica (6.5%) and Icterohaemorrhagiae (6.5%). Among all serotypes which were recognized in region, male and female reacted to 81.81% and 18.19% of them, respectively.

**Conclusion:** Because in Mazandaran Province, men and women work together at rice field, also urban peoples still relate to agriculture, it's acceptable that genus and living place have not significant variance with prevalence of leptospirosis. In order to as certain that the effect of contact with animals has no significant variance with the prevalence of leptospirosis, it needs more investigations, direct sampling from animals or their urine and comparison of them to the results of this study.

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Incidence of mycobacteria and fungi in clinically suspected urinary tract infection of immunocompromised patients – A tertiary care hospital study

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**Background:** Urinary tract infections are among the most common infections especially in hospital settings. Most of these infections are caused by bacterial agents. Mycobacterial and fungal agents of urinary tract infections although less frequent in immunocompetent individuals but they are more common and severe in immunocompromised individuals.

**Objectives**

Detection of Mycobacteria and Fungi in urine specimens of clinically suspected urinary tract infection of immunocompromised patients and evaluation of different tests for detection of Mycobacteria and Fungi in urine specimens.

**Methods:** Study was done in Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India, from October 2005 to September 2007. Urine samples of 50 patients with clinical suspicion of Tubercular and Fungal etiology of Urinary tract infection of immunocompromised patients [Diabetes mellitus (14), AIDS (13), Renal transplant recipient (09), Malignancies (07), End stage renal disease/ Chronic renal failure (06), Systemic lupus erythematosus (01)] & 50 apparently immunocompetant patients were taken as diseased control. For Mycobacteria microscopy (Ziehl-Neelsen staining), Culture (Lowenstein Jensen Medium and BACTEC medium), PCR and NAP test were done. For Fungus microscopy (Direct wet mount, Gram's stain), Culture (Sabouraud's Dextrose Agar), Germ tube test, Corn meal agar, Sugar Fermentation & assimilation test were done. Culture was considered as gold standard.

**Results:** Out of 50 immunocompromised patients, 7(14%) were positive for mycobacteria [MTBC 5(10%), MOTT 2(4%)] and 10(20%) were positive for fungi [*C. albicans* 4(8%), *C. glabrata* 4(8%) and *C. tropicalis* 2(4%)]. Out of 50 apparently immunocompetant patients (diseased control), 4(8%) were positive for mycobacteria [MTBC 3(6%), MOTT 1(2%)] and 3(6%) were positive for fungi [*C. albicans* 2(4%) and *C. tropicalis* 1(2%)]. Sensitivity and specificity were 71.42%, 90.69% of Ziehl-Neelsen stain, 88%, 100% of Lowenstein Jensen culture, 57%, 100% of PCR for Mycobacteria, 80%, 100% respectively for wet mount for fungi.

**Conclusion:** Incidence of mycobacterial/fungal infection in immunocompromised patients is more than to immunocompetent individuals and with immunocompromised conditions, urinary tract infection should be screened for mycobacterial and fungal pathogens.

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Molecular analysis and risk factors for nasal colonization with methicillin-resistant *Staphylococcus aureus* among community individuals attending a Family Health Care Program in Rio de Janeiro, Brazil

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**Background:** *Staphylococcus aureus* has been the most prevalent pathogen in hospital infections worldwide, including Brazil. Many factors contribute to the world dissemination of hospital endemic multiresistant MRSA strains, enabling the recent emergency of Community Acquired Methicillin-Resistant *S. aureus* (CA-MRSA) infections. Molecular methods have been used to investigate its role in community infections to address the emergency of outbreaks of new CA-MRSA clones.

**Methods:** We selected subjects with diabetes and/or Hypertension and their relatives, from different areas of the city of Niterói, in Rio de Janeiro, attending the Family Health Care program by Health Foundation of Rio de Janeiro, Brazil. A total of 519 subjects from the communities underwent swabbing of both nares. Colonies of interest were phenotyped and all methicillin resistant *S. aureus* isolates were genotyped to search for *mecA* gene, characterize pulsed-field gel electrophoresis profile and *SCCmec* type.

**Results:** Among all subjects, 357 were colonized with *S. aureus* (68.79%). Of *S. aureus* isolates, 37 (10.36%) were methicillin resistant. Women and men presented a prevalence of approximately 68%. However, when this isolates were compared for MRSA colonization, women presented a higher prevalence (11.11%) than men (7.24%). Individuals aged 60 years or more, had higher estimated probabilities of nasal *S. aureus* colonization (75.76%). However, subjects aged from 12 to 19 years and 40 to 59 years presented the highest methicillin resistant prevalence rates (10.57%). In addition, regions with poor sanitation conditions showed the highest level of *S. aureus* colonization. The analysis also revealed that 67% of the individuals carrying some metabolic syndrome were colonized by *S. aureus*.

**Conclusion:** The study suggests, corroborated by many authors, that subjects presenting diabetes and hypertension are found to be more colonized by *S. aureus* than other subjects. Hitherto, factors such as increase of the cost of treatment, the potential loss of productivity of infected patients, the possibility of untreatable infections and the increased mortality, indicate the need for a prevention of MRSA infections.

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The prevalence of *Staphylococcus aureus* nasal colonization among Korean children attending day care centers

**J.-H. Lee**<sup>1</sup>, Y. M. Kim<sup>2</sup>, E. Y. Cho<sup>1</sup>, K. B. Hong<sup>1</sup>, J. Lee<sup>1</sup>, E. H. Choi<sup>1</sup>, H. J. Lee<sup>1</sup>

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**Background:** There is an increasing incidence of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infection worldwide. Nasal colonization with *S. aureus* is a known risk factor for subsequent infection. The aim of this study was to investigate the prevalence and antimicrobial resistance of *S. aureus* colonizing anterior nares of children attending day care centers in Korea

**Methods:** Nasal swabs were obtained from both nostrils of the 425 children attending 7 day care centers located in Seoul, Korea. Nasal swab specimens were cultured in enrichment broth, and plated on mannitol salt agar to grow *S. aureus*. Antimicrobial susceptibilities were determined by disk diffusion method according to CLSI guidelines. The D-zone test was performed to detect inducible macrolide-lincosamide-streptogramin B (MLSBi) resistance. To identify risk factors for acquisition of MRSA, a standardized questionnaire was completed by the caregivers at the time of obtaining the specimens.

**Results:** Of the 425 children, 124 (29.2%) and 40 (9.4%) children were colonized with methicillin-sensitive and methicillin-resistant *S. aureus*, respectively. Only 2 out of the 40 children colonized with MRSA had been hospitalized within 1 year. *S. aureus* isolates were resistant to penicillin (98.2%), erythromycin (43.9%), clindamycin (1.2%), ciprofloxacin (0.6%), gentamicin (7.3%), chloramphenicol (1.2%), and tetracycline (3.7%). Inducible MLSBi resistance was found in 41.5% of *S. aureus* isolates by the D-zone test. All isolates were susceptible to vancomycin, trimethoprim-sulfamethoxazole, and rifampin.

**Conclusion:** MRSA carriage rates among Korean children attending day care centers were high. These findings necessitate the continued surveillance of the carriage rate and antimicrobial resistance patterns of *S. aureus*.

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Leptospirosis: analysis of the notification records and investigation on the year of 2007, of the municipal district of Salvador-Bahia-Brazil, 2009

**S. Cerqueira, J. F. Silva**  
Federal University of Bahia, Salvador, Bahia, Brazil

**Background:** The leptospirosis is an acute infectious disease that attacks men and animals. It is caused by microorganisms belonging to the genus leptospire. Leptospire is endemic in Brazil and the largest incidence commonly occur in areas with larger precipitation rainfall subject to the occurrence of inundations and of precarious socioeconomic conditions. It is a disease of compulsory notification in Brazil and it feeds the System of Information of Offences of Notification - SINAN. The notification is accomplished by the assistant units through the completion of a record that contains the patients personal data, and post-epidemic investigation. It collects important data such as race, occupation, disease related to the work, signs and symptoms, risk factors and data regarding the hospitalization, final classification of the disease, confirmation criterion and evolution among others

**Methods:** Study is grown through quantitative type - descriptive with the objective of identifying the fields that frequently left out in the notification records and investigation of the leptospirosis to discover the consequences of those actions for the evaluation of the data in the municipal district of Salvador-Bahia-Brazil. The completion of the listed fields were analyzed regarding partner-demographic data, occupation, pregnant, disease related to the work, hospitalization, final classification, confirmation criterion and evolution.

**Results:** It was verified that the data related to the conditions partner - demographic, beginning of the symptoms, investigation date and closing of the case frequently they were filled out accurately and correctly. The fields that presented smaller percentile of completion are: race, occupation, work-related diseases, hospitalization, final classification, confirmation criterion and evolution.

**Conclusion:** The lack of registrations in the fields disables the description of the epidemic profile of the population reached by the leptospire and as consequence the epidemic surveillance can't take measures to control and prevent it efficiently.

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Lassa fever awareness and practices in a Nigerian rural community

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**Background:** Lassa fever is one of the causes of morbidity and mortality in Nigeria. The knowledge, attitude and practice by the people is largely undetermined. This information is an important requirement for the design of a strategic plan for the control of the disease. This necessitated the study in Ebhodiza, Uhie-Ekpoma, a community of about 500 persons.

**Methods:** By means of a cluster sampling technique, one hundred and sixty (160) interviewer administered structured questionnaires were distributed to the respondents while one hundred and forty seven (147) of them were retrieved, giving a response rate of 91.9%.

**Results:** Farming was predominant occupation (61%). Only 32% had up to primary school level of education. Thirty-six percent (36%) of the respondents have heard about the disease, mainly through the electronic media. Fifty-one percent (51%) identified rats as the main source of infection of Lassa fever, while 14% of respondents said that the disease can be spread from person to person.

The study revealed that only 31% of the people had a correct knowledge of Lassa fever, and 32% had poor attitude and engage in practices that favor transmission of the disease. Most (72%) of the respondents do not use any means to control rats in their households. Forty-three percent (43%) of respondents encouraged bush-burning.

There was a significant relationship between the level of education of respondents and knowledge of Lassa fever ( $X = 3.4$ ,  $P < 0.05$ ). However, the relationship between the occupation of respondents and practices was not significant ( $\chi = 3.2$ ,  $P > 0.05$ ).

**Conclusion:** It is therefore recommended that the three tiers of government and non-governmental organizations (NGOs) in Nigeria should intensify effort in adequately educating the populace through enlightenment campaigns most especially through the mass media and religious groups on the mode of transmission of the disease, its signs and symptoms and encourage community participation in health education and environmental sanitation.

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Serological update of the Chikungunya epidemic outbreak in Italy

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**Background:** Chikungunya (CHIKV) is an arthropod-borne virus belonging to the *Alphavirus* genus in the *Togaviridae* family. Epidemic Chikungunya virus (CIKV) infections result common limited to several countries of the tropical areas. The only epidemic of CHIKV that aroused outside the tropical areas was the small epidemic outbreak occurred in the Emilia Romagna region, Italy, during summer of 2007. Local transmission has been made possible by the enormous population of *Aedes albopictus*. On the basis of these facts, a serological screening of chikungunya fever was initiated by Centro Riferimento Regionale Emergenze Microbiologiche (CRREM) of Bologna to determine the specific CHIKV IgG and IgM titres in confirmed CHIKV case.

**Methods:** Serum obtained from the 172 CHIKV infected persons were collected and tested for the presence of CHIKV antibodies. Specific antibodies titres were analyzed by an indirect immunofluorescence assay for the detection of anti-CHIKV IgG and IgM by using a commercial EUROIMMUN assay.

**Results:** During the outbreak occurred in Emilia-Romagna on 2007, all serum from laboratory confirmed CHIKV infections were collected and tested as follows. Of these samples, 108 (62,8%) were CHIKV positive both in IgM and IgG, 27 (15,6%) were only IgM positive, 12 (6.9%) were IgG positive only. In opposite, 25 (14,5%) were negative both for IgM and IgG, but tested PCR positive. Moreover the 71,4% of the IgM positive samples had a titre comprised between 1/100 and 1/1600. In addition the 85,8% of the IgG positive samples had a titre ranging from 1/100 to 1/6400.

**Conclusion:** Here we report the first sero-prevalence study for specific CHIKV-antibodies in a population outside the tropical geographic areas. In addition, this study was performed on all the autochthonous CHIKV infected persons. The results obtained clearly demonstrated the high level of antibody response to CHIKV in the acute phase, or immediately after, infection. Studies are presently on going in order to define the persistence of CHIKV specific antibodies.

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Sero-prevalence of West Nile virus in north-eastern Italy

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**Background:** West Nile virus (WNV) is a member of the flavivirus family and is transmitted in natural cycles between birds and mosquitoes, particularly *Culex* spp. Human and horses are susceptible but dead-end hosts. Firstly identified in tropical Africa, WNV infection has been evidenced in northern Africa, Israel, India and Australia. WNV spread in America since 1999 and has been the cause of outbreaks and sporadic cases in central eastern and Mediterranean Europe for more than 45 years. Of people infected with mosquito-borne WNV, 80% develop asymptomatic infections, 20% develop symptomatic infections and <1% develop severe neuro-invasive disease. The first human cases of neuro-invasive WNV infection was identified in 2008 near Ferrara province where had been identified several WNV-positive horses and birds. After this first case of WNV infection in Emilia-Romagna, a sero-epidemiological survey was started to ascertain the sero-prevalence of specific antibodies in the blood donor population and the relative risk in population.

**Methods:** Serum obtained from 10.000 blood donors resident in Ferrara province were collected between September 2008 to April 2009, and tested for specific WNV. Virus-specific IgG were detected in serum specimens by enzyme-linked immunosorbent assay (ELISA). To confirm results of positive IgG samples and to determine the effectively titers in the serum, an Immuno Fluorescent assay (IFA) was performed.

**Results:** Between a period of 8 months, since autumn 2008, 10.000 serum obtained by blood donors in Ferrara province were collected and tested as described in previously. 70 of the 10.000 serum analyzed resulted positive in IgG. This data showing an incidence of 0.7% in a blood donors population.

**Conclusion:** After the outbreak of WNV infections in Italy during the 2008, a sero-prevalence study was performed to determine the WNV mosquitoes-related infections in a blood donors in Italy. We report the first study of human sero-prevalence in Europe involving a high number of persons. The first data of the study show an incidence of 0,7% and suggest the large diffusion of WNV in the area studied. In addition, the high incidence prospect a necessary surveillance in blood and organ donors, performed by NAAT in this part of Italy.

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A retrospective laboratory analysis of clinically diagnosed Lassa fever cases in a tertiary hospital in Nigeria

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**Background:** Lassa fever (LF) is associated with a wide spectrum of clinical manifestations and its clinical diagnosis is often complicated by the fact that the initial symptoms of the disease are indistinguishable from a number of other infections seen in endemic areas. Over the years, many outbreaks and deaths from LF have been reported in Nigeria mostly associated with no or inadequate laboratory testing. Consequently, the accuracy of clinical diagnosis is therefore of interest.

**Methods:** Data including blood specimens and background information were collected from 267 consented in- and out-patients clinically diagnosed to have LF at the Irrua Specialist Teaching Hospital, Nigeria. Blood specimens were tested for Lassa virus RNA using Reverse transcriptase polymerase chain reaction (RT-PCR). Data derived were analyzed using EpiInfo 6.04a version.

**Results:** Comparison of clinical diagnoses with laboratory test results showed a low specificity of clinical judgement. Of the 267 clinically diagnosed cases, LF was laboratory-confirmed in 62 (23.2%), of which 35 (56%) were rightly treated with Ribavirin. On the other hand, 57 (28%) of the 205 LF negative patients were erroneously treated with Ribavirin. However 35 (56%) of these patients recovered, 18 (29%) died and 1(2%) was referred to another hospital for further attention. Patients that tested positive to LF were more likely to report vomiting, bleeding and have fever, sore throat, headache, abdominal pain and malaise than those that did not ( $p < 0.05$ ).

**Conclusion:** Until now, the diagnosis of LF is mainly clinical, and was considered sufficient for treatment decision. Unfortunately, clinical diagnosis of LF by health workers is hardly achievable especially in the absence of haemorrhage. Ribavirin can only help if it is administered early in the course of illness. In the above elucidated challenge, prompt diagnosis is clearly needed for early commencement of treatment. The place of appropriate laboratory support in the effective and prompt diagnosis cannot be over-emphasized in the management of LF in all endemic countries of West Africa.

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Syphilis among young women: a population based survey in central Brazil  
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**Background:** Syphilis remains an important public health problem worldwide, despite preventive measure and effective treatment available for decades. The evolving epidemiology, changing risk groups, and social environments present challenges for syphilis control and further elimination. Population based syphilis prevalence studies are scarce among adolescent and youth, considered a vulnerable group for sexually transmitted diseases (STD). To estimate the prevalence of syphilis in adolescents and young females, sexually active in central part of Brazil.

**Methods:** The study was a population-based cross sectional study conducted among 15 to 24 years old sexually active females attending Family Health Program (~ 14.000 families registered at FHP), in three medium sized cities (~10.650 to 75.600 inhabitants) of Goias State- Central Brazil, from 2007 to 2009. Potential participants were randomly selected from a nominal list of youth registered at local FHS. Community health agents invited the participants for an interview at the local health center. The demographic data and sexual behavior profile were investigated by confidential interview. For those sexually active, a serum sample was collected for syphilis diagnosis using two screening tests (VDRL and ELISA) followed by a confirmatory test (FTA Abs).

**Results:** 1073 participants were interviewed, among them 698 (65.0%) were sexually active. The median age of the first sexual relation was 15.9 years (dp=2.1), 73.5% of the participants referred inconsistent condom use, and 38.8% had at least one previous pregnancy. Eleven women were positive on screening test (VDRL or ELISA) for syphilis. One sample was positive on screening and on the confirmatory test, yielding a syphilis prevalence of 0.14% (CI95% 0.00-0.79).

**Conclusion:** The overall prevalence of syphilis was lower than the expected for pregnant women and blood donors, in Brazil. However, adolescents and young women, of the evaluated cities, reported high risk sexual behavior for SDT, pointing out for the need of preventive strategies towards syphilis control and future elimination.

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Clinical features of *Plasmodium malariae* in Venezuela

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**Background:** Despite the increased mass of knowledge in different aspects of malaria, *P. malariae* is the least studied of the four species that infect humans; in part because of its low prevalence. There is evidence that *P. malariae* infections are vastly underreported. We undertook a prospective cohort study to investigate the clinical features of *P. malariae* infections in endemic areas in Venezuela.

**Methods:** National malaria parasite data per year was obtained from the national malaria surveillance system. A prospective cohort study assessing the clinical and parasitological profiles of malaria infections was conducted among patients attending the malaria post at the Centro de Investigación de Campo Francisco Vitanza (CICFV) in Tumeremo, Sifontes Municipality, Bolivar State, Venezuela. A pre-piloted clinical record form was used to capture information on each malaria episode. Malaria blood films were stained with 4% Giemsa and read using microscopy (1000X). 100 microscopic thick film fields were inspected before a slide was being declared malaria negative. For malaria-positive smears parasite species were identified and densities recorded as number of parasites per 200 WBC.

**Results:** Between 2005 and 2007, there were 120 cases of *Pm* reported in Venezuela - CICFV diagnosed 71. Detailed clinical data was available among 59 cases (83%). All *Pm* cases occurred in communities located along the Venezuelan-Guyanese border. Mean age was 28 years (1-79). All cases reported fever and/or history of fever in the last 2 days (100%), other signs/symptoms included: headache (92%), chills (86.4%), sweating (83.1%), osteomuscular pain (78%), weakness (66%), and poor appetite (64%). Cough and difficult to breath was reported in 61% and 37%, respectively. No nephritic syndrome was found. Median parasitemia was 660 parasites/uL (range 48-17604 uL).

**Conclusion:** For our knowledge, this is the first report on the clinical features of *P. malariae* in Venezuela. Fever, chills and headache were the most common signs/symptoms reported. Interestingly, as seen in *P. falciparum* in Africa, respiratory symptomatology was present among *Pm* cases. Nephrotic syndrome was not reported. Parasitological profiles and socio-economical data were collected, describing a case with high parasitemia. Further research is necessary in order to describe the full clinical spectrum of *Pm* infections in the Amazon basin.

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Severe malaria surveillance and treatment delays in Canada

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**Background:** Malaria remains the principle life-threatening infection for travellers to endemic regions and severe malaria has a 20% case fatality rate. The Canadian Malaria Network (CMN) provides intravenous anti-malarial therapy and collects surveillance data on all malaria cases utilizing parenteral therapy.

**Methods:** Examines epidemiological features and treatment delays associated with cases of malaria reported to CMN between June 2001 and July 2009.

**Results:** Over the eight years 131 malaria cases were reported (an average of 15 reports per annum). Species distribution: *P. falciparum* 90%, *P. vivax* 3% , *P. malariae* <1% and 4% unknown. 87% of cases were acquired in Africa and a majority of cases presented in Ontario (51%) and Quebec (30%). Overall, there was an equal distribution of gender. Only 31% of the cases reported seeking pre-travel advice and 28% indicated any chemoprophylaxis use. 55% of cases were non-Canadian born, 63% adults, 30% minors. Most indicated immigration as the reason for travel. 45% of cases were Canadian born, 76% adults and 20% minors. Visiting friends/relatives was the major reason for travel.

Median delay between arrival in Canada and symptoms was 6 days; between symptoms and first physician visit was 3 days. The Median delay between the 1st physician visit and diagnosis or between diagnosis and request for malarial treatment/administration was found to be 0 days. In 41% of the cases, a delay of one or more days occurred between diagnosis and treatment.

**Conclusion:** The time delay in consulting a physician and the low rate of individuals seeking pre-travel advice indicates gaps of knowledge among persons arriving to, or returning from endemic regions. The inappropriate use of chemoprophylaxis and delays in treatment requests indicates gaps of knowledge with respect to the treatment provider. Canadians who plan to visit endemic regions should be instructed to seek pre-travel advice regarding malaria. Non-Canadians immigrating to Canada should be instructed to seek medical attention if they experience fever to ensure early diagnosis and treatment.

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Leptospirosis in Mazandaran province, northern Iran, 2008-2009

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**Background:** Leptospirosis is one of infectious diseases cause by pathogenic leptospira. This is an important zoonotic disease. Most of human morbidities occur by contact with patient animals' urine contaminant water and soil. This disease in special occupation (such as rice and cane farm workers, veterinarian, laboratories staff, slaughter house personal and herdsman) is seen more. This evaluation accomplished to determine morbidity rate of this disease in patient who referred to Mazandaran Province leptospirosis diagnostic center in Pasteur institute of Iran, Amol Research Center, Dept. of Parasitology.

**Methods:** Suspect patient blood samples along with filled out questionnaires by infection disease specialists from different hospitals of Mazandaran Province (such cities as Sari, Babol, Qaemshahr, Amol and ....) were send to Pasteur institute of Iran, Amol Research Center, Dept. of Parasitology during March 2008-March 2009. After separating samples sera, serologic study was done by Indirect Immunofluorescent Antibody Assay (IFA) and Cut-off for this method was  $\geq 1/80$ .

**Results:** From 157 suspect samples, 36 (22.93%) were female and 121 (77.07%) were male. From 36 female patients, of them 2 farmer and 4 housekeepers who also work in farms, were serological positive. From 121 male patients, 32 of them, 18 (78.26%) farmers, 2 (8.69%) employees, 2 (8.69%) self employees and 1 (4.36%) herdsman were serological positive.

**Conclusion:** Analyses of questionnaires showed that leptospirosis had a high prevalence in farmers and herdsman. So it can be considered as an occupational disease. More occurrence of leptospirosis in male than female is explainable duct a working of more men than women in farms.

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Epidemiologic comparison of Human *Brucella suis* and *Brucella melitensis* infections

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**Background:** *Brucella suis* is endemic in feral swine across the state of Florida, and with the eradication of *Brucella abortus* in cattle, has emerged as the primary cause of human and animal Brucellosis in the state. *Brucella melitensis* is not present in Florida but is endemic in Mexico. Many Mexican immigrants return to visit friends and family in Mexico regularly, where they may be exposed to *Brucella* through consumption of unpasteurized milk products or domestic animal contact. Although *B. suis* has a worldwide distribution, most epidemiologic case studies describe demographics, clinical presentation and other epidemiologic information from patients infected with *B. melitensis* and *B. abortus*. This study provides an epidemiologic comparison of 36 *B. suis* and 11 *B. melitensis* infected patients reported to Florida Department of Health.

**Methods:** Human surveillance data from 1997-2008 were reviewed including clinical symptoms and signs, time from symptom onset to treatment and prescribed treatment. Thirty-six patients culture positive for *B. suis* (32 patients), epidemiologically linked to a confirmed patient (1 patient) or serologically confirmed per national guidelines (3 patients) were compared to 11 *B. melitensis* culture positive patients reported during the same time-frame.

**Results:** Most *B. suis* patients were male (92%), white (94%) and non-Hispanic (81%) with an average age of 43 years (range 18-78). Endocarditis or aneurysm was reported in 4 (11%) of *B. suis* patients, two with fatal results. Of the 22 patients with medical treatment information all were prescribed doxycycline, 14 (66%) rifampicin and 13 (62%) an aminoglycoside; 4 (11%) patients relapsed. *Brucella melitensis* patients were 55% male, 55% white and 73% Hispanic. Average age was 37 (range 4-76). Gastrointestinal signs and/or abdominal pain were reported in 4 (36%) of patients, as was single joint or bone pain. Only 4 (44%) of patients were prescribed doxycycline, 2 (22%) rifampicin and 1 (11%) and aminoglycoside; 3 (27%) patients relapsed.

**Conclusion:** Patient demographics, clinical presentation and clinical management were different for infections with different *Brucella* species and warrant further study to improve disease prevention strategies and treatment recommendations. Physician education, standardized guidance for convalescent monitoring, and improved testing to reduce incidence of disease relapse are needed.

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Shigellosis in Central American countries

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**Background:** Shigellosis is an important cause of morbidity and mortality around the world. *Shigella* spp is transmitted person to person through the faecal-oral route, also by contaminated food and water. The infection is considered as a childhood disease but it also represents an important cause of diarrhea in adults, particularly among tourists who travel to endemic areas. The symptoms range from mild watery diarrhea to severe bacillary dysentery with fever, abdominal pain, blood and mucus in stool samples. The indiscriminate use of antimicrobial drugs is responsible for the increasing resistance among *Shigella* spp. strains worldwide. Even though shigellosis could be a prevalent infection in the region, there are insufficient studies from Central America.

**Methods:** Fecal specimens from 3045 children up to 10 years old from San José, León, San Salvador and Panama, hospitalized due to severe diarrhea were collected and analyzed at local laboratories using standardized bacterial culture. Samples were inoculated in selective media and analyzed by biochemical tests and serology. Antibiotic susceptibility test was performed to *Shigella* positive strains by the Kirby-Bauer Method (Costa Rica, Nicaragua and El Salvador) and the Biomerieux Vitek Systems (Panama).

**Results:** The frequency of *Shigella* in Central American countries was 9.4%(16/170) in Costa Rica, 3.5%(18/508) in Nicaragua, 8.9% (95/1071) in El Salvador and 8.4%(109/1296) in Panama. Eight strains of *S.dysenteriae* (4 from El Salvador y 4 from Panama), 165 of *S.flexneri* (8 from Costa Rica, 14 from Nicaragua, 95 from El Salvador y 109 from Panama), 55 of *S.sonnei* (5 from Costa Rica, 4 from Nicaragua, 20 from El Salvador and 26 from Panama) and 7 of *S. boydii* (El Salvador) were found. Resistance of the *Shigella* isolates to 7 out of 12 antibiotics tested such as Ampicillin, Cephalotin, Gentamicin, Erythromycin, Chloramphenicol, Tetracycline and Trimetoprim-sulfamethoxazole was observed.

**Conclusion:** *Shigella* spp is a common pathogen in the Central American region. *S.sonnei* and *S.flexneri* species were the most common agents. In Costa Rica and Nicaragua only *S. flexneri* and *S. sonnei* species were isolated meanwhile in El Salvador and Panama *S. dysenteriae* and El Salvador *S.boydii* were also found.

Emergence of resistant-*Shigella* strains to common antibiotics was observed, in particular with Trimethoprim-sulfamethoxazole (more than 60% of strains), Ampiciline, Tetracycline, Erythromycin and Cephalotine.

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Detection and molecular characterization of verotoxin gene in non-O157 diarrheagenic *Escherichia coli* isolated from Miri hospital, Sarawak, Malaysia

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**Background:** Non-O157 diarrheagenic *Escherichia coli* are typical *Escherichia coli*. Infections with non-O157 *Escherichia coli* are now increasingly recognized in many countries. The virulence profiles of most of the non-O157 *E. coli* are unknown. Therefore, easy detection, isolation, and characterization of non-O157 *Escherichia coli* isolates are essential for improving our knowledge of these organisms.

**Methods:** A total of 32 non-O157 diarrheagenic *Escherichia coli* isolated (Miri hospital, Sarawak, Malaysia, 2001) from the patients with diarrhea were examined for the detection of verotoxin (VT) gene. For this purpose, two sets of primers (VT1 and VT2) were used for PCR method. VT1 probe was prepared which was used for the Southern hybridization study. Bacteriophage induction was carried out using mitomycin C. Nucleotide sequencing was made from the VT1 gene fragment isolated from an *E. coli* isolates using PCR protocol.

**Results:** The PCR protocol used here produced a fragment of 348 bp of VT1 gene and 584 bp of VT2 gene, respectively. It was shown that 13 *E. coli* isolates (40%) carried VT1 gene whereas none was found to carry VT2 gene using PCR protocols. Plasmids were detected in all the VT1-positive isolates. From the Southern hybridization study, VT1 probe showed homology with the chromosome of all 13 VT1-positive isolates indicating the VT1 gene to be located on chromosome. Bacteriophage induction as carried out using mitomycin C showed that none of the VT1-positive isolates harbor any lysogenic bacteriophage. However, nucleotide sequencing made from the VT1 gene fragment isolated from an *E. coli* isolates using PCR protocol showed 97% homology with the known VT1 gene which proves to be the similar gene carried by *E. coli* O157 and other ancestors.

**Conclusion:** The PCR method used here was sensitive, specific and reliable. VT1 was found to be the most common verotoxin among the *E. coli* strains isolated from clinical sources in Malaysia and this VT1 gene is located in the chromosome of the *E. coli* isolates. This study improves the knowledge of a highly significant emerging pathogen non-O157 *E. coli*.

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Isolation and characteristic distribution pattern of *cagA+* *Helicobacter pylori* in dental plaque of dyspeptic patients

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**Background:** *Helicobacter pylori* is one of the most common human pathogens which colonize in stomach. Recently the oral cavity has been proposed as a reservoir for *H. pylori* but not much evidence from the presence of *cagA+* *H. pylori* in dental plaque is available.

The aim of this study was to investigate the possibility of isolation of *cagA+* *H. pylori* in dental plaque of dyspeptic patients and to study the distribution pattern of *cagA+H. pylori* in different areas of oral cavity.

**Methods:** One hundred (100) patients with dyspepsia, attending the routine endoscopy were examined and samples from sub gingival plaque of molar and incisor areas of patients were taken, placed in suitable transport media and immediately sent to lab before 4 h. All samples cultured on modified brucella blood agar and all the susceptible colonies were tested by urease, catalase, oxidase and gram stains. Also after DNA extraction process, two sets of primers, highly specific for *ureC* and *cagA* were used for PCR.

**Results:** *H. pylori* was detected in dental plaque samples of 40 ( 40%) of patients by culture on enriched brucella agar media , 100% of these samples were positive by PCR of *ureC* gene and 25 samples ( 62.5%) were also positive for *cagA* gene. Results of PCR for *ureC* gene on different areas of dental plaque, also showed that sub gingival plaque of molar areas had the most microbial load of *H. pylori* (73%, 29 cases) in comparison of incisor sites (27%, 10 cases).

**Conclusion:** According to our findings, it's estimated that dental plaque, especially sub gingival plaque of molar areas, can be a suitable reservoir for *H. pylori*. Being out of reach of oxygen and difficult access of this site for cleaning tools of oral cavity can confirm this thesis.

Also it was shown in this study that *cagA + H. pylori* can be isolated from dental plaque of dyspeptic patients , so maybe *H. pylori* can be transmitted by oral route and oral cavity may act as a good source of keeping and re-infecting of stomach of dyspeptic patients after antibiotic therapy

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Multiresistant *Acinetobacter spp* in a burn unit in Argentina

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**Background:** Multiresistant *Acinetobacter spp* has emerged as an important microorganisms in critical areas like burn units.

**Objetives:** Infections caused by multiple drug resistant (MDR) *Acinetobacter spp* was studied in our burn unit to evaluate epidemiological, clinical features, outcome, use of colistin and adverse effects related with this antibiotic.

**Methods:** A prospective chart review of pediatric patients admitted to our tertiary burn unit between January 2005 and December 2006 with isolation of multiresistant *Acinetobacter spp* was performed. During the study period, 26 p were evaluated with isolation of *Acinetobacter spp*.

**Results:** The mean age of the patients was 66.5 months (range: 2 to 168 m), 17 patients (65%) were male. The burnt surface was between 10% and 87% (median 42.5%). Full thickness was present in 10 p (39%). Burn wound sepsis was the most frequent focus in 10 patients (39%). In 8 patients (30%), burn wound infection occurred without sepsis. Intravascular catheter-related bacteremia was found in three patients (11%) and bacteremia in one (4%). Two patients had pneumonia (8%), and two urinary tract infection (8%). All patients were treated with colistin according susceptibility test.

The time of colistin treatment was between 10 and 71 days (median 21days).

None of the children developed adverse effects attributable to colistin. The outcome was favorable in all patients.

**Conclusion:** Multiresistant *Acinetobacter spp* has emerged as a new threat in burn units. Burn wound sepsis was the most common foci. Colistin was a safe drug for the treatment. The outcome was favorable in all patients.

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Investigation of *Helicobacter pylori* in dyspeptic patients and its relationship with Iranian life style  
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**Background:** *Helicobacter pylori* infection is recognized as an important player in the development of chronic gastritis, peptic ulcers, and gastric carcinoma. The aim of this study was to evaluate the association between *H. pylori* infection and clinical symptoms in patients referred to a referral academic hospital (Shahid Beheshti hospital).

**Methods:** A total of 303 patients with dyspeptic symptoms underwent endoscopy in the academic hospital. Clinical data were collected for each patient and gastrointestinal symptoms were evaluated by using the Gastrointestinal Symptom Rating Scale Questionnaire. During endoscopy two biopsy specimens were taken from the antrum for histological evaluation.

**Results:** Among the 303 patients, 263 (86.8%) were found to be positive for *H. pylori*, of whom 43% were male and 57% female. The prevalence of *H. pylori* infection in patients with anorexia, nausea, heart burn and belching was 98 (32.3%), 89 (29.4%), 132 (43.6%) and 171(56.4%), respectively. Likewise, we found 112 patients with abrupt pain and 46 with pain by consumption of tea were *H. pylori*-positive. Also 98 (32.3%) patients with a positive family history of gastroduodenal diseases had *H. pylori* infection. Among them, 20 of 41 patients with a family history of gastric cancers and 50 from 73 with a family history of gastroduodenal ulcers were infected by *H. pylori*.

**Conclusion:** There was an increased risk of *H. pylori* infection in 60-69 aged patients (OR= 0.27, 95%CI, 0.086-0.87) compared to young people with aged 20-29. *H. pylori* was not associated with, marital status, education and ethnic group of the patients (P > 0.05). We found no significant difference in the prevalence of *H. pylori* infection according to ethnicity (P > 0.05). Also we did not observe any relationship between *H. pylori* infection and family history. It was observed that some symptoms such as belching, patients with abrupt pain and pain with consumption of tea was significantly associated with *H. pylori* infection, whereas there was no difference between consumption of other foods and other clinical symptoms.

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Cytokine profile in patients with severe gram negative sepsis

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**Background:** Cytokine-related systemic intravascular inflammation may represent a common pathogenic link between initial insult and multisystem organ failure. Interactions between different cytokines in sepsis are complex and not well understood. The primary aim was to study the effect of standard therapy on serum levels of IL1 $\beta$ , IL-6, IL-16, and TNF- $\alpha$  and CRP levels in patients with severe gram negative sepsis/septic shock.

**Methods:** In a prospective study, 60 patients with severe gram negative sepsis or septic shock (as per ACCP/SCCM Consensus Conference definitions) were studied for 28 days or survival. All standard therapy was given as per the hospital protocol. Samples for cytokine estimation were drawn at enrolment and again at the end of 4 weeks. Serum cytokine levels were assayed using commercially available immunoassay kits for IL1 $\beta$ , IL-6, TNF- $\alpha$ , IL-16 and hsCRP, as per the specification of the kit.

**Results:** In the study group 40 (66.7%) patients survived while 20 (33.3%) patients had expired before completing 28 days of follow up. Diabetes (26.7%), chronic kidney disease (13.3%) and hypertension (11.7%) were the most frequent underlying diseases. 43 patients (71.6%) were culture positive for gram negative organisms. E.coli (35%), Acinetobacter (18%) and Klebsiella (13%) were amongst the commonest isolated organisms. Stepwise regression analysis revealed that metabolic acidosis alone accounted for a 11.7% risk and in combination with nervous system dysfunction a 20.6% risk of death ( $p=0.014$ ). Levels of cytokines – IL1 $\beta$ , TNF $\alpha$ , IL-6, IL-16 and hsCRP were significantly higher than the normal range at the time of admission. Statistically significant decline in the day 28 cytokine levels was seen in IL-6 and IL-16 ( $p<0.05$ ) while there was no change in IL1 $\beta$ , TNF $\alpha$  and hsCRP. There was no correlation between cytokine levels and individual organ dysfunction, although IL1 $\beta$ , TNF alpha and CRP levels were significantly elevated in patients with multisystem organ failure. There was poor correlation between serum cytokines and survival except for hsCRP.

**Conclusion:** Serum proinflammatory cytokines are markedly elevated in severe sepsis/septic shock due to gram negative sepsis. However a cause and effect relationship between cytokine levels and outcome could not be established.

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*Burkholderia pseudomallei* musculoskeletal infections (Meloidosis) in India: Are the orthopaedic surgeons aware?

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**Background:** Melioidosis, an infection due to gram negative *Burkholderia pseudomallei*, is an important cause of sepsis in East Asia especially Thailand and northern Australia. It usually causes abscesses in lung, liver, spleen, skeletal muscle and parotids especially in patients with diabetes, chronic renal failure and thalassemia. Musculoskeletal melioidosis is not common in India even though sporadic cases have been reported mostly involving soft tissues.

**Methods:** During a two-year-period, we had five patients with musculoskeletal melioidosis. All patients were diagnosed on the basis of positive pus culture.

**Results:** All patients presented with multifocal osteomyelitis, recurrent osteomyelitis or septic arthritis. One patient died early because of septicemia and multi-organ failure. All patients were treated by surgical debridement followed by a combination of antibiotics; (ceftazidime, amoxy-clavulanic acid, co-trimoxazole and doxycycline) for six months except for one who died due to fulminant septicemia. All other patients recovered completely with no recurrences.

**Conclusion:** With increasing awareness and better diagnostic facilities, probably musculoskeletal melioidosis will be increasingly diagnosed in future.

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Spectrum of hepatic dysfunction in enteric fever

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**Background:** Liver involvement in enteric fever can be in the form of asymptomatic biochemical alterations or hepatitis like presentation. This condition has been described as "Typhoid/Salmonella Hepatitis". In India, where enteric fever is endemic, hepatic involvement either clinical or biochemical is not uncommon.

This study was undertaken in order to observe the hepatic manifestations in enteric fever and their clinical significance.

**Methods:** A prospective study was done from November 2005 – April 2007 at Kasturba Hospital, Manipal (Karnataka), India. Patients diagnosed to have enteric fever based on the isolation of *S typhi* or *S. paratyphi* in blood or bone marrow cultures or a rising (four fold) titer of Widal agglutinins on serial estimation were studied with particular reference to liver function abnormalities.

Patients satisfying any 3 of the following criteria were classified as Salmonella hepatitis;

1. Jaundice
2. Hepatomegaly
3. Elevated liver enzymes > 2 times normal.
4. Prolonged Prothrombin Time
5. Abnormal histopathology

**Results:** 111 patients with enteric fever were included in the study, of which 56 patients (50%) were diagnosed with typhoid fever and 55 patients (50%) were found to have paratyphoid fever. The most common abnormality observed was deranged liver enzymes more notably in patients presenting beyond the second week of illness;

AST levels were abnormal in 93 patients (82%), range (16 - 757 U/L), average value 118.8 U/L.

ALT levels were abnormal in 80 patients (72%), range (7 - 500 U/L), average value 100.3 U/L.

ALP levels were abnormal in 21 patients (20%), range (25 -1153 U/L).

Hyperbilirubinemia was present in 13 patients (11%).

We observed 13 cases of Typhoid hepatitis (11.7%).Jaundice, hepatomegaly and abnormal biochemical tests were present in all of these patients.

The antibiotic sensitivity patterns were similar in both hepatitis and non hepatitis group of patients with all hepatitis patients showing a good response and normalization of liver functions with treatment.

**Conclusion:** Liver involvement is common in enteric fever with elevated transaminases as the most common abnormality noticed. Hepatitis with jaundice is a rare presentation of enteric fever; however in tropics recognition of Salmonella hepatitis in patients presenting with fever and jaundice is important and should be differentiated from common ailments presenting in a similar manner like viral hepatitis, malaria and leptospirosis.

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Verotoxin gene in non-O157 diarrheagenic *Escherichia coli* can be plasmid-mediated

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**Background:** Infections with diarrheagenic non-O157 *Escherichia coli* are now increasingly recognized in many countries. Verotoxins were first identified in *Shigella dysenteriae*, where it is chromosomally encoded but the genes for its production are readily transmitted between *E. coli* strains by toxin encoding bacteriophages. VT1 and VT2 genes are encoded on two different lysogenic lambdaoid temperate bacteriophages inserted into the chromosome in *E. coli*.

**Methods:** A total of 30 non-O157 diarrheagenic *Escherichia coli* isolated (University Hospital, Kuala Lumpur, Malaysia, 2003) from the patients with diarrhea were examined for the detection of verotoxin (VT) gene. For this purpose, two sets of primers (VT1 and VT2) were used for PCR method. VT1 probe was prepared which was used for the Southern hybridization study. Bacteriophage induction was carried out using mitomycin C. Nucleotide sequencing was made from the VT1 gene fragment isolated from a 7.2 kb plasmid of an *E. coli* isolate using PCR protocol.

**Results:** The PCR protocol produced a fragment of 348 bp of VT1 gene and 584 bp of VT2 gene, respectively. Twelve *E. coli* isolates (40%) carried VT1 gene whereas none was found to carry VT2 gene. Plasmids were detected in all the VT1-positive isolates. VT1 probe showed homology with the chromosome of 10 VT1-positive isolates after Southern hybridization indicating the VT1 gene to be located on chromosome. Interestingly, VT1 probe also showed homology with the 7.2 kb plasmid of 2 VT1-positive isolates indicating the VT1 gene to be located on a plasmid.

Bacteriophage induction as carried out using mitomycin C showed that none of the VT1-positive isolates harbor any lysogenic bacteriophage. However, nucleotide sequencing made from the VT1 gene fragment isolated from the 7.2 kb plasmid of an *E. coli* isolates using PCR protocol showed 97% homology with the known VT1 gene.

**Conclusion:** In this study, we believe to report for the first time in the world that VT1 gene to be located on a 7.2-kb plasmid of non-O157 *E. coli* and there is no evidence of a lysogenic phage present in it. VT1 was found to be the most common verotoxin among the *E. coli* strains. The PCR method used here was sensitive, specific and reliable.

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Vertebral column damage due to brucellosis

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**Background:** Brucellosis is zoonotic infection very common in Albania (and Balkans region), because of uncontrolled infection of animals, especially in sheeps and goats. The evaluation of clinical, laboratory and vertebral imaging findings is important to assess the efficacy of the treatment applied.

**Methods:** The author has studied 12 patients, 20-74 years old, hospitalized in Infectious Diseases Department of UHC during the period Jan. 2002 - Oct. 2009. They were all diagnosed with Brucellosis confirmed by serological tests: RB, Wright and ELISA (IgM, IgG). They all, had also, vertebral lesions seen in MRI or CT examination. The treatment protocol consists of a combination of three antibiotics and steroidal and non steroidal antiinflammatories administered over a three months period. Then the MRI or CT examination was repeated.

**Results:** Totally, 11 cases (91%) were considered to be in the subacute form of the disease, while 1 case (9%) in the acute form. All of them had back pain and difficulties in walking. According to CT or MRI findings in 3 cases (25%) the region affected was the L2-L3 level, in 5 cases (41%) the L3-L4 level, in 2 cases (16%) the L4-L5 level and only in one case (8%) the damage was seen in C2-C3 level. Also, in one case there were simultaneously lesions in T11-T12 and L2-L4 vertebral levels. In 3 cases (25%) oedema of paravertebral soft tissue was observed, without abscess formation. Narrowing of intervertebral spaces was seen in 11 cases (91%), associated with a destruction of vertebral bodies surfaces, but the spinal cord was intact. Pain was considerably relieved after treatment in 11 cases (91%) (the patients with lesion in one vertebral level) and partially relieved in 1 case (9%) (the patient with two vertebral level lesions) According to the second MRI or CT examination, in all cases fibrotic changes in vertebral bodies were seen associated with discal space reduction.

**Conclusion:** The author draws special attention to vertebral column lesions due to Brucellosis; adequate treatment improves clinical signs and symptoms and imaging findings.

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Carbapenem-resistant *Enterobacteriaceae* in a tertiary care cancer hospital

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**Background: Background :** Infection with carbapenem-resistant *Enterobacteriaceae* (CRE) or carbapenemase-producing *Enterobacteriaceae* is emerging as an important challenge in health-care settings. Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is the species of CRE most commonly encountered. CRKP strains are resistant to many available antimicrobial agents, and infections with CRKP have been associated with high rates of morbidity and mortality, particularly among persons with prolonged hospitalization and those who are critically ill and exposed to invasive devices (e.g., ventilators or central venous catheters).. The most important mechanism of resistance for CRKP is the production of a carbapenemase enzyme, *bla*kpc. The gene that encodes the *bla*kpc enzyme is carried on a transposon, which increases the risk for dissemination. Detection of CRE is difficult because some strains that harbor *bla*kpc have minimal inhibitory concentrations (MICs) that are elevated but still within the susceptible range for carbapenems.

This study was undertaken to assess the presence of carbapenem-resistant *Enterobacteriaceae* in immunocompromised patients who are more vulnerable to infections.

**Methods: Material & Methods:** A total of 5121 clinical samples received during the study period in the Dept. of Microbiology, were included in the study. All the samples were processed, identified and antimicrobial susceptibility testing performed as per standard microbiological procedures. Imipenem resistance in *E.coli* and *K.pneumoniae* was confirmed by E-test, Modified Hodge test and double disk synergy test.

**Results: Results :** Of the 5121 clinical samples received , there were 275 isolates of *E.coli* and 154 isolates of *K.pneumoniae*. A total of 23 carbapenem-resistant *Enterobacteriaceae* were isolated from 12 patients. Of the 23 isolates, 14(61%) were *E.coli* and 9(39%) were *K.pneumoniae*.

**Conclusion: Conclusions :** The emergence and spread of CRKP and other types of CRE is an important event in a series of worrisome public health developments in antimicrobial resistance among gram-negative bacteria and underscores the immediate need for aggressive detection and the implementation of urgent control strategies.

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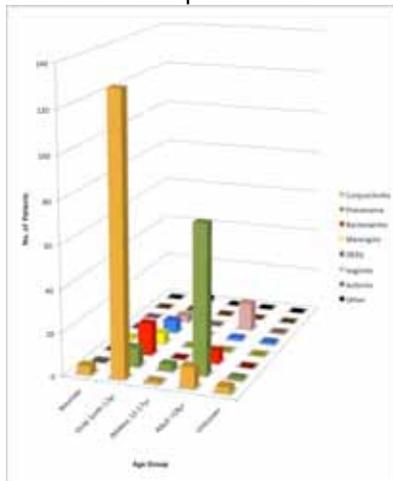
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Infections due to *Haemophilus influenzae* encountered in Jamaica: Analysis of 286 cases at the University Hospital of the West Indies  
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**Background:** Infections due to and antibiotic susceptibility of *Haemophilus influenzae* (HI) varies in different areas. There are a large number of reports on HI infections from many developed countries. Published data from developing countries, however, are few and far between. There are very few such reports from countries in the Caribbean. In this report, we examine 286 cases of HI infections encountered in 3 years (2000-2002) at the University Hospital of the West Indies (a 534 bed Tertiary Care Teaching Hospital) in Kingston, Jamaica.

**Methods:** The isolates were identified by standard methodology including tests for requirement for X and V factors (Manual Clin Microbiol, 6th ed. p 556-565). Antimicrobial susceptibilities were determined by CLSI disc diffusion method. The disc susceptibility results were further validated by test for MIC by E-test using *Haemophilus influenzae* ATCC 49217 as control with some isolates. Nitrocefin discs (BBL) were used to test for betalactamase production.

**Results:** There were 286 patients with HI infections during the period, 127 (44.4%) males and 159 (55.6%) females. The age ranged from 1 day to 89 years and the mean age was 16.8 years. There were 28 invasive (sterile site) infections (mostly meningitis and bacteraemia) all of which were due to type b strains and 258 non-invasive infections, the vast majority of which were conjunctivitis (148) and pneumonia (88). Pneumonia was the most common infection in adults and conjunctivitis the most common in children. These two infections together accounted for over 80% of all HI infections in the hospital during the period. The rate of resistance to front-line antibiotics were: ampicillin 14.3%, chloramphenicol 1.5%, amoxicillin-clavulanate (augmentin) 2.6%, erythromycin 12.7% and cotrimoxazole 44.2%. All ampicillin resistant isolates were betalactamase producers.



**Pattern of infection due to *Haemophilus influenzae* in various age groups at UHWI 2000 - 2002**

**Conclusion:** The changing epidemiology of HI infection in recent years underlines the need for periodic surveillance of both invasive and non-invasive HI infections especially in developing countries where *Haemophilus influenzae* continues to remain a major cause of morbidity and mortality. The high prevalence of cotrimoxazole resistance in this hospital which may be due to a very high use of co-trimoxazole here is similar to those reported from this region (Microb Drug Res 7:403-411, 2001).

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Incidence of human Brucellosis in Gevgelia region, Macedonia

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**Background:** In Macedonia of 2008 year to enroll 9500 diseased sheeps and goats of Brucellosis. That is too much potential to appear of human Brucellosis. The same year to records 213 new diseased peoples of human Brucellosis who is significant health problem many years ago.

**Methods:** It used descriptive-epidemiological method, biochemical and serological analysis (BAB, Wight, Coombs) to follow up a ten years period to disease (1998-2008) in General hospital-Gevgelia, Macedonia.

**Results:** In the department of Infectious diseases-Gevgelia for period of ten years are treated totally 135 diseased persons. The last year to appear one outbreak of human Brucellosis to one livestock farm with 15 diseased peoples. In the season (march-september) to enroll 108 (80.0%) patients. The structure of diseased peoples moreover was sheep-breeders and their own family, farmers, housewives and veterinary surgeons. Most frequently age group was 35-45 years of 62 (45.0%) patients. The smallest number of patients to enroll 2005 year (6). The last year have to biggest number of patients (20). Clinical manifestation of illness was pains at knees, hips, shoulders e.t.c. Not often clinical manifestations was high temperature, night perspiration, weakness, exhausting. More frequently complications was hepatitis brucellogenes (22,2%), orhyepidydymitis (5,9%), endocarditis (1,5%) and bronchopneumonia (0,7%). Therapeutical treatment was introduced during of six weeks with dual therapy with (Rifampicin, Doxiciklin/Co-trimoxsol).

**Conclusion:** The very much numbers of diseased animals (sheeps and goats) to 9500 of last year subsequently to introduce very high infectious potential to appear of human Brucellosis at Republic of Macedonia. Risk group to obtaining human Brucellosis was farmeres and their own family, house-wives whos to handles whit animal products, veterinary surgeons e.t.c. Most frequently complication was hepatits brucellogenes (22.2%) with auspicious course. To preventing to this zoonosis should complete vaccination and eradication to diseased stock.

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Risk factors for death amongst children of five years and younger, with invasive non-typhoidal *Salmonella* infection, 2004 – 2008, South Africa

**V. Dermaux**, A. Sooka, S. Meiring, N. Govender, J. Moyes, C. Cohen, K. Keddy

National Institute for Communicable Diseases for GERMS-SA, Sandringham, South Africa

**Background:** In Sub-Saharan Africa, non-typhoidal salmonellae (NTS) are an important cause of bacteraemia, with case-fatality ratios (CFR) amongst hospitalized children ranging from 4%-27%.

**Methods:** NTS isolated from normally-sterile sites from January 2004 through December 2008 were reported to a national, laboratory-based surveillance system. In-hospital outcome, HIV infection status and other clinical data were collected at sentinel sites. Incidence rates for 2007 amongst HIV-infected and uninfected children  $\leq 5$  years were calculated, assuming that the age-specific HIV prevalence amongst tested children was similar to those not tested for HIV. Risk factors for death were evaluated using logistic regression. Nosocomial infection was defined as infection identified from specimens collected  $> 2$  days after admission.

**Results:** The highest incidence of NTS was amongst children aged  $< 1$  year (19.8 per 100,000) and 1 year old children (6.2 per 100,000) as compared to older children (1.1 per 100,000 for 2 to 5 years old age group). The overall incidence was 88.3 (per 100,000) amongst HIV-infected children and 2.7 (per 100,000) amongst HIV-uninfected children.

HIV-infected children with invasive NTS infection had a 3.4-fold increased odds (95% confidence interval [CI95%] 1.9–6.3) of dying compared to HIV-uninfected children (30%, (50/166) HIV-infected versus 9%, (10/112) HIV-uninfected,  $p < 0.001$ ). The CFR was higher amongst patients aged  $< 1$  year (22%, 88/399) compared to those aged 1-5 years (16%, 40/248) (OR 1.5 CI95% 1.00–2.2,  $p = 0.066$ ).

CFR varied by province: 6% (7/111) Western Cape, 20% (64/319) Gauteng, 24% (31/128) KwaZulu-Natal and 29% (26/91) other provinces,  $p < 0.001$ .

Nosocomial infections accounted for 19% (96/496) of infections and were associated with a 1.8-fold (CI95% 1.1– 3.0) higher risk of death compared to community-acquired infections (CFR 29% (27/94) nosocomial versus 19% (73/392) community-acquired,  $p = 0.030$ ).

On multivariable analysis, controlling for province, HIV-infected children with invasive NTS had a 3 times greater odds of death as compared to HIV-uninfected children  $p = 0.002$  (OR 3.0 CI95% 1.7–5.5).

**Conclusion:** The incidence of NTS was highest in children aged  $< 1$  year and HIV-infected children. HIV-infected children were also at increased risk of death. Access to prevention of mother-to-child transmission, HIV testing and highly active antiretroviral therapy should be prioritised.

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Changes in the epidemiology of *Haemophilus influenzae* invasive disease, in Portugal, after the introduction of the Hib vaccine

**M. P. Bajanca-Lavado**, C. Betencourt  
National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal

**Background:** *Haemophilus influenzae* (Hi) is responsible for a number of human diseases ranging from chronic infections to meningitis. Most of the isolates are non-encapsulated (NC), although it is known until now six serotypes (a-f). Hi serotype b (Hib) vaccine was adopted in Portugal since 1994 and obligated since the year 2000 to children less than five years old. Our aim is to analyse the serotypes of Hi invasive strains in parallel with possible changes in antimicrobial susceptibility, before and after the introduction of vaccine.

**Methods:** We collected 104 invasive Hi isolates (50 CSF, 52 blood cultures and 2 pleural fluids) from 1989 to 2000, and 111 (22 CSF, 81 blood cultures and 8 pleural fluids) from 2001 to 2008, from several Hospital Laboratories in Portugal.

Fifty two strains (50%) were isolated from children (<18 years old) in the first period and 34 (32.7%) after the vaccination period.

Identification of Hi was confirmed by standard procedures. Susceptibility testing was performed by a microdilution assay, according to the CLSI guidelines.  $\beta$ -lactamase production was determined with nitrocefin. Serotyping was performed by PCR.

**Results:** In relation to ampicillin resistance,  $\beta$ -lactamase producing was detected in 28.8% of the strains in the period 1989-2000, and 12.6% between 2001-2008. Five strains (5.2%), all isolated in the pos vaccine period, had MIC values to ampicillin and clavulanic acid of 2 mg/L, which indicates BLNAR possible. In relation to serotype characterization, in the pre-vaccine period, 68.3% of the strains were Hib and 31.7% were NC; after the introduction of the vaccine, 12.6% were Hib, 79.3% NC, and 8.1% non-b-type (2.7% a and 5.4% f).

**Conclusion:** Considering the two periods 1989-2000 and 2001-2008 we observe a rise in NC strains and a decline in serotype b, in invasive disease in Portugal. Non-b-type strains were characterized only after the introduction of the vaccine. In this way, we document changes in invasive infections following the introduction of the vaccine in Portugal, with an emergence of NC and an increase of serotypes other than b. We also expect a new rule in the virulence mechanisms of these strains.

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Analysis of clinical outcome in patients with multidrug-resistant *Acinetobacter baumannii* bacteremia

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The catholic University of Korea, Seoul, Korea, Republic of

**Background:** Multidrug-resistant *Acinetobacter baumannii* (MDRAB) has been increasing reported worldwide, and the therapeutic options are limited. The aim of this study was to investigate clinical characteristic, risk factors for mortality and outcome among patients with MDRAB bacteremia.

**Methods:** A retrospective study was performed at a tertiary care hospital in Korea from January 2005 to July 2009. Only first bacteremic episode from one patient was included in the study. Antibiotic therapy was considered to be appropriate if the isolated strain was sensitive, in vitro, to initial antibiotic, which were administered within 48 hours after blood culture sample obtained. Multidrug-resistant *Acinetobacter baumannii* (MDRAB) was defined as resistance to all commercially available antibiotics except colistin.

**Results:** Seventy-seven patients (40 male, 37 female) with *Acinetobacter baumannii* bacteremia were included. The presumed sources of bacteremia that caused bacteremia were lung(n=24,31.2%), catheter(n=21, 27.3%), intraabdomen(n=13, 17%), postoperative wound(n=6, 7.8%), urinary tract(n=5,6.5%), and unknown(n=8, 10.4%). Forty-three patients(55.8%) were infected with MDRAB. The overall 30-day mortality in patients with *Acinetobacter baumannii* bacteremia was 33.7%(26/77), and was higher in MDRAB patients than non-MDRAB (62.5% vs 20.8%,  $p<0.05$ ). According to univariate analysis, diabetes mellitus, central venous catheter, mechanical ventilation, hospital day until bacteremia, inappropriate antimicrobial therapy, resistance to imipenem and resistance to multidrug except colistin were associated with 30-day mortality ( $p<0.05$ ). Multivariate analysis using logistic regression model showed that significant risk factors for 30-day mortality were mechanical ventilator (OR=5.6, 95% CI 1.24-.24.83,  $p=0.02$ ), and resistance to multidrug (OR=40.77, 95% CI 3.95-420.85,  $p=0.001$ ).

**Conclusion:** Patients with MDRAB bacteremia showed higher mortality rate than non-MDR bacteremia, although underlying status play an important role in clinical outcome. The efforts to minimize the emergence of antimicrobial resistance should be important.

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Clinical profile and antimicrobial resistance pattern of *Enteroaggregative E.coli* isolated from patients with diarrhoea, using a multiplex PCR assay in a tertiary care hospital in North India  
**S. pahil**, N. Taneja, G. Singh, M. Sharma  
Post Graduate Institute of Medical Education and Research, Chandigarh, India

**Background:** *Enteroaggregative Escherichia coli* (EAEC) is an increasingly recognized enteric pathogen which can cause both acute and persistent diarrhoea in children, traveller's diarrhoea and diarrhoea in immunocompromised patients. The identification of EAEC by conventional techniques is technically demanding and time consuming. The genetic and phenotypic heterogeneity also makes the diagnosis of EAEC difficult. Therefore a single assay multiplex polymerase chain reaction (PCR) can be useful for the identification of enteroaggregative *Escherichia coli*. To identify both typical and atypical EAEC with a high sensitivity and specificity we selected three targets including *aggR*, CVD432 and *astA*. The EAEC plasmid gene regulator, *aggR* has 27.5-82% EAEC detection rate. The antiaggregation protein transporter gene, CVD432 (now *aat*), has a sensitivity of 15-89% and specificity of 99%. The enteroaggregative heat-stable enterotoxin gene *astA* has been reported in 29.8-44% EAEC strains and is most frequently detected among atypical EAEC.

**Methods:** Multiplex PCR was established for three genes *aggR*(254bp), CVD432(194bp) and *astA*(94bp). Antimicrobial susceptibility was performed on the confirmed isolates by Kirby Bauer method as recommended by CLSI.

**Results:** Out of a total of 185 isolates of *E.coli* 29% (53/185) showed presence of *Enteroaggregative E.coli*. Virulence gene such as *aggR* was present in 69.8% (37/53) isolates. Genes such as *east* were positive in 43.3% (23/53) of isolates and CVD was present in 56.6% (30/53) of isolates. Majority of the strains were resistant to most first line antibiotics showing high resistance for cotrimoxazole(84%), amoxicillin(81%) and norfloxacin(77%).

**Conclusion:** *Diarrhoeagenic E.coli* is a well established cause of significant morbidity and mortality worldwide. The identification of EAEC by conventional techniques is technically demanding and time consuming. Multiplex PCR assays targeting various virulence genes of EAEC can easily be adopted for the routine diagnosis. In our study, 29% of cases were found to be caused by EAEC which were confirmed by multiplex PCR. Such a rapid technique can be extremely useful in investigation of acute diarrhoea outbreaks due to EAEC. Drug resistance in EAEC is an increasing cause of concern and most of isolates were resistant to all first line antibiotics.

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Phenotypic and molecular characterization of *Pseudomonas aeruginosa* isolates expressing high-level betalactamic resistance in cystic fibrosis patients

**M. D. C. Batlle**, J. Ayala  
Academy of Sciences, Havana, Cuba

**Background:** Most cystic fibrosis (CF) patients suffers from chronic and fatal pulmonary infections caused by strains of *Pseudomonas aeruginosa* (*Ps. aeruginosa*). Resistance to extended spectrum Cephalosporin in this pathogen is associated in most cases with the over production of Cephalosporinase. The b-lactamases of *Ps. aeruginosa* destroy antimicrobials with: carboxipenicillin, aminopenicilin and cephalosporin The purpose of this study was to determine the presence of b lactamases genes (*bla pse--1*, *ampC* and *bla oxa*) in strains resistant. The nucleotide sequences by theses gene were determined in three resistant strains to beta lactam antibiotic.

**Methods:** In our study 65 *Ps. aeruginosa* isolations were obtained from 15 cystic pediatric patients attending in William Soler, in Havana during five years. Were characterized by Micro dilution broth (MIC), Api System, Phagotyping and serotyping and Pulse Field gel Electrophoresis. High level Ceftazidime resistant isolate were confirmed by PCR detection of *bla pse--1*, *ampC* and *bla oxa* gene. Amplicons resulting from PCR determining regions of these genes were purified with a Quia Quick spin PCR purification kit. The nucleotide sequences were determined with an automatic Sequencer ABI PRISM 377 DNA SECUENCER

**Results:** Mutations in the beta lactam resistance determining region of the *ampC* gene were detected in 3 strains Ceftazidime resistant. High level by MIC to Ticarcillin, Aztreonam and Ceftazidime was associated with mutations at codon 180 and 195 of the AmpC gene. The codon 180 Amino acid change Pro to Arg and the codon 195 the aminoacid change Glu to Lys in the three strains. The epidemiological date indicated that such resistant strains probably emerge independently, possibly following selection during treatment with beta lactam drug.

**Conclusion:** Two main clusters were detected by PFGE in our study, with common clonal relation among strains Piperacillin, Tobramicin and Meropenem were active against most of the strains tested, and is a good choice of antibacterial drug for treating CF infections. The results this work will be very helpful to guide empirical antimicrobial therapy and the implementation of infection control in cystic fibrosis patients in our country.

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Epigenetic regulation of lymphocytes response to LPS

**E. Melo<sup>1</sup>**, M. Camargo<sup>2</sup>, R. Curi<sup>2</sup>, I. Velasco<sup>1</sup>, F. Soriano<sup>1</sup>

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**Background:** Recent evidence suggests that many inflammatory genes are regulated by epigenetic modifications to individual promoters. This level of control allows a single gene to be expressed or silenced according to its function and to the previous conditions suffered, irrespective of other genes induced by the same receptor, and therefore is “gene-specific”. The aim of the study is analyze in LPS tolerance the epigenetic via used to regulate gene expression (demethylation or hypermethylation) in lymphocytes response.

**Methods:** Lymphocytes were cultured in DMEM (control C). Tolerance was reproduced in culture lymphocytes exposed to LPS (T group) (24 and 48h). NAB or 5aza were added once (NAB1 and 5aza1) for 24 h . After this period LPS was added to the media. The production of IL-6, IL-10, IL-1, TNF, and MIP-2 were measured by enzyme-linked immunosorbent assay (ELISA). RT-PCR was performed to verify the differential mRNA expression. Primer sequences were designed using GenBank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov) ).

<b>Results:</b>	Control	LPS high dose	Tolerant	NAB1	NAB2	AZA1	AZA2
ELISA							
TNF	±500pg/ml	±20000pg/ml	±1000 pg/ml	±1000pg/ml*	±1000pg/ml#	±2000pg/ml&	±10000pg/ml\$
MIP	±50pg/ml	±3000 pg/ml	±100pg/ml	±4000pg/ml	±5000pg/ml	±4000pg/ml&	±2000 pg/ml\$
IL-1	±70pg/ml	±6000 pg/ml	±200pg/ml	±3000pg/ml	±5000pg/ml	±4000pg/ml	±1000 pg/ml\$
IL-6	±58pg/ml	±9000 pg/ml	±40pg/ml	±3000pg/ml*	±6000 pg/ml	±4500pg/ml&	±3000 pg/ml\$
IL-10	±50pg/ml	±2000pg/ml	±100pg/ml	±1500pg/ml*	±4000pg/ml	±1500pg/ml&	±4500pg/ml
RT-PCR							
TNF	±100	±40000	±1000	±8900†	±70000‡	±20000	±71000
IL-1	±150	±30000	±2500	±3000	±4500	±5300	±6000Δ

**ELISA**

NAB1, \* p< 0.05 compared to NAB2

NAB2, # p<0.05 compared to LPS high dose

AZ1 & p < 0.05 compared to AZ2

AZ2, \$ p<0.05 compared to LPS high dose

**RT-PCR**

NAB1, † p< 0.05 compared to NAB2

NAB2, ‡p<0.05 compared to LPS high dose

AZ2, Δ p<0.05 compared to LPS high dose

Results are expressed as means ± SEM. Comparisons among the groups were performed by using paired t-test.

**Conclusion:** Tolerance is regulating the expression of the inflammatory genes differentially: 1-the epigenetic mechanism involved in regulating the expression of the genes: MIP2 and IL1 is the demethylation of histones, 2- however, the epigenetic regulation of IL6 and IL10 is through hypermethylation and 3-the gene: TNF is regulated by demethylation and hypermethylation.

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Clinical and phylogenetic characteristics of *Escherichia coli* urinary tract infections  
**S. Y. Lee**, J. H. Kim, J. K. Hur, J. H. Kang  
The Catholic university of Korea, Seoul, Korea, Republic of

**Background:** We aimed to investigate the clinical and phylogenetic characteristics of *Escherichia coli* Urinary Tract Infections (*E. coli* UTI)

**Methods:** We enrolled patients with culture-proven *E. coli* UTI, who were admitted at the study hospital from September 2008 to August 2009. We investigated clinical data of patients with *E. coli* UTI and characteristics of isolated *E. coli* strains. The phylogenetic groups were classified using triplex polymerase chain reaction (PCR), and the distribution of nine virulent genes was determined by multiplex PCR.

**Results:** A total of 47 patients have participated in this study. Thirty (63.8%) were under 6 months; eight (17.0%) were between 6-12 months; and nine (19.1%) were over 12 months. We compared two age groups between under 6-month and over 6-month. In the age group under 6-month, higher proportion of male ( $P=0.002$ ) and group B2 strains ( $P=0.020$ ) were observed. In contrast, higher proportion of female and group non-B2 strains were observed in age group over 6-month. In the comparison of phylogenetic groups, group B2 showed higher distribution of virulent genes, while group D included more strains resistant to trimethoprim/sulfamethoxazole (TMP/SMZ) than other groups.

**Conclusion:** We showed the age group-specific difference in the distribution of phylogenetic groups; more male and group B2 strains in age group under 6-month, while more female and group non-B2 in age group over 6-month. However, further evaluation including larger number of patients will be necessary to confirm above thesis in future molecular epidemiological studies.

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Serogroup W135 meningococcal invasive disease in Argentina

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**Background:** Meningococcal invasive disease (MID) is endemic/ hyperendemic/ epidemic at La Plata city, Buenos Aires, Argentina and its surrounding areas. First isolation of *N.meningitidis* W135 (NmW135) in Argentina was made in 1981 in this location and, since then, sporadic cases occurred. MID is a rare disease in persons older than 15 years of age in our country. The aim of this study is to describe the clinical presentation, age group and fatality rate for NmW135 MID.

**Methods:** We prospectively studied 707 inpatients with MID in a 13-year period; children accounted for 99.4% (n=703). Blood and CSF cultures and isolation were made by standard methods, identification with DIFCO serum, and for the last three years PCR gen (crgA) by M. Taha method (J Clin Microbiol 2000; 38:855-857) at Microbiology Laboratory, Sor María Ludovica Children's Hospital, La Plata, Argentina.

**Results:** First period (1996-2001): 419 MID total cases and 1.2% (n=5) were caused by NmW135. Age group (range): 3 months-9 years old. Clinical presentation: sepsis and purulent meningitis; no fatalities occurred. Second period (2002-2008): 288 MID total cases and 5.9% (n=17) were caused by NmW135. This serogroup emerged in the year 2007 (n=6) and the peak occurred in 2008 (n=10). Age group (range): 1 month-58 years old. Clinical presentation was bacteremia (5.9%), bacteremia and pneumonia (5.9%), bacteremia and arthritis (5.9%), fulminant sepsis (23.5%), and sepsis with purulent meningitis (58.8 %). Considering both periods, 4 adults were affected; age (range): 21 to 58 years old. The global fatality rate was 17.6% but in adults it was 50%.

**Conclusion:** We alert for the emergence of MID caused by NmW135 with an unusual frequency in 2007. According to the National Reference Laboratory at the National Institute of Infectious Diseases Dr. Carlos G. Malbran, NmW135 increased from 1.3% (1998-1999) to 11.6% (2006-2007), achieving 38.9% in 2008. Clinical and microbiological laboratory data are necessary for epidemiological surveillance to detect early changes in frequency and Nm serogroup incidence. The mechanism by which a strain causes sporadic or epidemic cases and the importance of a change in serogroup strains remain unknown.

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**Type: Poster Presentation**

Salmonella prosthetic joint infections: An uncommon entity

**R. GUPTA**, S. M. Boger, L. R. Leddy, H. D. Schutte, C. E. Marculescu, H. A. Demos  
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**Background:** *Salmonella* Prosthetic Joint Infection (PJI) is a rare entity, with twenty cases reported in the literature to date. *S. typhimurium* is the most commonly isolated species.

**Methods:** We conducted a medline search for all cases and series of *Salmonella* prosthetic joint infections published between 1967-2008. We found twenty cases reported in the literature, which are summarized in Table I.

**Results:** We present two cases of *Salmonella* PJI seen at our institution between 1995-2009. Both cases had an involvement of a total hip arthroplasty (THA). A preceding diarrheal illness was only identified in one case. *Salmonella livingstone* was isolated from intraoperative cultures in this case; *S. enteritidis* from the second patient. Symptoms at presentation in both cases were fever and hip pain. Both cases presented in the late post-operative period. The surgical therapy included resection of prosthesis in one and, debridement with retention in the other, followed by directed prolonged intravenous antimicrobial therapy. The outcome in one patient is favorable at two months of follow-up. The other patient died from an unrelated complication after eight weeks of therapy.

**Conclusion:** *Salmonella* spp is an uncommon cause of PJI. To our knowledge, this is the first reported case of *Salmonella livingstone* PJI. Identifiable risk factors for *Salmonella* PJI are uncommon. A combination of surgical and medical modalities is required for successful management of such infections

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Genetic analysis by pulsed-field gel electrophoresis of Enteropathogenic *Escherichia coli* (EPEC) strains isolated from Peruvian children

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**Background:** Enteropathogenic *Escherichia coli* (EPEC) are a leading cause of infantile diarrhea in developing countries. The aim of this study was to determine the genetic diversity of EPEC isolated.

**Methods:** 182 EPEC strains isolated from a cohort diarrhea study in Peruvian children were characterized for the Pulse Field Gel-Electrophoresis (PFGE). The strains were distinguished between typical (tEPEC) and atypical (aEPEC) EPEC using PCR for *bfpA* gene.

**Results:** A dendrogram of 182 EPEC strains (98 from diarrhea cases and 84 from controls; 35 were tEPEC and 147 were aEPEC) based of XbaI-digested DNA pattern was created with the InfoQuest (Bio Rad) software using Dice similarity indices. PFGE, which revealed 171 distinct pulsed-field patterns (PP) based on a difference of a single band as a basis for discrimination between isolates, were arranged in revealed 28 clusters (I-XXVII; 1-44 strains per group) with general similarity of 80% according to the Dice similarity index. Four strains were not typeable by PFGE because it repeatedly produced only a smear of degraded DNA after PFGE. Each cluster gathered strains belonging to different date of isolated, sample type (isolated from diarrhea cases or control) or EPEC type (tEPEC or aEPEC). Two exceptions were cluster IX and XIX, which gathered only strains (6 and 8 respectively) isolated from diarrhea cases. The clusters XXVI, XXVII and XXVIII shown the most divergent pattern and constituting a separated groups from other strains analyzed and are formed exclusively by one strains each group (aEPEC isolated from diarrhea in all cases).

**Conclusion:** The 182 strains showed 28 different PFGE patterns. A high degree of polymorphism among different strains was observed. The characteristics as sample type (diarrhea or control), date and EPEC type do not observed closely related in the dendrogram.

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Antibody subclasses as a tool for *Helicobacter pylori* antigen characterization

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**Background:** *Helicobacter pylori* is a bacteria that colonizes about 50% of the world population. *H. pylori* infection is associated with severe disease including peptic ulcer and gastric cancer. Several factors have been proposed as important for this variability. Among the factors that possibly participate in this variable disease outcome is the host's immune response. Antibody subclass markers could be important markers for disease outcome and could be used as interesting markers of the development and polarization of immune response. Therefore, we characterized the differences in subclass antigenic recognition according to *H. pylori* associated disease and source of antigen.

**Methods:** IgG1 and IgG2 titres were determined by ELISA in 44 sera of patients with different *H. pylori* associated diseases (Gastric cancer, peptic ulcer and dyspepsia). *H. pylori* antigens were obtained from 12 different clinical isolates from similar associated diseases.

**Results:** When the IgG1 and IgG2 titres were analyzed, we found higher IgG2 titres across all titre determinations unrestrictive of pathology. In particular, peptic ulcer patients showed higher IgG2 /gG1 titre ratios. Source of antigen also had an effect on these determinations, as dyspepsia derived antigens revealed higher IgG1 titres across all screened sera.

**Conclusion:** There was an increase in IgG2 titres in all sera, in concordance with a Th1 response. This increase is more represented in peptic ulcer patients. Antigen source was found to be a variable in these determinations, which represents a novel characteristic of subclass-associated antigenic analysis.

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Age-specific seroprevalence of hepatitis A in SARI (northern part of IRAN)

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**Background:** Hepatitis A virus (HAV) is an enteric, viral, infectious disease still endemic in many developing countries such as Iran. Improved sanitary condition has generally resulted in a significant decline in the incidence of hepatitis A. However, a low incidence of infection results in increased HAV susceptibility. The present study investigates the seroprevalence of hepatitis A virus antibodies in relation to age to clarify the current HAV status and HAV susceptibility in the city of Sari, northern part of Iran.

**Methods:** A total of 1034 Serum samples collected during 2007 from Iranian urban and rural individuals ranging in age from 1- 25 years , were tested for anti-HAV IgG antibody using a commercial enzyme immunoassay kit. Subjects were investigated using a standardized social and medical history questionnaire. In data analysis, we use X<sup>2</sup> (chi-square) and fisher exact test.

**Results:** 1034 subjects were enrolled including 620 urban and 414 rural people. The overall seroprevalence rate was 38.8%. There were 192 children under 5 years old and 330 subjects between 5 and 15 years and 512 subjects between 15 to 25 years old. . There was significant correlation between ages, parent's educational levels and home condition with anti HAV antibody levels.

**Conclusion:** The surveillance of anti-HAV antibody prevalence is useful for implementing preventive measures and for controlling the spread of HAV. It seems that immunization against HAV will be needed in early future in our Population.

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Association of polymorphism of HLA-DRB1 13 and HLA-A 33 alleles with outcome of hepatitis B virus infection

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**Background:** Hepatitis B virus (HBV) infection is a major public health problem worldwide. The mechanism of susceptibility to chronic persistent HBV infection is not well clarified, while the outcome of HBV infection mainly depends on the host immune response. Different HLA class 1 and 2 alleles may play roles in HBV infection outcome. The current study aimed to determine association between HBV infection outcome and HLA-A and DRB1 genotyping in North part of Iran.

**Methods:** Ninety-four HBV infected patients were enrolled in this study. First HLA-A and DRB1 alleles were analyzed by using low resolution PCR sequence-specific-primer (PCR-SSP) and then we used high resolution PCR-SSP method for subtyping HLA-A\*33 and DRB1\*13 alleles which were significantly related to the outcome of HBV infection.

**Results:** The frequency of A\*33 allele in persistent group was higher than in recovered group (9.37% vs. 0%,  $P < 0.008$ ) and sub typing showed HLA-A\*3303 and HLA-A\*3301 in 75% (allele frequency: 7.3) and 25% (allele frequency: 2.34) of persistent HBV infected cases respectively. The frequency of DRB1\*13 allele was lower in persistent group than in recovered group (3.13% vs. 11.67%,  $P < 0.03$ , OR = 0.22, 95%CI 0.06-0.82), and HLA- DRB1\*1301 and HLA-DRB1\*1303 were found in 66.7 % ( allele frequency: 4.89) and 33.3% (allele frequency: 3.45) of cases respectively. HLA-A\*3303 and DRB1\*1301 were the predominant subtypes of HLA-A\*33 and DRB1\*13 at high resolution PCR-SSP method

**Conclusion:** Host HLA polymorphism is an important factor to determining the outcome of HBV infection. HLA-A\*3303 and DRB1\*1301 were the predominant subtypes of HLA-A\*33 and DRB1\*13 alleles in Iranian HBV infected patients.

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Hepatitis A epidemiology in a Brazilian fire department

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**Background:** In developing countries, the first contact with hepatitis A virus (HAV) occurs early in life. In recent years, the improvement on sanitation conditions in some developing areas resulted in an increase of proportion of the population that reaches adulthood without acquired immunity. In Brazil, although hepatitis A is considered an endemic disease, data published in recent investigations carried out in the State of Rio de Janeiro revealed a shift in the hepatitis A epidemiological pattern. Consequently studies must be conducted to define the seroprevalence of anti-HAV antibodies in different populations and a programme to vaccinate non immune people must be created.

**Methods:** To find out the seroprevalence of anti-HAV antibodies in a population professionally exposed to water contact (flooding, sea and fresh water) living in Rio de Janeiro, Brazil. A secondary objective was to evaluate the cost-benefit of performing serological screening prior to vaccination.

Participants were military fireman from the State of Rio de Janeiro, that filled in a socioeconomic questionnaire. Blood specimens were obtained for anti-HAV serological test (ELISA). The results were analyzed using t-student test and 2 test, as well as a logistic regression.

**Results:** Nine hundred and thirty one participants were enrolled in the study. The median age of the population was 38 years and the overall seroprevalence of anti-HAV was 78.5%. The distribution of positive tests according to age groups were 47,8% in 20 - 25 years, 53,4% in 26-30 years, 69,4% in 31-35 years, 87,2% in 36-40 years, 88,6% in 41-45 years and 91% in > 46 years.

**Conclusion:** Military fireman from Rio de Janeiro have a high level of immunity against HAV. The results of cost benefit showed that the population which has a prevalence of up to 27%, should be vaccinated without serological screening. As the lower prevalence of this group was 47.8%, everyone should undertake serology before being vaccinated.

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Association of exon 9 but not intron 8 VDR polymorphisms with occult HBV infection in south-eastern Iranian patients

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**Background:** Occult hepatitis B infection (OBI) is characterized as a form of hepatitis in which despite of absence of detectable hepatitis B surface antigen (HBsAg), HBV-DNA is present in patient's peripheral blood. Investigators believe that divergent genetics and immunological parameters are varying in resistant individuals from patients. Vitamin D3 and it's known receptor seem to be involved in anti-viral immune responses. Therefore, because OBI is a form of viral infection, the aim of this study was to investigate the association between polymorphisms in intron 8 and exon 9 of vitamin D receptor (VDR) with OBI.

**Methods:** In this experimental study, the plasma samples of 3700 blood donors were collected and tested for HBsAg and anti-HBs using ELISA. The HBsAg-/anti-Hepatitis B core+ (anti-HBc) samples were selected and screened for HBV-DNA using PCR. HBV-DNA positive samples assigned as OBI cases and PCR-RFLP was performed to examine the polymorphisms in intron 8 and exon 9 of VDR.

**Results:** Results of the current study demonstrated that 352 (9.5%) of 3700 blood samples were HBsAg-/anti-HBc+. HBV-DNA was detected in 57/352 (16.1%) of HBsAg-/anti-HBc+ samples. Our results showed significant difference in T/T allele of exon 9 of VDR but any differences were also observed in the other examined alleles.

**Conclusion:** The polymorphisms in T/T allele of exon 9 of VDR is possibly associated with OBI thus it can be concluded that VDR and its functional polymorphisms can probably have a relation with sensitivity and resistance of immune system to HBV in OBI patients

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IL-12 +1188 and IFN-g +874 polymorphisms is not associated with these cytokines serum level in occult HBV infected patients

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**Background:** Occult hepatitis B infection (OBI) is identified as a form of hepatitis in which despite of absence of detectable HBsAg, HBV-DNA is observed in peripheral blood of patients. The main aim of this study was to investigate the association between polymorphisms in +874 of IFN-g and +1188 of IL-12 with their serum level in patients suffering from OBI

**Methods:** In this experimental study, plasma samples of 3700 blood donors were tested for HBsAg and anti-HBc by ELISA. The HBsAg-/anti-HBc+ samples were selected and screened for HBV-DNA by PCR. HBV-DNA positive samples assigned as OBI cases and ARMS-PCR techniques was performed to examine the polymorphisms in IL-12 and IFN-g. The serum level of IL-12 and IFN-g was also detected by ELISA.

**Results:** Results of this study demonstrated that, 352 (9.5%) out of 3700 blood samples were HBsAg-/anti-HBc+ and HBV-DNA was detected in 57/352 (16.1%) of HBsAg-/anti-HBc+ samples. Our results showed that groups showed significant difference in CC allele of +1188 region of IL-12 and no difference was observed in the other evaluated genes. Our results also showed that the alleles of +1188 region of IL-12 and alleles of +874 of IFN-g were also not associated with serum level of cytokines

**Conclusion:** According to the results of this study it may be concluded that the polymorphisms in +1188 region of IL-12 and +874 region of IFN-g would not affect the expression of both cytokines at serum level in OBI patients

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Occult hepatitis B virus infection in ART-naïve HIV-infected Indian patients

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**Background:** Hepatitis viruses are frequently found as a co-infection with HIV due to shared routes of transmission. Globally the most common method of diagnosis is serology, which can only detect active HBV infection while the occult HBV infection may be missed. We estimated the burden of HBV infection in patients who were tested positive for HIV at a tertiary care centre in north India. We also attempted to determine the prevalence and clinical characteristics of occult HBV among these treatment-naïve patients and compare their demographics features with other HIV patients.

**Methods:** During a period of 6 years between January 2002 to December 2007 837 HIV positive patients were tested for serological markers of HBV (HBsAg) and HCV IgM in our laboratory. For comparison 428 apparently healthy, HIV-negative organ donors were also included in the study. Data on demographics, sexual behaviour, medical history, laboratory tests viz., serum ALT and CD4 count of these patients were recorded. A sub-group of 53 HBsAg negative samples from HIV positive patients were randomly selected and assessed for anti-HBs, anti-HBc total (IgG+IgM) and HBV-DNA using a highly sensitive qualitative PCR and analysed retrospectively.

**Results:** Overall, 7.3% of HIV positive patients showed presence of HBsAg as compared to 1.4% in the HIV negative control group. The prevalence was higher (8.55%) in males than females (3.39%). Overall occult HBV with detectable HBV-DNA was found in 37.5% patients; being 45.5% in anti-HBs positive patients and 30.7% in patients positive for anti-HBc antibodies alone.

**Conclusion:** High prevalence of HIV-HBV co-infection was found in our patients. The most reliable method to diagnose occult HBV co-infection in HIV seropositive patients is the detection of HBV DNA.

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Treatment of chronic hepatitis C: The efficacy and predictors of response to therapy

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**Background:** The current standard for therapy of chronic hepatitis C (CHC) is combination of pegylated interferon alpha (PEG IFN- $\alpha$ 2a or PEG IFN- $\alpha$ 2b) and ribavirin (RBV). It is universally accepted that achievement of Sustained Virological Response (SVR) means successful treatment. Certain factors are good predictors of treatment outcome and they provide optimal, individual therapeutic approach.

**Objectives:** Evaluation of efficacy of CHC treatment and predictors of treatment response.

**Methods:** 194 patients with CHC were treated with combination therapy (PEG IFN- $\alpha$ 2a + RBV). Virological examinations: anti-HCV antibodies (ELISA), serum HCV RNA levels and HCV genotypes (PCR technique). Liver biopsy specimens were staged in accordance with the Metavir score. Predictor with influence on treatment outcome were estimated by multivariable logistic regression analysis.

**Results:** There were more patients of male gender (59.3%), older than 40 years (58.2%), with high basal HCV-RNA level ( $> 800.000$  IU/ml, 63%), with G1 HCV (67%) in treated group of patients with CHC. Bridging fibrosis and cirrhosis (F3, F4) were found in 54 (27.8%) of patients. SVR was achieved in 69.2%, relapse was found in 11.9% of patients. 19.8% of patients had no response on therapy. Predictors for achievement of SVR were: non-1 HCV genotype  $p=0.002$ , OR 5.9 95% CI (1.9-18.0), achievement of early virological response (EVR)  $p=0.000$ , OR 0.92, 95% CI (2.73-35.97), and patients younger than 40 years  $p=0.005$ , OR 3.66 95% CI (1.48-9.0). Predictors for failure of therapy for patients who didn't respond to treatment were: G1 HCV  $p=0.005$ , OR 3.9, 95% CI (1.5-10.3), high basal viral load  $p=0.013$ , OR 3.45, 95% CI (1.29-9.19) and interrupted continuity of treatment  $p=0.002$ , OR 4.49, 95% CI (1.72-11.76).

**Conclusion:** The current standard antiviral therapy was efficient in 127/194 (69.2%) of our patients. The most important predictors for successful treatment (SVR) were: non-1 HCV genotype, achievement of EVR for G1 HCV and patients age. Predictors for failure of treatment were: G1 HCV, high basal viral load, older age ( $>40$  years), presence of cirrhosis and discontinuity of treatment.

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Occult hepatitis C virus infection among chronic liver disease patients in the United Arab Emirates

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**Background:** Viral Hepatitis C (HCV) is one of the major world wide health problems. WHO estimates that about 3% of the world's population has been infected with HCV and that some 170 million are chronic carriers at risk of developing liver cirrhosis and/or liver cancer. Occult hepatitis C virus (OCV) infection is characterized by presence of HCV infection with undetectable hepatitis C antibodies or/and RNA in serum. Scanty information is available about the etiology of viral chronic liver diseases in the United Arab Emirates (UAE). Therefore a study was carried out for detecting and genotyping of HCV & OCV in patients with chronic liver disease (CLD) in UAE.

**Methods:** A total of 142 serum samples from CLD patients and 139 healthy individuals were tested by polymerase chain reaction (PCR) & ( RT-PCR ) in determining HCV RNA & HBV-DNA in serum samples. ELISA tests were used to detect HCV & HBV markers in patient sera. Liver biopsies were taken from CLD patients for immunohistochemistry ( IHC ) staining.

**Results:** The results indicated the prevalence of HCV, Occult HCV, dual (HBV &HCV) and HBV infections at a rate of 43.7%, 27.5%, 19.0% and 12.7% in CLD patients respectively. Healthy subjects were positive for anti body HCV and HBV-DNA at a rate of 3.6% and 2.2% respectively ( P=0.001). The most prevalent genotypes and subtypes of HCV infection in CDL patients were genotype 4 (28.3%),Where as the prevalence of other genotypes ,3a,1a, and 3b,1b, 5, 2, 3a & 3b, 2a and 1a & 1b were in the range of 25.3 to 1.3% . IHC results for HCV in biopsy tissues revealed that 84.6 (11/13) were positive .

Histopathological studies of liver biopsies revealed that patients with HCV infection were more likely to have necroinflammatory activity and fibrosis than patients without HCV infection.

**Conclusion:** Occult HCV is relatively frequent among patients with CLD in the UAE.

Phylogenetic tree showed that genotype 4 appears to be the dominant genotype Where as the prevalence of other genotypes that circulating in UAE population.( 1b, 5, 2, 3a & 3b, 2a and 1a & 1b ) were less dominant than genotype 4

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Clinical features of acute viral hepatitis A complicated with acute renal failure

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**Background:** Although acute hepatitis A is usually self-limited, the clinical manifestations can vary from mild to severe liver dysfunction. However, little is known about risk factors and outcome predictors for acute renal failure (ARF) in acute hepatitis A. The objective of this study was to identify the simple clinical predictors for acute renal failure (ARF) and its clinical course.

**Methods:** The study and control groups consisted of patients who did or did not develop acute renal failure, respectively, after acute hepatitis A from January 2006 to June 2009. A total of 396 patients were enrolled in this study. We conducted a retrospective analysis of the incidence, risk factors, and outcomes of patients with acute hepatitis A complicated with ARF.

**Results:** Thirty patients (7.6%) developed ARF. Older patients and males were more likely to ARF during hepatitis A. ARF patients were more likely obese, heavy alcoholics and having fulminant course.

The ARF patients had a higher white blood cell count, higher levels of AST, ALT, total bilirubin and CRP, lower levels of albumin, more frequent coagulopathy, and lower platelet count compared with the others. On multivariate analysis, male gender [Odds ratio (OR) 9.616, 95% confidence interval (CI) 1.307-70.000,  $P=0.026$ ], the presence of hypertension [OR 5.91, 95% CI 1.407-24.824,  $P=0.015$ ] and fulminant hepatitis [OR 57.95, 95% CI 10.399-322.928,  $P<0.001$ ] were independent risk factors of hepatitis A-associated ARF.

Of the 30 patients with ARF, 23 (76.7%) patients fully recovered; 14 (46.7%) patients recovered with conservative care only, and nine (30%) patients required short-term hemodialysis. The two groups of spontaneous recovery from ARF ( $n=14$ ) and hemodialysis/death ( $n=16$ ) showed significant differences in platelet count at its worst value, creatinine at its worst value, albumin at admission, INR at admission and fulminant hepatic failure. Albumin level at admission higher than 3.6 mg/dL [OR 13.183, 95% CI 1.064-163.403,  $P=0.045$ ] and INR at admission less than 1.6 [OR 14.969, 95% CI 1.346-166.447,  $P=0.028$ ] were independent predictors of spontaneous recovery of ARF during acute hepatitis A.

**Conclusion:** Acute renal failure associated with acute hepatitis A is not a rare complication. Male gender and hypertension are risk factors of ARF. Patients complicated with ARF, higher albumin level and shorter INR at admission could a favorable prognostic factor.

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Prevalence of isolated hepatitis B core antibody among injection drug users in Central Province of Iran

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**Background:** Prevalence of hepatitis B virus (HBV), hepatitis C virus (HCV) and co-infection of HBV and HCV are high among injection drug users. Hepatitis B may be presented only with isolated Hepatitis B core antibody (anti-HBc). This study aimed to determine the prevalence of isolated anti-HBc among injection drug users in central province of Iran and comparison of its prevalence with healthy blood donors.

**Methods:** A total of 531 voluntary blood donors with mean age of  $36 \pm 10.18$  years (range 16–60 years) and 153 injection drug users with mean age of  $30.66 \pm 5.92$  years (range 20-50 years) were enrolled in this study. All of the injection drug users were prisoners. Hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), anti-HBc, Anti-HIV and hepatitis C antibody (anti-HCV) were tested in all subjects.

**Results:** Of the 531 blood donors, 11 subjects (2.1%) had isolated anti-HBc and of 153 injection drug users, 12 cases (7.84%) showed isolated anti-HBc. All of 12 injection drug users with isolated anti-HBc were also HCV positive.

Serologic profile of 2 groups was shown in table 1.

Variable	Blood donors	Injection drug users	P value
HBsAg	0.4%	7.2%	0
Anti-HBs	31.8%	43.8%	0
Anti-HBc	11.5%	35%	0
Anti-HCV	0.2%	59.5%	0
Anti-HIV	0%	5.9%	0

**Conclusion:** Our study showed that prevalence of isolated anti-HBc among injection drug users was 3.73 fold in comparison with blood donors. This study supports that injection drug use and incarceration is contributing to increase prevalence of isolated anti-HBc.

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**Final Abstract Number: 53.011**

**Session: Hepatitis**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Persistence of protective antibodies against Hepatitis B virus among vaccinated health workers, Al-Hussein Hospital, Salt, Jordan, 2008

**S. Sheikh Ali**<sup>1</sup>, M. Jaghbir<sup>2</sup>

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**Background:** Health workers (HWs) have occupational exposure to blood or body fluids; unvaccinated HWs or those vaccinated with low hepatitis-B surface antibodies ("anti-HBs" <10UI" are at higher risk of Hepatitis-B infection they could be focus for dissemination of infection. Duration of protection after fully vaccination is not known. The objective is to assess the immunogenicity "anti-HBs ≥10mIU/ml" post vaccination among HWs.

**Methods:** Two stage study; cross-sectional and follow-up prospective-cohort . Voluntary HWs who met the inclusion criteria were surveyed and tested primary for hepatitis-B core antibodies and hepatitis-B surface antigen, only those with negative results were tested for "anti-HBs"; fully vaccinated HWs with negative anti-HBs were voluntary enrolled in the second stage, retested for anti-HBs after 45 days from a booster dose.

**Results:** 252 HWs participate in the study; 10% of those don't meet the inclusion criteria, primary study sample was 226 HWs, 81% were fully and 19% were partially vaccinated. There was a significant association between the number of vaccine doses and the presence of anti-HBs, p-value-0.0002. There is a significant difference regarding the presence of anti-HBs among fully or partially vaccinated HWs; P-value-0.003. To verify "persistence", statistics were limited to 182 fully vaccinated HWs; was found significant association between the time period from the last administrated doses, age at vaccination, duration of practice and the persistence of antibodies, P-value-0.00004, 0.040, and 0.010 respectively. There were no significant differences regarding the vaccination site, Fisher exact: 0.7018. There is no association between the "place of working, profession, and frequency of exposure to blood or needle stick injury" and the persistence of antibodies. 8% of participants in second stage didn't response by reforming the anti-HBs. 65% of HWs who were exposed to a needle stick injury report no post exposure preventive measures was taken after they had reported the injury.

**Conclusion:** The Hepatitis-B prevalence among HWs was 10%; the non-response rate to vaccine was 8%; the probability for antibodies-persistence is declining by time; probability of antibodies persistence is highly significant if the period from the last administrated dose is less than three years and increases with a booster dose. The older age the vaccine was administrated the higher was the risk to non response to vaccine.

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**Type: Poster Presentation**

Regional and ethnical aspects of viral hepatitis B in pregnant women in Slovakia  
P. Kristian, I. Schreter, Z. Paralicova, **P. Jarcuska**, L. Siegfried, S. Porubcin  
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**Background:** The estimated prevalence of HBsAg in Slovak population is <2% with supposed regional differences and higher prevalence of HBV infection in Roma population. Pregnant women constitute a special subgroup, that is subject to routine HBsAg screening because of the risk of vertical transmission, and also pregnancy can modify the course of hepatitis B infection. The aim of this study was to determine the prevalence of HBV infection in pregnant women in districts of eastern Slovakia with diverse prevalence of Roma population and to determine the subset of women with chronic hepatitis B and increased risk of vertical transmission.

**Methods:** We have analyzed data from 9 regional Departments of Clinical Microbiology from January 2008 till June 2009. We have evaluated the portion of HBsAg positive findings from the overall examined samples and among pregnant women. Available sera from HBsAg positive pregnant women were also screened for ALT, HBeAg and HBV DNA.

**Results:** Overall, 44,912 sera samples were examined, with 10,739 from pregnancy screening. The number of HBsAg positive samples overall and during pregnancy was 803 (1.79%) and 251 (2.34%) respectively. Comparing districts with higher (>4%) and lower (<3%) prevalence of Roma population, there was no notable difference in the overall HBsAg prevalence (1.85% vs. 1.68%), however in the subgroup of pregnant women (2.69% vs. 1.25%) this difference was highly significant ( $p<0.001$ ).

HBV DNA was evaluated in 158 pregnant women, 19 patients had viral load >105 copies/ml, 27 in the range of 104-105 copies/ml and 112 patients <104 or negative. HBeAg positivity was confirmed in 17 of 146 examined samples (11.6%) and elevated ALT in 11 of 69 cases (15.9%). Twelve out of 58 patients with normal ALT (21%) had viral load >104 copies/ml, 3 of them were simultaneously HBeAg positive.

**Conclusion:** The prevalence of HBV infection in pregnant women in eastern Slovakia exceeds the overall estimated Slovak prevalence, and in districts with higher Roma population confirms presumed higher prevalence of HBV infection in this population. Majority of infected women are HBsAg carriers, but we have confirmed several cases of pregnant women with high viral load and increased risk of vertical transmission. We consider evaluation of ALT in pregnancy as insufficient for ruling out chronic hepatitis B. This study was supported by VEGA grant No.1/0050/08.

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**Final Abstract Number: 53.013**

**Session: Hepatitis**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

New approaches in in-vitro diagnosis of Hepatitis C infections: The diagnostic performance of new hepatitis C virus core antigen detection test

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**Background:** There are problems currently in the diagnosis of hepatitis C infections We aimed to investigate the diagnostic performance of new HCV Ag (Abbott Diagnostic, Germany

**Methods:** All of the study cases were applied to the İstanbul University Microbiology and Clinical Microbiology Department and to the Düzen laboratory between February 2008-March 2009. Molecular (real-time PCR) and serological methods (Micro-ELISA) were applied to the all of the cases, 123 HCV RNA-positive and anti-HCV positive cases, 48 HCV RNA negative and anti-HCV negative cases were included as the study and negative control groups, respectively. We evaluated sensitivity, specificity, positive predictive (PPV) and negative predictive (NPV) values, accuracy and kappa values of antigen detection HCV Ag kit which includes monoclonal anti-HCV antibodies against HCV core antigen coated on the solid phase.

**Results:** Sensitivity, specificity, PPV, NPV, accuracy and kappa values were evaluated as 94.3%, 97.9%, 99.1%, 87%, 95.3% and 88%, respectively for HCV Ag kit.

Viral load distribution and HCV Core Ag results of HCV RNA Positive Patients

HCV RNA (IU/ml)	HCV RNA Positive Patients	HCV Core Ag Positive Patients	Negative Patients
0-1000	4	0	4
1001-2000	2	0	2
2001-10000	2	2	0
10001-100000	13	12	1
100001 and upper	102	102	0
Total	123	116	7

**Conclusion:** As a result, the diagnostic performance parameters of recently developed HCV-Ag kit have not satisfactory results because false negative ratio was 5% and NPV was 87%. We suggest that this situation is related with insufficient analytic sensitivity especially for HCV cases which have a 2000 IU/ml and below viral load. We suggest that the re-evaluation of the sensitivity of this kit will be useful for blood screening.

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**Final Abstract Number: 53.014**

**Session: Hepatitis**

**Date: Thursday, March 11, 2010**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

No detected hepatitis B virus-DNA in thalassemic patients infected by hepatitis C virus in Kerman province of Iran

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**Background:** Occult hepatitis B infection (OBI) is characterized as a form of hepatitis in which despite of absence of detectable hepatitis B surface antigen (HBsAg), HBV-DNA is present in patient's peripheral blood. This project was aimed to investigate the prevalence and clinical impact of occult HBV infection in thalassemic patients with chronic HCV infection

**Methods:** In this cross-sectional study we have totally examined 60 patients suffering HBV and HCV infections by PCR and RT-PCR methods, respectively, in Kerman province of Iran. ELISA technique (RADIM, Italy) was used to detect anti-HBc, anti-HBs and HBsAg. The serum level of liver enzymes (SGOT, SGPT, DB, TB and ALK) were analyzed in the HCV infected patients (MAN, IRAN). Statistical analyses performed using t-test and Chi-square

**Results:** We found that 27 cases (out of 60) were infected by HCV but HBV-DNA was not seen in HCV infected patients. Our findings also showed that none of samples were HBsAg positive but 9 (33%) (out of 27) HCV-RNA positive patients were anti-HBc positive and 11 (40.7%) were positive for anti-HBs. We found that SGOT, SGPT, DB, TB and ALK are above normal in 27 (100%), 19 (70.3%), 12 (44.5%), 15 (55.5%) and 15 (55.5%) RNA-HCV positive patients, respectively. The prevalence of hepatitis C infection is very high in thalassemic patients and based on other studies our results showed that the prevalence of HCV infection in Kerman is more than other provinces of Iran.

**Conclusion:** In contrast with other studies HBV-DNA in these patients could not be detected, hence, it seems that occult HBV infection isn't frequent in Iranian thalassemic patients who suffering from chronic HCV infection.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Genotypes and reverse transcriptase variability in asymptomatic chronic hepatitis B virus

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<sup>1</sup>University of Basque Country, Bilbao, Spain, <sup>2</sup>Basurto Hospital, Bilbao, Spain, <sup>3</sup>Basurto Hospital. University of Basque Country, Bilbao, Spain

**Background:** Genomic mutations presented during hepatitis B virus (HBV) reverse transcription could explain its genetic diversity and account for genetically distinct eight genotypes which show distinctive geographical distribution. The main objectives of this study were to determine the prevalence of hepatitis B virus genotypes in asymptomatic chronic hepatitis B patients without treatment, and to identify mutations associated to nucleos(t)ides analogues.

**Methods:** Twenty two serum samples from asymptomatic chronic hepatitis B patients without treatment were analyzed using INNO-LIPA HBV Genotyping assay and INNO-LIPA HBV DRv2 assay (Innogenetics). Nucleic acid was isolated and quantified using real time PCR assay COBAS Taqman HBV test. HBsAg, anti-HBs, HBeAg and anti-HBe were determined by ADVIA Centaur.

**Results:** Genotype D was the most prevalent (45.5%) followed by genotype A (27.3%) and genotype E (9%). There were four coinfection cases (18.2%) (two D/H, one E/F and one A/D genotypes). Both patients infected with genotype E were from sub-Saharan area.

In our study, all the patients were positive for HBsAg and negative for HBeAg. HBV-DNA levels were quantified and the mean was 3.4 log<sub>10</sub> copies/ml (minimum 1.07 and maximum 7.04). Among the twenty two HBV chronic inactive carriers, in only one case was detected a single mutation in rtV214A position, which has been associated to adefovir dipivoxil treatment. In this case, patient was infected with genotype D. In the remaining patients no mutations were identified at reverse transcriptase domain.

Risk factors for HBV acquisition were as follow: vertical (27.3%), transfusion (13.6%), sexual (9.1%), tattoos (4.5%), interfamilial (4.5%) and unknown (41%).

**Conclusion:** INNO-LIPA is a convenient tool for detection HBV genotype and resistance mutation. Genotype D was the most prevalent accompanied with genotype A in asymptomatic patients, but also coinfection genotypes are frequent. A resistant mutation is rare in asymptomatic patients but it was detected in one patient, indicating a possible predisposition to adefovir dipivoxil therapy resistance. HBV reverse transcriptase gene sequencing analysis provides additional insight as part of monitoring program that includes genotypic analysis and quantification of circulating virus.

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**Session: Hepatitis**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Antiviral treatment with interferon alpha and ribavirin influences serum markers of liver fibrosis in children with chronic hepatitis C

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University of Medical Sciences, Poznan, Poland

**Background:** Liver fibrosis largely influences the course and prognosis of chronic hepatitis C (CHC). Although percutaneous liver biopsy is a valuable tool in the evaluation of liver injury, it poses significant drawbacks that limit repeated use of this technique. The aim of this study was to assess chosen serum markers of liver fibrosis – laminin (LAM), hyaluronic acid (HA) and transforming growth factor beta 1 (TGF-beta-1) with their relation to histopathological findings as well as their alterations after antiviral treatment with recombinant interferon alpha (IFN-alpha) and ribavirin (Rib) in children with CHC.

**Methods:** Study group included 68 children, age range 6-18 years (mean 13,34±3,27 years) with diagnosed CHC. History data were analysed. Diagnosis was confirmed by the presence of HCV-RNA in serum by RT-PCR. All children underwent histopathological evaluation of liver biopsy specimen according to Ishak scoring system. Serum sample and liver biopsy specimen were collected the same day. LAM, HA and TGF-beta-1 were measured in serum by enzyme linked immunosorbent assay kits. Children were treated with IFN-alpha (3 MU/m<sup>2</sup> 3 x/week in s.c. injections) and Rib (orally, twice daily) for 48 weeks. Serum markers of fibrosis were also assessed at the end of the treatment.

**Results:** Length of infection was 7.34±4.03 years (range 2-13.5 years). Mean ALT activity was 60,82±65,02 IU/l. Increasing mean serum TGF-beta-1 (p=0.029) and decreasing mean LAM level (p=0.039) was found in the groups of children with increasing stage of fibrosis. Level of LAM was defined a risk factor of significant fibrosis (95%CI 18.05; 67.87, p=0.001), while level of TGF-beta-1 of significant (95%CI: -33.51;-1.73, p=0.030) and progressed liver fibrosis (95%CI -36.09; -3.37, p=0.019). Conducted antiviral treatment with IFN-alpha and Rib resulted in significant decrease of TGF-beta-1 (51.42±30.68 vs. 35.24±31.50 ng/ml, p=0.032) and increase of LAM (60.26± 52.38 vs. 83.70±46.25 ng/ml, p=0.002) in serum. No significant alterations were detected in the control group of untreated children.

**Conclusion:** Level of liver injury in children with CHC varies in severity. TGF-beta-1 and LAM are not only proportional to the stage of fibrosis but also seem to be a good risk factor for the development of significant and advanced fibrosis. Their alterations during antiviral treatment allow monitoring the progression of liver fibrosis.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Incidence of HBeAg/anti-HBe and HBs/anti-HBs seroconversion after antiviral treatment in children with chronic hepatitis B

P. Kemnitz, **W. Sluzewski**, A. Mania, M. Figlerowicz, A. Kowala-Piaskowska, M. Michalak  
University of Medical Sciences, Poznan, Poland

**Background:** Chronic hepatitis B (CHB) is still a significant clinical problem in the whole world. It is estimated that 2 billion people had contact with hepatitis B virus (HBV) and 400 million suffer from CHB or its remote consequences.

The study was aimed at remote analysis of the incidence of seroconversion in HBeAg/anti-HBe and HBsAg/anti-HBs after antiviral treatment and their relation to age at infection as well as evaluation of aminotransferase activity and chosen biochemical parameters.

**Methods:** The study included 310 children (220 boys and 90 girls) with diagnosed CHB who underwent antiviral treatment with interferon-alpha (IFN-alpha) or lamivudine. 177 children were treated with IFN-alpha, 25 with lamivudine and 107 children were treated with lamivudine after prior lack of response to IFN-alpha treatment. Mean age at HBV infection diagnosis was  $4.14 \pm 3.23$  years. Mean age at the beginning of observation was  $6.93 \pm 3.69$  years. Mean length of follow-up was  $7.11 \pm 2.39$  years.

**Results:** In 222 children (71.6%) seroconversion in HBeAg/anti-HBe was present - on the average  $6.69 \pm 3.02$  after the diagnosis and  $2.19 \pm 1.47$  after the introduction of antiviral treatment. The group of children treated with IFN-alpha was introduced  $3.72 \pm 2.12$  years and lamivudine  $6.38 \pm 3.71$  years after the diagnosis of HBV infection. Reverse relation was found between the incidence of seroconversion and the age at diagnosis ( $r = -0.30$ ;  $p < 0.001$ ).

ALT and AST activity at HBV diagnosis was compared in children with and without seroconversion. Children with HBeAg/anti-HBe seroconversion had higher aminotransferase at the diagnosis of HBV infection in comparison with patients without seroconversion.

Viral load in children with seroconversion revealed significant alterations in the following years of the follow-up period in children with seroconversion ( $p < 0.0089$ ). Similar changes were not present in children without seroconversion.

**Conclusion:** Age at infection seems to be a significant factor influencing the incidence of seroconversion. ALT and AST activity at the diagnosis is a good prognostic factor of HBeAg/anti-HBe and HBsAg/anti-HBs seroconversion.

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**Session: Hepatitis**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Seroprevalence and associated risk factors of hepatitis B virus among street children in Isfahan, Iran (2005-2007)

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**Background:** Millions of children throughout the world live on the street. They are at higher risk of viral diseases (eg.hepatitis B virus) because of physical, sexual and drug abuse. The aim of this study was to evaluate the prevalence of hepatitis B virus (HBV) and its risk factors in Isfahan street children.

**Methods:** In a cross-sectional study, we selected 399 street children by simple sampling method in June 2005-March 2007 from correctional centers in Isfahan,Iran. After getting information and giving concept form,data on sociodemographic characteristics and life time risk factors were obtained during a structured interview and a venous blood sample was taken for HBV testing.The tests for HBs Ag, HBs Ab and HBc Ab were doen by ELISA method and data were analyzed by SPSS software version 15 used t-test,  $\chi^2$ , Mann Whitney and logistic regression.

**Results:** We studied 271 (68%) boys and 128 (32%) girls. Their mean age was  $12.74\pm 3.27$  years. In our study, poverty was the main motivation for street children (68% of participants). 91.7%, 94.7% and 96.7% of the participants had no history of smoking, drinking alcoholic beverages or drug abuse, respectively. 34.4% of girls and 4.4% of boys ( $P=0.001$ ) were sexually abused and 68% of girls and 17.3% of boys ( $P=0.001$ ) were involved in physical fighting. HBs Ag, HBs Ab and HBc Ab seroprevalence were 3.3%, 14.4% and 2.1% respectively. No significant statistical difference was between HBV-seroprevalence and its probable risk factors.

**Conclusion:** According to prevalence of HBV markers in the same age group in Isfahan general population,it seems that street children have had more HBV infection and less protection. Educational programs for safer behaviors, improve quality of life, and hepatitis B vaccination in those who are non-immune are recommended for them.

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**Session: Hepatitis**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Prevalence of HBeAg positive cases and abnormal aminotransferase tests in HBV chronic carriers in Kashan, Iran

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**Background:** Hepatitis B virus (HBV) infection and its sequelae are major global health problems. It is estimated that 400 million people worldwide are HBV carriers. The natural history of hepatitis B is complex and is influenced by many factors, including age at infection, viral factors (HBV genotype, viral mutations, level of HBV replication), host factors (gender, age, and immune status), and exogenous factors such as concurrent infection with other hepatotropic viruses or alcohol. This study was conducted to evaluate HBsAg persistence, related risk factors of HBV infection and detect of HBeAg positive cases and abnormal liver enzyme tests among chronic HBV carriers in Kashan.

**Methods:** This descriptive study was performed in HBsAg positive blood donors. They were invited to retest HBsAg. A questionnaire consisting demographic and history of risk factors of HBV infection were filled by interviewing and then 5cc blood was taken and HBsAg was measured by ELISA method. Who had HBsAg positive test without history of acute hepatitis during the previous year, were known as chronic carriers. and HBeAg, hepatic aminotransferases and alkaline phosphatase were measured.

**Results:** Of 150 HBsAg positive blood donors 124 (83.7%) were male and 26 (17.3%) female. Persistence and clearance of HBsAg was seen in 132 (88%) and 18 (12%). No significant statistical correlation between sex, age, marital status, history of icter, endoscopy, tattoo, transfusion, with persistence of HBsAg was found.

In 132 HBsAg + chronic carriers the prevalence of HBeAg positive cases was 18.2%. There was abnormal aminotransferase level including: 35 (26.3%) AST, 16 (12%) ALT, 26 (19.5%) ALKP. Significant statistical correlation was seen between ALT and HBeAg but there was no statistical correlation between AST and ALKP with HBeAg.

**Conclusion:** Considering the lower chance of clearance of HBsAg and detect of correlation between HBeAg positive and abnormal ALT, and also detect of suspected cases of chronic hepatitis, follow up seems to be essential in HBeAg positive cases.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Positive association of high risk behaviors and Hepatitis B infection

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**Background:** Hepatitis B is one the most infectious diseases, without seasonal distribution, accounting for more than one million deaths annually due to chronic hepatitis, cirrhosis and hepatocellular carcinoma. We aimed to investigate the seroprevalence of the HBV infection in Bandar Abbas, Iran and determine the association of respective risk factors with its occurrence.

**Methods:** We conducted a cross sectional community-based study of 1152 apparently healthy individuals aged 8-65 years for the serological HBsAg screening. A standard questionnaire was administered to all subjects. Unpaired t-test for continuous data and the  $\chi^2$  test for categorical data were performed

**Results:** A total of 1152 individuals were assessed for hepatitis B surface antigen (HBsAg), which was 2.1% in overall. There was no significant difference between males and females with respect to HBsAg seropositivity (male, 2.2% vs female, 2.1%,  $P > 0.05$ ). Singles showed a prevalence of 1.7%, while HBsAg seropositivity in married participants was 2.3%. Subjects with tattooing practices reflected significant higher prevalence (7.5%) compared to those without tattooing (1.7%,  $P < 0.0002$ ). History of drug abuse remained significantly associated with HBsAg seropositivity (4.9% vs. 1.4%,  $P < 0.0001$ ).

**Conclusion:** HBsAg seropositivity was found 2.1%. High risk behaviors associated significantly with seroprevalence of HBsAg. Of these, tattooing emerged more crucial compared to the risk factors identified in out in other regions.

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**Type: Poster Presentation**

Biodynamics of HCV infection in haemodialysis patients in Pahang

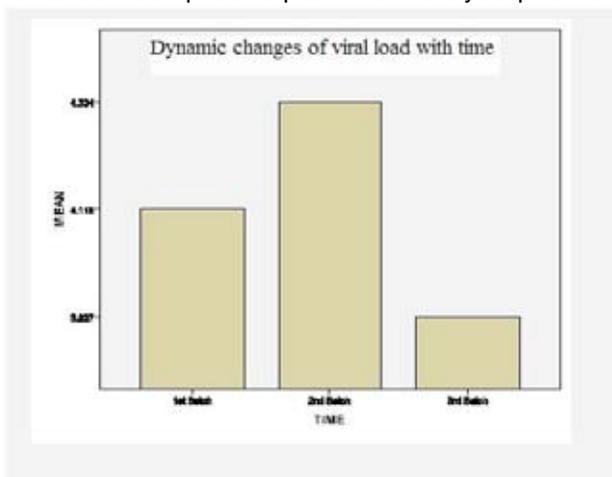
**M. Mustafa**, M. S. Abdul Majid, H. A. Binti Hamzah, M. H. bin Hasmaini

Faculty of Medicine, International Islamic University Malaysia, Kuantan, Pahang, Malaysia

**Background:** Hepatitis C is a global disease, WHO has described it as a “viral time bomb”. In Malaysia, the sero-prevalence is 1.6%. HCV infection is frequent in patients undergoing maintenance haemodialysis, with prevalence between 8 and 10%. Hepatitis C has an adverse effect on both patient and graft survival in those who get renal transplants. There are relatively scarce reports on the natural fluctuation in viral load and alpha interferon ( $\alpha$ -IFN) level in patients on chronic hemodialysis.

**Methods:** A longitudinal short-term three months study where 27 chronic hemodialysis patients infected with known HCV genotypes were recruited from seven hemodialysis centers in Pahang. Serum samples were collected monthly, both pre- and post-hemodialysis sessions, over a period of three months. Viral RNA was extracted from serum using QIAamp Viral RNA Extraction kit (Qiagen). The HCV viral load was measured using one step reverse transcriptase qPCR (Applied Biosystems) targeting the 5' HCV non-coding region. The serum  $\alpha$ -IFN level was measured using commercial ELISA kit (Amersham, UK). Six biochemical liver function tests (AST, ALP, TP, albumin, ALT and TB) were also done for all pre-hemodialysis samples.

**Results:** All patients showed persistent low level viral load that varied significantly over the study period ( $P = 0.001$ ). HCV genotype 1 viral load was significantly higher than that of genotype 3. The difference between pre- and post-haemodialysis viral load was statistically insignificant. No significant correlation between viral load and liver function status was noted. No correlation was observed between pre-haemodialysis serum  $\alpha$ -IFN level and pre-haemodialysis viral load. The difference between pre and post-haemodialysis plasma  $\alpha$ -IFN levels was statistically insignificant.



**Viral load variation in three batches of sera taken one month apart**

**Conclusion:** HCV infected haemodialysis patients experience significant fluctuation in viral load with time but no correlation with biochemical evidence of liver injury. HCV genotype 1 is associated with higher viral load as compared to genotype 3. Single-step rt qPCR assay has the potential for rapid HCV genotyping.

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**Session: Hepatitis**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Runs test and linear trend test of the time series of primary hepatocellular carcinoma mortality in Haimen City, 1969-2007

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**Background:** To evaluate the trend and contributing factors of time series changes of primary hepatocellular carcinoma (HCC) mortality between 1969 and 2007 in Haimen City.

**Methods:** A five-year retrospective survey was conducted in Haimen City in 1973 to verify the cause of death among all city population between 1969 and 1973. The birth/death reporting system was also established and was defined as one of the nation's pilot city for vital statistics by Ministry of Health and Ministry of Public Security. In 2006 it was accepted by the International Union Against Cancer (UICC) as a member institute. This paper describes the time series changes of HCC mortality in Haimen City between 1969 and 2007. Runs test was performed as stability test for the time series and linear trend test was performed as significance test.

**Results:** HCC mortality increased from 33.18 per 100,000 in 1969 to 49.32 per 100,000 in 1979, with an annual increasing rate of 4.04%. Between 1979 and 1989 the mortality increased from 49.32 to 70.64 per 100,000, with an annual increasing rate of 3.66%. Between 1989 and 1999 the mortality decreased from 70.64 to 54.94 per 100,000, with an annual increasing rate of -2.28%. Between 1999 and 2007 the mortality decreased from 54.94 to 50.09 per 100,000, with an annual increasing rate of -1.15%. The runs test showed an instable time series, and the linear trend test indicated a statistical significant decreasing trend of HCC mortality since 1990. It is unlikely that this decreasing trend is caused by random.

**Conclusion:** The decreasing trend of HCC mortality in Haimen City since 1990 is related with the intervention of environmental factors including 'changing drinking water, changing staple food and prevention of HBV infection' as major components.

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**Session: Hepatitis**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Predictive factors for sustained virological response in the treatment of patients with chronic hepatitis C

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**Background:** The goal of antiviral therapy in patients with chronic hepatitis C is to prevent progression of the disease. The most important treatment response is the absence of viral RNA six months after discontinuation the therapy, defined as the sustained virological response (SVR). Successful therapy depends on many factors that are in association with the virus itself and/or infected person.

The aim of the investigation was identifying some characteristics of the patients and hepatitis C virus as pre-treatment predictive factors for achieving SVR.

**Methods:** Total of 364 treatment-naïve patients was enrolled in the study (60.4% were males; age from 16 yr-65 yr, mean 42.18, SD ± 11.71). Liver cirrhosis had 21.7% of patients. Patients were treated with standard doses of pegylated interferon alpha and ribavirin during 6 to 12 months depending on the genotype in the period from January, 2004 to January, 2009. Source of infection was blood transfusion, intravenous drug abuse, accidental injury and unknown in 26.5%, 19.5%, 12 %, and 42% of patients, respectively. Genotype distribution revealed genotype 1, 3, and 4 in 56.8%, 26.7% and 5% of patients, respectively. The minority of patients had genotype 2 and/or mixed genotypes (1b3a, 1b4). Detection and quantification of viral RNA with commercial assay (Cobas Amplicor HCV Monitor v2, Roche Molecular Systems) was evaluated before treatment, after treatment and six months later. Electronic data base (SPSS for Windows V11.0) was used for statistical analysis.

**Results:** Total of 82.8% of patients were negative (HCV RNA < 50 IU/mL) after treatment while SVR was achieved in 68.8% of patients. Binary logistic regression analysis revealed intravenous drug abuse (p= .005) and genotype 3 (p= .003) as positive, whereas age older than 40 (p= .000), presence of cirrhosis (p= .039), and genotype 1 (p= .000) were negative predictor factors for SVR. In multivariate analysis, age older than 40 (p= .001) and genotype 1 (p= .000) were significant variables (Exp(B)= .351 vs. .233).

**Conclusion:** Successful virological response to therapy is expected in patients with genotype 3 and intravenous drug users. Presence of genotype 1 is the most important negative predictor for treatment of patients with chronic hepatitis C.

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**Type: Poster Presentation**

Is there any association between chronic Hepatitis C virus and celiac disease?

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**Background:** Celiac disease (CD) is an immune-mediated disorder associated with some autoimmune liver diseases, including primary biliary cirrhosis, autoimmune hepatitis and primary sclerosing. In addition, there are contradictory reports on association of chronic hepatitis C (HCV) and celiac disease. The aim of this study was to determine the prevalence of untreated celiac disease and Hepatitis C Virus in Iranian pregnant women and assess the outcome of pregnancy compared to non-celiac cases.

**Methods:** In this cross sectional study, 827 pregnant women were recruited for this study during the period of January 2007- February 2009. The mean age was 26.07 and SD 5.35 and mean pregnancy 5.4 months. Data were obtained through questionnaires distributed during pregnancy. The questionnaire included questions about GI symptoms and infections during pregnancy. They underwent a total IgA test and antihuman IgA class antitissue transglutaminase (tTGA) antibodies for celiac disease and samples also screened for anti-HCV antibodies by Third generation enzyme linked immunosorbent assay.

**Results:** From 827 pregnant women 27 (3.26%) had a positive serology for tTGA. Only two samples (0.24%) were anti-HCV positive and one of them had positive tTG-IgA. Eleven cases from 827 had IgA deficiency and none of them were positive for IgG-tTG. Out of the 27 diagnosed women, three had low birth weight babies and four had a history of miscarriage.

**Conclusion:** Routine screening of HCV in CD patients is not recommended, however, the presence of CD should be considered in the setting of clinical deterioration during the pregnancy.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Prevailing HCV genotypes and subtypes among HIV infected patients in Georgia

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**Background:** Recent analysis of ART program data in Georgia showed that end-stage liver disease is leading cause of death among HIV/HCV co infected patients on ART after initiation of ART program in 2005. Therefore, treatment of hepatitis C among HIV infected individuals is essential for sustaining success of ART in Georgia and to improve survival rates of HIV infected patients. HCV treatment response rate and sustained viral suppression after antiviral treatment largely depends on the HCV genotypes along with the influence of HIV co- infection itself. The objective of this retrospective study was to study the prevailing genotypes and subtypes of HCV virus in a cohort of HIV infected patients in order to obtain the preliminary data.

**Methods:** We analyzed 1490 HIV infected patients co infected with HCV virus. Among them 59% were HCV coinfecting. Measurement of HCV RNA viral load was done by COBAS TaqMan HCV-2.0 Test and HCV genotyping by reverse hybridization line probe assay using VERSANT HCV Genotype kit 2.0 respectively.

**Results:** Detectable HCV RNA was found among 680 patients (91.%). Less than 10 lu/ml was found among among 67 (9%). This numbers indicate low rate of self clearance of the virus among HIV persons. Greater HCV RNA levels were associated with a greater chance to be infected with HCV genotypes 1. Most prevalent genotypes were genotype 1 accounting for (41.6 %), followed by genotype 3 (34.7%) and 2 (17.6%), inter genotype recombinants were found among 5.8 %.

**Conclusion:** Study demonstrated high prevalence of HCV infection among HIV-infected patients and reveals 1 as predominant genotype. The differences of prevailing HCV genotypes among general population and HIV co infected group was probably attributed to the different methods for sample selection used. Another option can be possible influence of diverse transmission networks among HIV infected group. The high number of intergenotype recombinants might be results of continues parenteral exposure to different HCV genotypes during drug injection paraphernalia. Study highlights and strengthens the need for careful follow-up of HCV/HIV co infected patients, effective management and therapies against HCV in order to reduce liver related death rates in patients on ART.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Hepatitis B Virus (HBV) mutations during long-term therapy in chronic hepatitis B patients

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**Background:** Long-term lamivudine (LAM), adefovir (ADV) and entecavir (ETV) treatment has been found to induce the emergence of drug-resistant hepatitis B virus (HBV) in a significant number of patients with chronic hepatitis B infection. The aim of our study was to evaluate the LAM, ADV and ETV mutations detected in our patient group.

**Methods:** Fifteen patients diagnosed with chronic hepatitis B were enrolled in this study. The patient group consisted of those who had received two years of treatment with nucleos(t)ide analogues. Patients were evaluated based on virologic and serologic response to therapy, and were classified as patients with a detectable level of HBV DNA and undetectable HBV DNA (<1.08 log copies/ml). The mutations associated with HBV drug resistance were investigated in patients with detectable HBV DNA. Due to resistance, in this group of patients treatment was change.

**Results:** In our study group, four patients developed LAM-associated mutations (rtL180M+rtM204V, rtL180M+rtM204V/I, and 2 present rtM204I), three patients developed ADV-associated mutations (rtA181V+rtN236T; rtN236T and rtA181V), one patient developed ADV+LAM associated mutations (rtL180M+rtA181V+rtN236T) and other patient developed ETV-associated mutations (rtL180M+rtT184A+rtS202G+rtM204V). Beside the primary resistant mutations, various combinations of secondary and compensatory mutations conferring resistance to nucleos(t)ide analogues were detected in 5 (33%) patients.

**Conclusion:** HBV treatment with nucleoside analogues results in the development of mutants strains, leading to drug resistance. These data suggest an early development of ETV resistance in patients with prior LAM and ADV resistance. Therefore genotypic resistance is important in monitoring HBV treatment. In conclusion, optimization of therapy combining LAM and ADV may be a good choice for patients with hepatitis B who have resistance mutations to ADV or LMV.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Long term virological follow up of Hepatitis C (VHC) monoinfected patients who achieved end of treatment response (ETR)

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**Background:** Over the last few years a new concept has introduced a possibility a SVR may indicate cure. VHC-ARN clearance at 6 months after therapy discontinuation SVR ( sustained virologic response) is generally assumed as probable cure . The medical literature shows a significative reduction in the rate of progression to cirrosis and Hepatocarcinoma development in patients who achieved SVR compared to non responders and relapsers.

**Methods:** We retrospectively analysed a group of VHC patients that fulfilled histologic criteria for antiviral treatment from 1998 to 2009. We performed VHC ARN PCR determinations at baseline, during treatment, in the last week of antiviral treatment, every 6 months' until the 2<sup>o</sup> year after therapy discontinuation. and once a year further on.

We defined ETR as negative or undetectable ARN VHC PCR in the last week of antiviral treatment.

**Results:** In our study 38 patients achieved ETR (negative VHC PCR during the final week of IFN treatment) . Only 3 patients that achieved ETR showed early relapse in the next six months after stopping therapy (so they not achieved SVR). The rest of the patients are not detectable up to the last control., in the long term follow up.

**Conclusion:** In this small group of patients we didn't find relapses after 6 months following discontinuation of therapy, with a mean follow up of nearly 10 years

The period of highest risk of relapses was the next 12 months after the end of treatment.

The rate of relapses after 24 months of the end of treatment was very low, as estimated in different studies between 1 and 2%.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Seroprevalence of hepatitis A among children and adolescent from south and southeast region of Brazil

**G. Figueiredo**<sup>1</sup>, M. R. Cardoso<sup>2</sup>, R. MOREIRA<sup>3</sup>, L. Pereira<sup>4</sup>, U. Montarroyos<sup>4</sup>, C. Braga<sup>5</sup>, A. Stein<sup>6</sup>, G. Coral<sup>6</sup>, R. Ximenez<sup>4</sup>

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**Background:** The objectives were to estimate the prevalence of hepatitis A among children and adolescents from south and southeast region of Brazil and to identify individual-, household- and area-levels factors associated with hepatitis A infection.

**Methods:** This population-based survey was conducted in 2007–2008 and covered individuals aged between 5 and 19 years. A stratified multistage cluster sampling technique with probability proportional to size was used to select 5,054 individuals aged between 5 and 19 years living in the State capitals of 7 states in the study regions. The sample was stratified according to age (5–9 and 10- to 19-years-old) and capital within each region. Individual- and household-level data were collected by interview at the home of the individual. Variables related to the area were retrieved from census tract data. The outcome was total antibodies to hepatitis A virus detected using commercial EIA. The associations between HAV infection and independent variables were assessed using the odds ratio and corrected for the random design effect and sampling weight. Multilevel analysis was performed by GLLAMM using Stata 9.2.

**Results:** The prevalence of hepatitis A infection in the 5–9 and 10–19 age-group was 20,6 and 37,7%, respectively for the Southeast and, 18,9 and 34,5%, respectively for the South. A trend for the prevalence of HAV infection to increase according to age was detected in all sites. Multilevel modeling showed that variables relating to different levels of education were associated with HAV infection in all sites.

The multilevel model showed that individual, household- and area-level variables are independently associated with HAV infection. Age is an important predictor of outcome. Additionally, education and sewage disposal service variables that were collected through household-level interviews or retrieved from IBGE census tract data were associated with HAV infection in all sites.

**Conclusion:** The study sites were classified as areas with low endemicity area for hepatitis A infection. This multilevel model allowed for quantification of contextual predictors of hepatitis A infection in urban areas.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Household survey of hepatitis B infection and risk factor assessment in the from South and Southeast region of Brazil

**G. Figueiredo**<sup>1</sup>, R. MOREIRA<sup>2</sup>, L. Pereira<sup>3</sup>, M. R. Cardoso<sup>4</sup>, G. Coral<sup>5</sup>, A. Stein<sup>5</sup>, U. Montarroyos<sup>3</sup>, C. Braga<sup>6</sup>, R. Ximenez<sup>3</sup>

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**Background:** In 1989, the Brazilian government first implemented immunization against hepatitis B for infants and children in the western Amazon region and gradually expanded this to other regions. Vaccination of risk groups for the whole country started in 1992, and new groups have been successively added to the original list. Since 1998, the HBV vaccine has been incorporated into the immunization schedule for infants as a national policy and, in 2001, this was broadened to include children and adolescents.

A population-based survey was conducted in Brazil to estimate the prevalence of and risk factors for HBV infection from South and Southeast region of Brazil.

**Methods:** Random multistage cluster sampling was used to select individuals aged 13-69 years. Outcomes indicating HBV infection were anti-HBc and HBsAg. HBsAg-positive samples were tested for HBV-DNA and genotyped. Univariate and multivariate analyses were performed.

**Results:** Overall, 10,496 individuals were included; the prevalence of anti-HBc was: South 1,58% for 10-19 and 11,3 for 20-69 years old; Southeast 0,61% for 10-19 and 7,9 for 20-69 years old. HBsAg positivity was less than 0,5% in all areas. Genotypes 3A, 1b,1a and 2b were identified. Age were associate with HBV infection in all region and male were independently associated with HBV infection in Southeast.

**Conclusion:** Our survey classified the South and Southeast region of Brazil as low HBV endemicity areas. Our findings that age is risk factors for HBV reinforce the need for extensive HBV vaccine coverage among adolescents to prevent viral infection. All individuals belonging to the risk groups identified by our survey should be considered candidates for HBV vaccine and educational measures. Therefore, vaccination upon request for individuals without specific risk should be considered, in accordance with the current CDC recommendations. This survey shows the importance of generating population-based information to facilitate comprehensive vaccination and of developing educational strategies that address regional differences.

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**Type: Poster Presentation**

Age-dependent, differentiated prevalence of anti-HAV and anti-HBc antibodies among patients with chronic hepatitis C (CHC): New aspect of future vaccination perspectives against hepatitis A Virus

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**Background:** Hepatitis A is an acute, usually self-limiting disease of the liver, caused by hepatitis A virus. Patients with chronic liver disease are at increased risk of severe complications, related to hepatitis A, which, in some circumstances, may even lead to death. Therefore, it is postulated that HAV susceptible individuals ( anti-HAV seronegative subjects) should be vaccinated against HAV to prevent or diminish morbidity and mortality of the disease.

**Aim:** Evaluation of the prevalence of anti-HAV antibodies among patients in different age groups with chronic hepatitis C.

**Methods:** 133 patients (the mean age: 38.3 yrs ) with CHC were divided into two groups, according to age (Table 1). The control group consisted of 150 healthy young medical students (the mean age: 25.3 yrs ). The presence of anti-HAV and addition of HBV infection viral markers: anti HBc and HBsAg in blood sera of the patients and of the control group were assessed, using the standard EIA method (Cobas Roche ).

**Results:** The prevalence of anti HAV antibodies and HBV infection markers among the evaluated groups are presented in Table 1.

Group	Age years	Pt n	anti HAV		Anti-HBc		HBsAg	
			n	%	n	%	n	%
CHC	18-30	33	5	15.5	6	18.8	0	0
	31-73	100	47	47.0	27	27.0	2	2
Control	24-26	150	15	10.0	8	5.3	2	1.33

In the study groups, age-dependent, differentiated prevalence of anti-HAV and anti-HBc was observed among younger ( < 31 years) and older ( > 30 years) patients with CHC ( 15.5% vs. 47.0%; p=0.05 and 18.8% vs. 27%,p=0.05, respectively), which was higher than in healthy controls ( 10.0% and 5.3 %, respectively).

**Conclusion:** Patients below 31 with CHC present significantly lower anti-HAV and anti-HBc prevalence than older ones, what creates a substantial risk for fulminant liver failure and even death in case of HAV or HBV infection. Thus vaccination programmes against HAV and HBV are strongly recommended in these groups of patients.

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**Type: Poster Presentation**

Ultrasonography changes of the liver of patients infected with hepatitis C met in the Foundation of Tropical Medicine of Amazonas

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**Background:** 50% to 85% of the patients infected by hepatitis C virus will develop a chronic infection, 20% of them will suffer from cirrhosis and 1% to 4% will develop hepatocellular carcinoma. Morphologic and hemodynamic changes which occur in the liver and in the portal system can be evaluated by Doppler ultrasonography at an early stage and in a non-invasive way. The main purpose of this paper was to study alterations detected by ultrasonography examinations in hepatitis C patients assisted at Fundação de Medicina Tropical do Amazonas.

**Methods:** In an 8-month period, 50 patients anti-HCV positive whose ages ranged from 18 to 69 were submitted to Doppler ultrasonography examinations.

**Results:** Most of the patients were males (74%) at an average age of 47,1. Morphologic alterations were detected, such as increase of the volume of the liver (12%) mainly in the left liver lobe, increase of liver echogenicity (24%), splenomegaly (14%) and increase of echogenicity and gallbladder wall thickening (4%).

**Conclusion:** Hemodynamic alterations were noticed, mostly a reduction on the portal vein velocity (26%), alterations on the congestion index (12%), on the vascular hepatic index (16%) and impedance of hepatic and splanchnic arterial index.

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**Type: Poster Presentation**

Detection of acute HCV infection among different risk groups

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**Background:** To investigate acute HCV infection from the very first days of the infection among blood donors, injecting drug users (IDUs), health care workers and persons who had first life time sexual intercourse with the chronic HCV infected persons in orders to asses clinical laboratory variants of infection, viral replication kinetic, disease outcome.

**Methods:** Study was performed among ELISA negative persons in four groups: 7500 blood donors, 3200 IDUs, 70 exposed health care workers and 45 persons who had first life time sexual intercourse with the chronic HCV infected persons. All groups were investigated on HCV RNA by Real Time PCR (COBAS TaqMan Roche) using pool method and genotyped by line probe assay (Innolipa, Versant, Bayer) according to the manufacturer's instructions. The results were analyzed by age, sex, duration of infection, modes of transmission and other variables.

**Results:** Total of 21 patients with acute HCV infection were identified: 7 from blood donors, 9 from IDUs, 1 from health care workers and 4 from persons who had first life time sexual intercourse with the chronic HCV infected patients. All HCV RNA positive persons revealed within the study were followed on viral load dynamic at detection moment and at 2, 4 and 8 weeks and after 3 and 6 months from their possible exposure. Among them: 5 were symptomatic and 16 asymptomatic. Among 21 subjects: 10 had genotype - 1b, 5 - 2a/2c and 6 - 3a. Out of 21 patients 7 cleared the virus (5 were symptomatic: 1 - genotype 1b, 2 - genotype 2a/2c, 2 - genotype 3a, and 2 asymptomatic: 1 - genotype 1b, 1 - genotype 3a), while 14 developed chronic infection. In all patients viremia increased rapidly and reached a peak by week 4. After week 10 the viremia rapidly decreased. It became either undetectable by weeks 16-18 (viral clearance), or virus was not eliminated (chronic infection).

**Conclusion:** We found that HCV viral clearance was correlated with high viral titers, symptomatic form of disease and sexually transmitted HCV infection in female subjects. Study revealed no correlations between viral genotype and viral clearance rates.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Efficacy of 96 weeks adefovir dipivoxil treatment in HBeAg positive chronic hepatitis B patients with various baseline biochemical levels

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**Background:** Adefovir dipivoxil (ADV) has shown efficacy and safety in a broad range of populations with chronic hepatitis B over 48 to 96 weeks. This study reports the 96-week long-term efficacy data with ADV treatment in nucleoside-naïve HBeAg-positive chronic hepatitis B patients with various baseline biochemical levels.

**Methods:** Ninety-eight HBeAg-positive patients who had never received nucleoside treatment received 96-week ADV 10mg/d therapy. All patients had serum level of HBV load over 105 copies/ml and increased serum alanine aminotransferase (ALT) level. Based on serum ALT levels at baseline, all patients were divided into two groups, A (48 patients with serum ALT level less than 200 U/L) and B (50 patients with ALT level more than 200 U/L). Serum HBV load was measured with quantitative real-time-PCR. ALT activity, HBeAg, anti-HBe-antibodies, HBV-DNA level in serum were evaluated at baseline, week 12, 24, 48 and 96 during therapy.

**Results:** After 24 weeks of therapy, mean reduction of HBV-DNA level, the percentage of patients with HBV-DNA lower than 5 log<sub>10</sub> copies/ml and the percentage of patients with HBV-DNA level decrease of more than 2 log<sub>10</sub> copies/ml in group B were significantly higher than those in group A ( $P < 0.05$ , respectively). At the end of 24, 48 and 96 weeks, the patients in group B had higher rates of undetectable serum HBV-DNA levels and ALT normalization than those in group A (46% vs 25%, 74% vs 54.2%, 88% vs 70.8%; 52% vs 31.3%, 66% vs 43.8%, 82% vs 62.5%;  $P < 0.05$ , respectively). HBeAg seroconversion rate was significant higher in group B than those in group A (48% vs 27.1%,  $P < 0.05$ ). There was no evidence of adverse effect in patients treated for up to 96 weeks.

**Conclusion:** Adefovir dipivoxil is an effective treatment option for nucleoside-naïve patients with HBeAg-positive chronic hepatitis B, especially for those with high serum ALT levels at baseline. Adefovir dipivoxil treatment through 96 weeks was well tolerated and resulted in continued benefit for patients with HBeAg-positive chronic hepatitis B.

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The relationship between HBV precore region mutation and the variation of T-lymphocyte subpopulations in chronic HBV-infected individuals with normal liver function tests

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**Background:** To investigate peripheral T-lymphocyte subpopulation profile and its correlation with HBV precore region 1896 mutation in chronic HBV-infected (CHI) individuals with normal liver function tests.

**Methods:** HBV precore region 1896 mutation and distribution of T-lymphocyte subpopulations in peripheral blood were measured in CHI individuals with normal liver function tests. HBV markers were detected with ELISA. Serum HBV DNA load was assessed with quantitative real-time polymerase chain reaction. The results of peripheral T-cell subsets were compared among groups based on HBV precore region 1896 mutation status, HBeAg, age, sex and the quantities of HBV DNA.

**Results:** CHI individuals with normal liver function tests had significantly decreased CD4+ cells and CD4+/CD8+ ratio, and increased CD8+ cells compared with uninfected controls, all with  $P < 0.01$ . Comparing with HBV precore region non-mutation group, the patients with precore mutation had significant decreased CD4+ cells and CD4+/CD8+ ratio and increased CD8+ cells. Univariate analysis showed a similar pattern of these parameters was significantly associated with presence of serum HBeAg expression and high viral load, all with  $P < 0.05$  or  $0.01$ . The presence of HBeAg expression carried by the HBV precore region mutation positive group were not significant different than those observed in HBV precore region mutation negative group. There is a significant difference in the quantities of HBV DNA between the HBV precore region mutation positive group and negative group. No obvious differences of T-cell parameters and presence of precore mutation were observed among various age groups and sex groups, all with  $P > 0.05$ .

**Conclusion:** T-lymphocyte impaired balance was significantly associated with HBV precore region mutation in CHI individuals with normal liver function tests. Which with HBeAg expression and the HBV DNA replication affect each other and co-result in lower T-cell immune function and chronic infection persistent status.

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Patterns of hepatitis A infection by Brazilian regions: Results of the national household survey 2004-2009

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**Background:** Brazil has been classified as high endemic country and HAV vaccine is not implemented in the routine immunization calendar. The objectives were to estimate the prevalence of hepatitis A among children and adolescents by Brazilian regions and household/contextual risk factors association. This population-based survey was conducted between 2004 and 2009. It was a Ministry of Health and PAHO initiative with the Universities and Public laboratories located in the State capitals.

**Methods:** A stratified multistage cluster sampling technique with probability proportional to size was used to select around 6400 individuals aged between 5 and 19 years residents of the State capitals and the Federal District. The sample was stratified according to age (5–9 and 10- to 19-years-old) within each region. Individual- and household-level data were collected by home interview. Total antibodies to hepatitis A virus were detected by EIA. Frequencies of HAV infection by region and the age distribution of the susceptible population were estimated and odds ratio (95% CI) were calculated using GLLAMM.

**Results:** The prevalence of hepatitis A infection in the 5–9 and 10–19 age-group by region: North (28.7% and 67.5%); Northeast (41.5% and 57.4%); Midwest (32.3 and 56.0%); Federal District (33.8% and 65.1%); Southeast (20.6% and 37.7%); South (18.9% and 34.5%). There was increasing trend for the prevalence of HAV infection with age in all sites. The curves of susceptible populations differed from one area to another. Multilevel modelling showed that deprivation/low socio-economic status was associated with HAV infection in all regions.

**Conclusion:** This is the first national population-based survey to determine prevalence of hepatitis A (HBA) in all capital cities showing the shift pattern from high to intermediated/ low endemicity. Differences in age trends of infection were detected among settings. This multilevel model allowed for quantification of contextual predictors of hepatitis A infection in urban areas.

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**Type: Poster Presentation**

Acute liver failure in the Colombian pediatric population: Retrospective and prospective studies

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**Background:** Describe the epidemiological and clinical characteristics in the pediatric patients with Acute Liver Failure that were attended in the period between 1998 and 2009 in the different health institutions in Colombia.

**Methods:** A transversal study based on the revision of clinical histories and questionnaires carried out in 10 health institutions in 5 Colombian cities; including 2 Centers specialized in Transplants to evaluate the cases of Fulminant Hepatic Failure in patients under the age of 18 years of age presented between January 1998 and November 2009. FHF was defined as: 1). The elevation of AST and ALT aminotransferases and/or the elevation of conjugated bilirubin. 2). The absence of known chronic hepatic disease. 3). INR > or equal to 1.5 or PT > or equal to 15 seconds after the administration of vitamin K, if the patient presents encephalopathy or 4). An INR > or equal to 2.0 or TP > or equal to 20 seconds after the administration of vitamin K if encephalopathy is not presented. 5.) The degree of encephalopathy was stratified according to age and according to its classification.

**Results:** 47 clinical histories were revised and 46 subjects were included. The average age was 5.8 years of age (4 months of age – 16 years of age); of which 58.6% corresponded to the female gender; 28.35% (13/46) coming from rural areas. Jaundice was found in 100% of the subjects upon entry and encephalopathy in 76% (35/46). Liver failure was mortal in 32.6% (15/46), with the average age being 6, 5 years of age. Infection as a result of the Hepatitis A Virus was one of the first causes of FHF with 32.6% (15/46) as well as Indeterminate Hepatitis (32.6%) and 15.2% caused by a toxic reaction to the medications. The greatest proportion of deaths is associated to Indeterminate Hepatitis due to the forcefulness of the disease and the limited elements to understand its etiology. 76.6% of the transplant candidates obtained a successful transplant (67%); retransplant was carried out in 2 subjects, one died and the other managed to survive.

**Conclusion:** Acute Liver Failure is a frequent pathology among the pediatric ages in Colombia and is caused by the Hepatitis A Virus in up to 32.6% of the cases. A high percentage of patients need liver transplantation and in some cases have fatal consequences. Many of these cases are preventable with vaccination.

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**Type: Poster Presentation**

Hepatitis B seroprevalence and associated factors in pregnant women and their couples in 6 Amazonian indigenous populations in Peru 2007-2008

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**Background:** Since 1995, there are reports documenting High magnitude of Hepatitis B transmission, but information about associated factors is scarce.

**Methods:** From June 2007 to december 2008 a seroprevalence study was conducted in 6 Amazonian indigenous populations: Shispibo-Konibo, Ashaninka, Kandozi, Shapra, Chayahuita, Matsigenka. Objective: to determine the magnitude of Hepatitis B infection, and associated factors. Blood samples and written surveys were obtained. Informed consent was obtained from National, Regional and local Community leaders and participants. For Hepatitis B testing, HBsAg and Anti-HBc elisa tests were performed.

**Results:** 1274 pregnant women and 721 male couples were surveyed. Anti-HBc positive results: 522 women (42.06% [95%CI: 39.30% – 44.87%]) and 377 males (54.09% [IC95%CI: 50.31% – 57.84%]). HBsAg were positive for 11/393 Anti-HBc positive women (2.80% [IC95%: 1.41% – 4.95%]), and 15/312 Anti-HBc positive males (4.81% [IC95%: 2.72% – 7.81%]).

Anti-HBc positivity in women was associated to:

Associated factor	OR (95%CI)	P value
Male couple Anti-HBc positivity	7.15 (5.09–10.03)	0.000
Sex during menstruation	0.75 (0.59–0.96)	0.013
Male couple history of sex with other men	5.49 (2.38–12.64)	0.000
Being younger than 19 years	0.54 (0.40–0.72)	0.000
Sexual initiation before age 15 years	1.32 (1.05–1.66)	0.008
Male couple with more than 10 sex partners lifetime	1.94 (1.36–2.76)	0.000
Male couple with at least 1 more sex partner in last 12 months	1.65 (1.22–2.23)	0.000
Male couple with more than 20 sex partners lifetime	1.90 (1.22–2.96)	0.002

Anti-HBc positivity in men was associated to:

Associated factor	OR (95%CI)	P value
History of sex with other men lifetime	5.88 (2.26–15.28)	0.000
Being younger than 19 years	0.41 (0.18–0.88)	0.016
Sexual initiation before age 15 years	1.99 (1.38–2.89)	0.000
Having sex with women presenting vaginal secretion	1.60 (1.09–2.36)	0.010

**Conclusion:** Hepatitis B is hyperendemic in Amazonian indigenous populations studied. These results suggest sexual transmission as the main infection route of Hepatitis B in indigenous population in reproductive age. A national plan of Hepatitis B prevention and control in the Amazonian indigenous population in Peru is urgently needed to provide proper education, vaccination and treatment.

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Performance of serologic assays specific to IgM antibodies against hepatitis E virus: pan-genotypic evaluation

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**Background:** The routine use of a reliable assay to identify infection by hepatitis E virus (HEV) belonging to all 4 genotypes is desirable in clinical practice and disease surveillance. We evaluated assays developed to detect anti-HEV IgM by interrogating them with panels of specimens derived from patients known to be infected with HEV belonging to each specific genotype.

**Methods:** Two in-house assays (assigned I and II) and 4 commercial assays (III to VI) were evaluated. Assay I used as antigen a 56-kD protein expressed from open reading frame (ORF) 2 of the SAR55 HEV strain in a baculovirus system. Assay II used as antigens recombinant proteins (each 166 amino acids in length) derived from ORF2 of 4 HEV strains, each strain originating from a specific genotype. The commercial assays evaluated were purchased from International Immuno-Diagnostics (USA), MP-Diagnostics (Singapore), Diagnostic Systems (Russia) and Mikrogen Diagnostik (Germany). All 6 assays were of indirect (sandwich) format. A total of 194 sera comprised the test panels. The acute hepatitis E panel comprised 51 samples (15 from patients infected by HEV belonging to genotype 1, 4 to genotype 2, 15 to genotype 3 and 17 to genotype 4). The non-acute hepatitis E panel comprised 143 samples originating from patients with acute hepatitis A, acute hepatitis B and acute hepatitis C, as well as those convalescing from acute hepatitis E. Sensitivity, specificity and inter-assay agreement were determined.

**Results:** The sensitivity of the assays among patients infected with 4 HEV genotypes varied from 72% for Assay IV to 98% for Assays I, II and V. The specificity ranged between 65% for Assay I and 92% for Assays V and VI. Kappa values for inter-assay agreement ranged from 0.38 to 0.75.

**Conclusion:** The sensitivities and specificities of the 6 anti-HEV IgM assays varied substantially. Inter-assay agreements were mostly poor. Precise and accurate laboratory diagnosis of and surveillance for hepatitis E would require the use of anti-HEV IgM assays with the better performance characteristics.

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**Session: HIV: Pathogenesis**

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**Type: Poster Presentation**

CC-chemokine Co-receptor R5 genotypes in Ghanaian couples discordant for human immunodeficiency type 1 infection

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**Background:** The prevalence of HIV among heterosexual couples is high. In spite of this, there are instances in which one partner is HIV sero-positive and the other HIV sero-negative (described as discordant couple) despite being at risk for HIV infection. A number of factors are responsible among which is a deletion in the major coreceptor (chemokine receptor CCR5) for entry of HIV-1 into CD4+ T lymphocyte cells. Individuals homozygote for a 32 base pair deletion (CCR5 Delta 32) do not express the CCR5 receptor at their cell-surface and have a natural resistance to HIV-1 infection.

**Methods:** In order to determine the CCR5 genotypes of Ghanaian serologically discordant couples and the possible role of CCR5 Delta32 mutant allele in the lack of HIV-1 transmission, 32 couples (serologically discordant couples SDC and serologically concordant couples SCC) visiting the Fevers Unit (FU) of the Korle-Bu Teaching Hospital (KBTH) were enrolled. Couples were made to fill questionnaires on their behavioural characteristics and blood samples were taken for HIV-1 antibody testing and confirmation. HIV-1 negative serostatus of discordant partners was confirmed by polymerase chain reaction (PCR). The CCR5 genotypes were determined using DNA subjected to PCR amplification.

**Results:** Couples response to questionnaires revealed that they indulged in high risk behavioural practises. HIV antibody testing with PCR confirmation revealed 8 SDC and 24 SCC. One SDC seronegative individual (out of 8 SDC seronegatives) was heterozygous for the CCR5 Delta32 genotype. This was an important finding (although possession of CCR5 Delta32 heterozygous genotype may delay the acquisition of HIV-1 infection but cannot be implicated in natural resistance to HIV-1 infection) because most research scientist augment that the gene mutation does not exist in people of African decent. This was the first time that the CCR5 genotypes of Ghanaians were determined and also the first time that a heterozygous was found. The rest of the couples had wild type CCR5 genotype.

**Conclusion:** CCR5 Delta 32 mutant allele is unlikely to explain discordance in this cohort. There the need for a population based study to help ascertain the prevalence of CCR5 genotypes in the Ghanaian population. Counselling and follow-up must also be intensified in the group at risk group.

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**Type: Poster Presentation**

Endothelial Progenitors Cells and Microparticles in HIV-infected patients

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**Background:** HIV infection is an inflammatory disease and can cause endothelial dysfunction and increases the cardiovascular risk. It plays a critical role in coronary artery disease, but the assessment of the endothelial dysfunction has been problematical. Exposure to cardiovascular risk factors alters the regulatory functions of the endothelium that progresses from a quiescent state to activation, apoptosis and death. Recently, it has been shown that endothelial cells (EC) release microparticles (MP) on activation or apoptosis and that evaluation of MP can provide useful information on EC status in patients with an increased cardiovascular risk. Identification of circulating endothelial cells and microparticles has raised considerable interest as non-invasive markers of vascular dysfunction.

**Methods:** We studied these new markers of cardiovascular risk, as endothelial progenitors cells and microparticles in HIV-infected naive patients and compared with HIV negative controls matched for age and sex. Standard laboratory study included: lipid profile, glycaemia, C-reactive protein and apolipoprotein B. The endothelial progenitors (EPC) cells and microparticles (MP) were measured by flow cytometry. The EPC were identified using the following markers: CD34+ (surface protein), KDR (vascular endothelial growth receptor 2) for definition and CD133+ for immature lineage cells. The MP were characterized with CD31+ (MP platelet and endothelial derived), CD42+ (MP platelet derived) and CD51+ (MP endothelial derived).

**Results:** Thirty patients were included, 15 in each group, 73,3% were male with mean age 30,9 years. The lipid profile was significant only in the HDL-c and LDL-c between the groups. In the HIV-infected group we observed 0,01% of CD34+/KDR+ and it was not isolated CD34+/CD133+ neither CD133+/KDR+. In this group it was also observed more release of MP CD51+ and CD31+/CD42+ comparing to the control group, any MP CD31+/CD42- were found.

**Conclusion:** Our results suggest a possible imbalance between EPC and MP. This new finding in HIV-naive patient may be associated with increased cardiovascular disease in the long term follow-up of these patients, and can be aggravated after antiretroviral therapy.

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**Type: Poster Presentation**

Analysis of longitudinal *nef* sequence variation throughout HIV-2 infection

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**Background:** Human Immunodeficiency Virus type 1 (HIV-1) and type 2 (HIV-2) may cause a severe immunodeficiency in humans (AIDS). However HIV-2 is more frequently associated with lower levels of transmission and disease progression, compared with HIV-1 infections. The role of *nef* gene *in vivo* during the development of AIDS has been clearly demonstrated in simian immunodeficiency virus infected *Rhesus* macaques model, but the determinants which play a role in the pathogenesis of HIV are relatively poorly understood. However, even less is known about the role of *nef* in HIV-2 infections.

**Methods:** In this study, it was analyzed the variation of 48 *nef* gene sequences, obtained from samples taken between 1994 and 2009, corresponding to 17 HIV-2-infected individuals with different clinical stages of infection. The sequences obtained by Nested PCR were classified by phylogenetic analysis and the functional protein motifs, described as important in CD4 and MHC-I downregulation and in viral infectivity were also analyzed.

**Results:** In all individuals were identified *nef* sequences from group A of HIV-2, which encoded possible functional and complete protein. There was a greater conservation of residues in the Nef sequences of individuals in the symptomatic stage (63%), comparatively to individuals in the asymptomatic stage (19%). While some functional motifs (MGxxxS1, DDDD93, RR137 and DD205) and also residues (G128, I141 and L142) remained conserved, others (YSRF39, LRAR21, PxxP101, EE185) revealed changes during the follow-up period. The PxxP motif exhibited wide inter-individual variation *in vivo* from an HIV-1-like tetra-proline motif (PxxP)<sub>3</sub> to disruption of the minimal core PxxPLR motif. The disruption was observed in 11 sequences exclusively from asymptomatic individuals (p=0.021). The sequence motif variation towards tetra-proline configuration was observed in 2 symptomatic individuals during time of infection. The results also revealed the existence of a negative selective pressure, as well as codons under positive pressure in the sequences.

**Conclusion:** In this HIV-2-infected individuals studied, it was observed a need for a greater degree of Nef protein conservation in a symptomatic phase. Sequences altered and potentially critical for the Nef function *in vivo*, in earlier stages of infection, may contribute at some level to a different pattern in viral pathogenesis and disease progression.

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**Session: HIV: Pathogenesis**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Prediction of R5, X4 HIV-1 coreceptor usage based on physicochemical properties of envelope V3- loop using artificial neural network

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**Background:** HIV-1 cell entry commonly uses, in addition to CD4, one of the chemokine receptors CCR5 or CXCR4 as coreceptor. Human immunodeficiency virus type 1 (HIV-1) isolates can be classified phenotypically according to the primary coreceptor used to enter the cells. Third variable region (V3- loop) of the gp120 subunit of the HIV-1 envelope protein has been identified as a major determinant of coreceptor usage. Knowledge of coreceptor usage is critical for monitoring disease progression and for supporting therapy with the novel drug class of coreceptor antagonists. Predictive methods for inferring coreceptor usage based on the third V3- loop region can provide us with these monitoring facilities while avoiding expensive phenotypic assays. In this study we applied an artificially neural network on physicochemical data, derived from V3- loop sequence to predict coreceptor usage of viral isolate.

**Methods:** At first authors has collected two reliable set of data that define the biochemical and structural conditions of co receptor usage of virus. In the next step we have developed an ANN models to predict virus coreceptor usage and compare the results of ANN models with MLR model. In the ANN model, two levels of 1 and 2 are used as output vector or goal, respectively for R4 and R5 and the physicochemical aspects are input variables, also it was in MLR model.

**Results:** The process of training and testing of this new model is done using a set of collected data. ANN model with  $R^2 = 0.99$  and RMSE = 15.18 in training stage and  $R^2 = 0.91$  and RMSE = 187.8 in testing stage is superior in predicting the coreceptor usage. Several statistical and graphical criterions are used to check the accuracy of the model.

**Conclusion:** The coreceptor usage values predicted by the ANN model satisfactorily compared with the measured data. The predicted values were also compared with those predicted using linear regression model. The presented methodology in this modeling is a new approach in estimating coreceptor usage and can be combined with other models and empirical methods of coreceptor usage determination.

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**Type: Poster Presentation**

Clinical and immunologic response of HIV patients on different ART regimens at Gondar University Hospital (GUH)

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I-TECH ETHIOPIA, ADDIS ABABA, ET, Ethiopia

**Background:** As HIV treatment program expands in resource poor countries, antiretroviral drug selection is based on WHO / National guideline criteria's. Like first line ART combinations for initiating treatment and second line ART combinations for treating first line treatment failure conditions. There are many combinations of first line drugs and it is important to define are all these first line regimens equally effective in real clinical set ups.

**Methods:** We conducted a retrospective chart review of the outcome of different ART regimens among 300 HIV- infected patients whose CD4 count was less than 200 cells/mm<sup>3</sup> on initiation of ART and has regular follow up at Gondar University Hospital in Gondar, Ethiopia between September 2008 and March 2009, who received standard first line combination ART regimens. Group 1 (N=100) include those treated with AZT, 3TC, EFV/NVP combinations, Group 2 (N=100) include those treated with D4T, 3TC, EFV/NVP combinations and Group 3 (N=100) include those treated with TDF, 3TC, EFV/NVP combinations.

**Results:** There were no difference between Group 1, 2 and 3 with regard to: IRIS [Ten (10%), Thirteen (13%) and Eleven (11%), P=ns] respectively; new opportunistic infections [Four (4%), Seven (7%), and Three (3%), P=ns] respectively; average CD4 increment by 30% [Eighty (80%), Seventy six (76%), and Eighty three (83%), P=ns] respectively and death [Six (6%), Five (5%), and Four (4%), P=ns] respectively. However, adverse drug side effect occurs more frequently in Group 1 and 2 than in Group 3 [Eighteen (18%) and Twenty (20%) versus Three (3%), P=0.03] respectively, which more frequently resulted poor adherence in Group 1 and 2 than Group 3 [Seventeen (17%) and Sixteen (16%) versus Three (3%), P=0.05] respectively.

**Conclusion:** This manuscript shows that in a retrospective review of HIV patients who are in different Antiretroviral drug combination regimens attending follow ups at GUH, the clinical and immunological responses for the different ART regimens are similar and comparable however it depicts differences in areas of drug side effects and adherence problems. Therefore different first line ART drug combinations selection in resource poor countries can be guided by drugs side effect and adherence issues.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Comparison of efavirenz and nevirapine based HAART regimens in 4187 patients with up to 6 years of follow up, a prospective, open label observational study

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**Background:** Non nucleoside reverse transcriptase inhibitors (NNRTI) based regimens are widely recommended as 1st line HAART and they are preferred in resource constrained settings due to high efficacy and low cost. Few studies compare effectiveness of efavirenz (EFV) and nevirapine (NVP) in large, prospective cohorts with extended follow up (f/u).

**Objective:** To determine the effectiveness, rate of discontinuation or change, toxicity and mortality of patients with EFV versus NVP based regimens as initial HAART in a nation wide cohort

**Methods:** Prospective, open label, f/u of patients enrolled in the Chilean AIDS Cohort (ChiAC) from October 2001 to March 2008. All subjects receiving at least one dose of EFV or NVP were included. Primary outcomes were survival, maintenance of initial HAART, reason for change of NNRTI, viral suppression and immune recovery.

**Results:**

Of 5120 patients initiating first HAART in ChiAC, 4187 started a NNRTI based regimen (plus 2 NRTIs); 3107 (74.2%) with EFV and 1080 (25.8%) with NVP. Median f/u was 2.7 years. At baseline the NVP group had a significantly less advanced stage (CDC classification) and higher median CD4 count (151 vs 86 cell/mm<sup>3</sup> in EFV,  $p < 0.001$ ), but similar viral load (VL) compared to the EFV group. Rate of change or discontinuation was significantly lower for EFV (12.8% vs 22.3%,  $p < 0.001$ ), due to fewer adherence problems or toxicity. Timing for change was similar in both arms (median 950 vs 1008 days for EFV and NVP respectively). There were no statistical differences in viral suppression rate ( $< 80$  cps/mL) at any time: 67.2, 74.9, 74.4, 65.1, 59.8% vs 63.6, 74.3, 72.7, 61.7, 59.4% at 6, 12, 24, 36 and 48 months for EFV and NVP respectively. At 12 months of f/u median CD4 cell count was similar for groups (242 for EFV and 250/mm<sup>3</sup> for NVP). Mortality for the total period was 2.89 and 2.85 per 100pts-year for EFV and NVP respectively ( $p = \text{NS}$ ).

**Conclusion:**

Efavirenz based HAART regimens were associated with similar viral and immune outcomes as Nevirapine based regimens, despite more severe disease at baseline in the EFV group. Discontinuation of NVP was more frequent, mainly due to toxicity and lower adherence, but not to viral failure

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**Type: Poster Presentation**

The economic impact of introducing HIV/AIDS guideline into Colombian National Drug Formulary. Cost-effectiveness analysis

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**Background:** Colombia has >165000 people infected with human immunodeficiency virus (HIV). In 2009, the World Health Organization (WHO) estimated only 40% had antiretroviral therapy coverage. Colombia's AIDS treatments Guidelines became available in 2006 and are currently being used by physicians in their daily practice. However, these guidelines have not yet been evaluated with respect to their relative costs and effectiveness.

**Methods:** A Markov model was developed using TreeAge® Pro-2009 and clinical experts. Success probabilities were derived from published randomized controlled trials. Drug costs were obtained from the 2009 Drug Price Guideline from Bogotá and the ISS 2008 Manual. Hospitalization costs were obtained from the West Kennedy Hospital, Bogotá 2008/2009 database, adjusted to 2008. One-way and two-way sensitivity analyses were performed to test the model's robustness by varying clinical success rates and costs of antiretrovirals. Probabilistic sensitivity analyses were also performed using Monte Carlo simulations.

**Results:** Based on our Markov model, AZT-3TC-efavirenz had the lowest cost of treatment (USD \$12.09 per day) and the highest rate of success (69%).

It was the primary cost-effective HAART for HIV/AIDS in Colombia. AZT-3TC-efavirenz dominated all other HAART treatments. Results were generally robust within ranges tested.

**Conclusion:** In Colombia, antiretroviral therapy will lead to major survival benefits and is cost-effective by World Health Organization criteria. The availability of second-line regimens will further increase survival, but their cost-effectiveness depends on their relative cost compared with first-line regimens.

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**Type: Poster Presentation**

Prevalence of metabolic abnormalities and metabolic syndrome in a cohort of hispanic patients on HAART

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**Background:** Metabolic abnormalities are a well known side-effect of HAART. However, some studies show similar prevalences of metabolic syndrome in patients on HAART and the general population. According to different studies, prevalence of metabolic syndrome in the general population ranges from 23.7% to 24.6% using the ATP III definition. The aim of this study was to determine the prevalence of metabolic abnormalities and metabolic syndrome in a cohort of hispanic patients on HAART attending an ID Clinic in Buenos Aires, Argentina

**Methods:** Retrospective cross-sectional study. The records from the patients were reviewed to determine prevalence of the metabolic syndrome (as defined by the NCEP ATP III Update), and the prevalence of metabolic abnormalities.

**Results:** The cohort was composed of 78 hispanic patients on stable HAART for at least 6 months (mean age: 40.89 ± 8.55 years [range: 22-64]; 9% were women). Mean CD4: 583 ± 282 cells/mm<sup>3</sup>; median VL: 50 (IQR: 50-50).

The general prevalence of the metabolic syndrome was 24.7% (95% CI 15.6-36.8). The prevalence among patients on a protease inhibitor(PI)-based regimen (n=15) was 26.7%, versus 24.2% among those without PI (p=NS). In this last group, those on efavirenz (n=43) had a prevalence of 26.2% compared to 14.3% those on nevirapine (p<0.05).

The age-specific prevalence in the 30-39 years group was 22.2%, and 32.1% in the 40-49 years group.

The prevalence of hypertriglyceridemia was 47.4%, but differed according to therapy group (PI, EFV or NVP; mean values (mg/dL): 240 ± 322.76; 165 ± 109 and 150 ± 80.4; respectively), and increased with age group (20-29: 33%, 30-39: 45.7%, 40-49: 50%).

General prevalence of low HDL was 37.3% (women: 42%, men: 38.2%); there was no difference between patients with or without PI; but in this last group, prevalence among those on EFV was 42.5% vs 21.41% in those on nevirapine (p<0.05).

Hypertension prevalence was 39.5%.

**Conclusion:** In this cohort, prevalence of the metabolic syndrome was similar to the prevalence in the general population. However, patients on protease inhibitors and efavirenz had a higher prevalence than those on nevirapine (and the general population). Drug-induced hypertriglyceridemia seems to be one of the major drivers leading to the emergence of the metabolic syndrome among these patients.

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**Type: Poster Presentation**

Medication errors in patients receiving antiretroviral therapy at an urban hospital

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**Background:** Combination Antiretroviral therapy (ART) has decreased morbidity and mortality for persons with human immunodeficiency virus (HIV) infection. These complex regimens involve three or more agents, and there are many potential errors. The errors in prescribing ART may lead to adverse/toxic effects, treatment failure, and drug resistance. We evaluated medication errors occurring among patients receiving ART at the Miami VAMC to identify potential interventions to decrease errors.

**Methods:** This was a retrospective study utilizing a medical chart review to evaluate medication prescribing errors occurring among patients who had received ART during January 1 through December 31, 2007 at the Miami VAMC. Using the DHHS guidelines for HIV therapy as a source, we screened for the following prescribing errors: inappropriate dosing, use of inappropriate regimens/combinations, use of other medications and ART when contraindicated, failure to adjust for renal insufficiency, and insufficient monitoring. In addition, we evaluated the frequency of errors occurring during admission in comparison to the out patient clinic.

**Results:** The medical charts of 833 patients of whom 514 (62%) were receiving ART were reviewed. A total of 24 significant prescribing errors involving antiretrovirals were detected. The most common errors involved failure to adjust for renal insufficiency (54%) followed by use of contraindicated combinations with potential for drug interactions (46%). Overall, 17% of the errors occurred while the patient was admitted to the hospital. Although 75% (3) of the errors in the inpatient group were due to renal dosing as opposed to 55.5% (10) in the outpatient group; this was not statistically significant. Similarly, there was no statistical difference regarding contraindicated combination medications among both groups.

**Conclusion:** In this Patient population, the most frequent errors involved failure to adjust ART in the face of renal insufficiency and the co-administration of contraindicated medications with ART. These errors can be avoided by automated alerts in the computerized medical record used at this facility and emphasizing to physicians the importance of adjusting dosage or changing the ART to account for renal insufficiency. System-based interventions can be developed to prevent these errors before they occur.

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**Type: Poster Presentation**

Comparison of the effectiveness of low dose Indinavir/ritonavir (IDV/r) versus Atazanavir/ritonavir (ATV/r) based antiretroviral therapy in NNRTI experienced HIV-1 infected patients in India

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**Background:** Currently, data on the effectiveness of second line antiretroviral regimens using IDV/r and ATV/r along with two back bone nucleosides in resource poor settings is limited. It is important to compare the potency and efficacy of each regimen in order to establish utility on clinical outcomes. We sought to evaluate viro-immunologic determinants of response to IDV400/r BID and ATV300/r QD in pre-treated HIV-infected patients.

**Methods:** Prospective cohort study on 441 patients who experienced immunological or clinical failure to first line NNRTI-based treatment and were enrolled in a 96-week study follow-up. Based on the patients ability to pay, 321 (72.8%) and 120 (27.2%) patients were prescribed regimens containing low dose of IDV/r 400/100mg BID or ATV/r 300/100mg QD with 2NRTI, respectively. Successful immunologic response to treatment was defined by annual rise in absolute CD4 count of 100/cmm or more from baseline, while successful virologic response was defined by pVL<400 copies/ml at 12 or 24 weeks after initiation of treatment. Multivariate Cox Proportional Hazards Model was used to assess comparative effectiveness of treatment regimens with correction for intra-cluster correlation by means of the Robust Sandwich Estimator.

**Results:** Both the groups had similar age, sex and weight distribution. ( $p=0.302$ ,  $0.232$  &  $0.459$  respectively). A total of 63 patients (14.8%) had failed second line treatments, 53 patients (17.2%) were using IDV/r while 10 patients (8.5%) were on ATV/r. Patients who took IDV/r were more than twice as likely to experience treatment failure as compared to those on ATV/r (HR 2.18; 95% CI 1.14, 4.15). A significant treatment-response interaction was observed for patient weight and prior combination of NRTI use. Successful response was not different between the two groups when patients weighed <55 kg at baseline (log rank  $p$ -value=1.00) in contrast to the findings among individuals weighing  $\geq 55$ kg ( $p < 0.0001$ ). Patients with one NRTI recycled, ATV/r was found to be superior to IDV/r ( $p=0.01$ ) while among those with both NRTI recycled, there was no difference in response to either group ( $p=0.61$ ).

**Conclusion:** We found that Successful response to second line therapy was twice as likely in the ATV/r group; however, this difference was eliminated in patients less than 55kg.

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**Type: Poster Presentation**

NRTI resistance profile of the subtype F HIV-1 strains from children and adolescents treated with multiple antiretroviral drugs

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**Background:** The HIV epidemic in Romania is characterized by the existence of a large cohort of children and adolescents that have been infected in the late eighties. Most of these patients have been under treatment for the last ten years: all of them are currently on HAART but their majority has experienced a period (years) of either monotherapy (AZT) or bitherapy (AZT with 3TC or ddC). This study examines resistance profiles in the RT gene of HIV-1 strains obtained from these patients.

**Methods:** We have studied resistance tests on antiretroviral therapy of experienced children's and adolescents with HIV infection and AIDS hospitalized in National Institute of Infectious Diseases "Prof. dr. Matei Bals". On these patients we have evaluated: antiretroviral therapy, number of antiretroviral schemes, the type of resistance mutations selected on Nucleoside Reverse transcriptase Inhibitors (NRTI's) and the implications on to established of antiretroviral therapy, also.

**Results:** The most frequent mutations were: M184V/I - 78.8 %, T215F/Y – 74.4 %, D67N – 68.2 %, K219E/Q – 66.6 %, K70R – 56.9 %, M41L – 41.4 %. The thymidine analogue mutations (TAMs) were common and frequently associated with M184V. Within the TAM group, T215F/Y was frequently associated with D67N, K70R and/or K219E/Q. V75T/V was associated with K219E but only seldom with K219Q.

The multiresistention on experimented HIV infected children was 3.2 % (Q151M, 69ins, K65R).

**Conclusion:** The mutation associations in the RT gene of the subtype F HIV-1 strains are partly different from those communicated for the subtype B strains and therefore require further scrutiny.

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**Type: Poster Presentation**

Implementation of HLA-B\*5701 genetic screening prior to abacavir prescription in Georgia  
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**Background:** A hypersensitivity reaction to abacavir develops in approximately 2-8% of patients receiving this drug and is strongly associated with presence of the human leukocyte antigen (HLA)-B\*5701. Screening for HLA-B\*5701 reduces the risk of developing an abacavir hypersensitivity reaction. The carriage rate of HLA-B\*5701 has not been studied in Georgia before 2009. Objective of the study was to determine HLA-B\*5701 prevalence in HIV-infected patients in Georgia.

**Methods:** One hundred and sixty HIV-1 positive patients attending Georgian Infectious Diseases, AIDS and Clinical Immunology Research Center in 2009 within the national treatment and care program were recruited for the study. None of the patients had previously been treated with abacavir. Blood samples were collected and screened for HLA-B\*5701 prior to abacavir prescription. Statistical analyses were performed using SAS 9.1. Proportion with exact 95% confidence interval (CI) and median with interquartile range (IQR) are reported.

**Results:** Of 160 patients recruited 108 were male (67.5%) and 42 (32.5%) - female. Median age of subjects was 32 years (IQR 27-36). Nine patients tested HLA B\*5701 positive – 5.6% (95% CI: 2.6-10.4%). Out of these nine patients 7 were males (male prevalence: 6.5%, [95% CI: 2.6-12.9] %) and 2 females (female prevalence: 4.8% [95% CI: 0.6-16.2%]).

**Conclusion:** The first prospective study of HLA-B\*5701 prevalence in Georgia show similar results to the results of other studies conducted in Caucasians. Abacavir still remains one of the key drugs of antiretroviral regimens in Georgia and other resource limited countries. Therefore, prospective HLA-B\*5701 screening should be implemented in all settings where abacavir is widely used to guide selection of ART regimens and to reduce the risk of potentially life threatening hypersensitivity reaction.

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**Type: Poster Presentation**

Efficacy of multivitamins containing phosphatidyl choline in the management of hepatotoxicity from antiretroviral and/or antituberculous drugs

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**Background:** When hepatotoxicity develops in the course of treatment with anti-tuberculosis and anti-retroviral drugs, early improvement is important in order to continue specific treatment as early as possible.

We aimed to determine the efficacy, safety and effect on quality of life of phosphatidyl choline-containing multivitamin in the management of antiretroviral and anti-tuberculosis hepatotoxicity.

**Methods:** This was a randomized, single blinded placebo-controlled pilot study. Included in the study were subjects that had been enrolled to access drugs in the ARV programme of Jos University Teaching Hospital (JUTH), who had signed consent to participate in the parent PEPFAR protocol and have started ARVs or antituberculous drugs.

Subjects received phosphatidyl choline-containing multivitamin (Livolin forte®) or placebo (Vitamin B + Vitamin E as in Livolin forte). Patients had either been on antituberculous or antiretroviral medications. Subjects on each arm were reviewed fortnightly or earlier if they had worsening of symptoms. The liver function tests including ALT, AST improvements and deteriorations were noted and appropriate clinical decisions were taken according to standard guidelines during the four weeks of each recruitment. The data were analysed and displayed in proportions and appropriate representations.

**Results:** Sixteen subjects completed the study. Five and eleven subjects were on placebo and livolin respectively. The placebo recruitment was stopped after the fifth subject due to deteriorations in liver function with associated increased mortality in that group. Nine subjects were co-infected with Hepatitis B or C infection and one had triple infection of all three viruses. The baseline means ALT for the livolin group and placebo arms were 313.19mM/L and 239.76mM/L respectively. There was significant decline of the mean ALT in the livolin group to 49.21mM/L and 38.17mM/L compared to placebo group 227.74mM/L and 129.24mM/L at two and four weeks respectively. While joint pains reduced from 73.3% through 6.7% to 0% at the end of the study period in the Livolin group; the placebo arm showed a fluctuating course of 33.3%, 83.3% and 16.7% respectively.

**Conclusion:** Phosphatidyl choline-containing multivitamin demonstrated moderate benefits to HIV infected patients who develop hepatotoxicity to anti-tuberculosis or antiretroviral drugs when introduced early in their management.

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**Session: HIV: Therapeutics**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Prevalence of minor populations of drug-resistant HIV-1 in newly-diagnosed treatment-naïve individuals in Singapore

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**Background:** Primary drug-resistance in HIV-1, whether in major viral populations or minor populations, is a significant clinical and public health concern. Previous studies based on conventional direct sequencing of virus samples have shown that HIV-1 primary drug-resistance was insignificant in Singapore. But it is not clear whether this is also true to the minor HIV-1 populations of the samples because direct sequencing usually cannot detect resistance mutations in these populations. We performed this study to determine the prevalence of primary drug-resistance in minor HIV-1 populations.

**Methods:** Twenty-four PBMC-associated virus samples from 24 newly-diagnosed treatment-naïve patients were analyzed. PCR products of pol gene that encompass the entire protease gene and two-third of the reverse transcriptase gene were amplified using an in-house method. The PCR products were then column-purified for direct sequencing and gel-purified for clonal sequencing. The viral gene cloning was performed using the TOPO TA cloning kit (Invitrogen). DNA sequencing was performed on an Applied Biosystems 3130xl Genetic Analyzer.

**Results:** All the sequences of the 24 samples produced by direct sequencing were wild type, with no any known drug-resistant mutation detected. A total of 70 viral gene clones were obtained and sequenced for the 24 samples, with average 2.9 clones per sample. Clonal sequencing detected 4 drug resistance-associated mutations (PI, V82A; NRTI, M184V, K70E; NNRTI, K101E) from the viral clones of 4 samples; therefore the prevalence rate of primary HIV-1 drug-resistance in the minor HIV-1 populations was 16.7% (4/24).

**Conclusion:** Our data suggest that a considerable proportion of treatment-naïve patients are infected by drug-resistant HIV-1 virus in Singapore, but it was not detected by conventional direct sequencing. This finding has important implications for the clinical management and public health of HIV-1, and also suggests that more sensitive genotyping method should be employed to determine the baseline of HIV-1 primary drug-resistance.

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**Type: Poster Presentation**

Prevalence of HIV-1 primary resistance among HIV chronically infected patients in Brazil's Southeast region

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**Background:** The prevalence of HIV primary resistance mutations has increased overtime and transmitted mutations can persist in HIV-1 chronically infected patients. Studies have shown that it is cost-effective to perform primary resistance testing when one observes a prevalence of HIV-1 primary resistance above 5% in a certain geographical location. Nevertheless, such studies were conducted among acutely/recent HIV infected patients. Our study hypothesis was that HIV-1 primary resistance prevalence would be greater than 5% among HIV-1 chronically infected patients with indication for antiretroviral therapy in Brazil's Southeast (SE) region.

**Methods:** We have included 75 patients from 2 major cities: 47 from Rio de Janeiro and 28 from Santos. Our population profile was non-pregnant chronically HIV-1 infected patients with indication for antiretroviral therapy based on National Guidelines. HIV DNA was extracted and amplified from dried blood spots samples. HIV's *pol* gene protease and reverse transcriptase regions were sequenced. Antiretroviral resistance mutations algorithm which excludes frequent polymorphisms was utilized.

**Results:** Prevalence of HIV-1 primary resistance was 17,3%.

18 mutations were observed among 13 out of 75 included patients. Of these 18 mutations, 10 were related with NRTI's, 8 were related to NNRTI's and none to PI's. The most commonly detected mutations were: K103N (16.6%), T69D (11.1%), M41L (11.1%). Only 4% (3 individuals) had two class (NRTI and NNRTI) primary resistance mutations. The most prevalent HIV subtype was B (77.3%), followed by BF (33.3%), F (6.66%) and C (1.3%).

**Conclusion:** High primary resistance levels were observed according to WHO's definition (5-15%), suggesting being cost-effective to perform primary resistance testing in this region. The increased prevalence of NNRTI's mutations and the absence of M184V mutations are two findings observed in this study, contrasting with previous studies from Brazil.

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**Type: Poster Presentation**

Challenges of antiretroviral therapy among children aged 6 to 15 years in Kabarole district (Western Uganda)

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Makerere University School of Public Health, KAMPALA, Uganda

**Background:** HIV/AIDS is one of the leading causes of mortality among children in Kabarole district. It is however unknown how many children are on ART and there is very little documented evidence of the challenges faced by children aged 6 to 15 years on ART.

**Methods:** A cross-sectional study was conducted to assess the challenges associated with ART among children aged 6 to 15 years in Kabarole district. Quantitative data were collected using a semi structured interviewer administered questionnaire and qualitative using Key Informant interviews. Adherence levels were assessed using self report information from caretakers and children 10 years and above. Logistic regression models were used to estimate odds ratios (OR's) of adherence and their associated 95% confidence intervals.

**Results:** As at 30th April 2009, there were 478 children on ARV's in the district. Of the 332 children in the study, 51% were girls and 66.3% were aged 9-12 years. Using one month recall, 95.5% of the respondent's adherence was 100%, 1.8% had 95-99% and 2.7% had adherence levels of less than 95%. On 3-day recall, 98.5% had 100% adherence, 1.5% had less than 95% adherence. Major challenges were a low proportion of children on ART, 42.5% of respondents failed to get transport at least once, 51% were orphans, co-trimoxazole stock outs at government facilities, lack of follow up and inadequate support supervision .

**Conclusion:** Co-trimoxazole prophylaxis among children aged 6 to 15 years was found to be a plausible predictor of adherence. Challenges to ART are numerous and solutions to them require a multi-faceted approach.

**Recommendations:**

Co-trimoxazole prophylaxis should be prioritised in all ART clinics for children aged 6 to 15 years. There's need for collaboration between DHT and hospitals in support supervision of lower health units. Home visits and training of staff need to be reinforced.

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**Session: HIV: Therapeutics**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Survival, clinical, immunological and hematological outcomes of antiretroviral therapy among HIV-infected children attending a public clinic in Kinshasa, Democratic Republic of Congo

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<sup>1</sup>Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, <sup>2</sup>Ecole de Santé Publique, University of Kinshasa, Democratic Republic of Congo, Kinshasa, Congo, Democratic Republic of, <sup>3</sup>Kalembe Lembe Pediatric Hospital, Kinshasa, Democratic Republic of Congo, Kinshasa, Congo, Democratic Republic of

**Background:** To assess the mortality, immunological, hematological, and clinical outcomes of children initiating antiretroviral therapy (ART) during the first 5 years (November 2004 – October 2009) of a standardized HIV care and treatment program at the Kalembe-Lembe pediatric hospital in Kinshasa, DRC.

**Methods:** Retrospective analysis of routine clinical data from a cohort of 603 HIV-infected children  $\leq 17$  years of age at ART initiation.

**Results:** Children initiated ART at a median age of 6.4 [interquartile range 3.2–10.5] years. At ART initiation, 27.1% were on treatment for tuberculosis, 54.6% were in WHO stage III&IV, 52.8% had  $< 10$  g/dL hemoglobin, 63.0% had a CD4%  $\leq 15\%$  (severe immunosuppression), 39.6% had a weight-for-age Z score (WAZ)  $\leq -3$  (severe underweight), and 9.8% had previously received antiretrovirals. The first line regimens included stavudine or zidovudine, lamivudine, and nevirapine or efavirenz. During 1409.8 child-years of follow-up (median follow-up, 28.7 months), 61 children died: 38 during the first 90 days of treatment (early mortality rate, 28.0/100 child-years; 95% Confidence Interval (CI), 20.4–38.4) and 23 after 90 days (late mortality rate, 1.6/100 child-years; 95% CI, 1.9–2.5). The mean CD4% and WAZ rose rapidly from 11.6% and -2.57 at baseline to 25.0% and -1.92 at 12 months before stabilizing above 28.0% and around -1.80 between 18 and 48 months, respectively. The mean height-for-age Z scores (HAZ) and hemoglobin increased almost linearly from -2.23 and 9.8g/dL at baseline to -1.78 and 10.8g/dL at 12 months to -1.23 and 12.0g/dL at 48 months, respectively. Children under two years of age at ART initiation had greater and more sustained gains in weight, height, CD4% and hemoglobin compared to children who started ART when at least two years of age ( $p < 0.01$ ,  $p < 0.01$ ,  $p = 0.01$ ,  $p < 0.01$ , respectively).

**Conclusion:** Despite late presentation in our clinic, good clinical, immunological, and hematologic outcomes were obtained within the first 12 months and maintained through 48 months of ART among children who survived the first three months. The high early mortality rate reflects obstacles to health seeking behavior, early HIV diagnosis, and access to timely ART initiation in Kinshasa.

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**Session: HIV: Therapeutics**  
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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Apparent virologic failures and blips in a suburban HIV Clinic caused by laboratory errors in a commercial HIV-1 RNA PCR assay

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**Background:** The use of commercial assays for quantification of viral RNA is a cornerstone in management of patients with HIV infection. We recently observed an increase in blips and apparent virologic failure not explained by poor adherence or resistance and that resolved when our clinic changed laboratories.

**Methods:** We retrospectively collected data on patients who were receiving highly active antiretroviral therapy (HAART) from October 2004 until June 2006 and who had one or more HIV-1 plasma viral load (VL) > 1,000 copies/mL. We excluded patients who had interruptions in therapy or had known poor adherence to clinic visits or medications.

**Results:** Our clinic changed our reference laboratory in December 2005. In the previous 15 months, 38 patients on HAART had at least one VL > 1,000 copies/mL. Six patients had at least one VL > 10,000 copies/mL and 24 patients had more than 2 determinations > 1,000 copies/mL. In the 6 months following the lab change, 37/38 patients had VL < 400, 21/38 < 50, and only one patient had a VL > 600 copies/mL. One year later, 36/38 had no detectable plasma viral RNA. All patients had stable CD4 lymphocyte counts (mean=636 cells/mm<sup>3</sup>). Twenty-one patients had at least one genotype during this period; no new mutations we found.

**Conclusion:** We believe that systematic errors in a commercial laboratory contributed to apparent virologic failures and blips in this small group of patients.

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**Session: HIV: Therapeutics**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Antiretroviral resistance-associated mutations and genomic diversity in reverse transcriptase and protease gene among drug-naïve patients

**S. saini**<sup>1</sup>, P. Bhalla<sup>1</sup>, U. K. Baveja<sup>2</sup>, S. T. Pasha<sup>2</sup>, R. Dewan<sup>3</sup>

<sup>1</sup>Maulana Azad Medical College, New Delhi, India, <sup>2</sup>National Institute of Communicable Diseases, Delhi, India, <sup>3</sup>Maulana Azad Medical College, Delhi, India

**Background:** Antiretroviral drugs (ARV) are being provided by Govt. of India since 2004 under 3 by 5 initiative led by WHO-UNAIDS. Despite wide spread use of ARV, little information is available on the prevalence of transmitted resistance-associated mutations in HIV-1, particularly from north India. The present study was designed for molecular characterization of HIV-1 and identification of ARV resistance-associated mutations and naturally occurring genotypic variation in reverse transcriptase (RT) and protease region of pol gene of HIV-1

**Methods:** Seventy ARV drug-naïve HIV-1 sero-reactive patients, newly registered in ART clinic of Lok Nayak Hospital, were enrolled in the study after obtaining written informed consent. Plasma HIV-1 RNA levels were estimated by Amplicor HIV-1 Monitor test V 1.5 (Roche Diagnostics). HIV-1 pol gene, encompassing protease region (codons;1-99) and RT region (codons;1-232 to 1-239) was reverse transcribed, followed by nested PCR with self designed primers. The PCR product was sequenced with an ABI PRISM 3100 genetic analyzer system

**Results:** The median HIV-1 RNA levels were log<sub>10</sub> 4.6 copies/ml (IQR; 4.2-5.3). All patients were found to be infected with HIV-1 subtype C. Only one (1.4%) study sequence harbored resistance-associated mutations in RT region, showing NRTI resistance mutation (M184I). No mutation at major resistance positions in protease region was detected. High rate of polymorphisms were observed at codons; 35, 39, 40, 48, 60, 121, 122, 123, 135, 166, 173, 174, 177, 185, 200, 204, 206, 207, 208, 211, 216, 217, 233 & 239 in RT gene and 12, 15, 18, 19, 36, 38, 41, 63, 69, 70, 89 & 93 in protease gene

**Conclusion:** The overall prevalence of transmitted resistance-associated mutations was found to be 1.4%, lower than the alert cut off of 5% defined by WHO. HIV-1 subtype C exhibited enormous polymorphism in RT and protease regions of pol gene as compared to subtype B

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**Session: Infection Control, Nosocomial Infections and Critical Care**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Clinical, radiological and microbiological corroboration to assess the role of endotracheal aspirate in diagnosing ventilator-associated pneumonia in an intensive care unit of a tertiary care hospital, India

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**Background:** Early and accurate diagnosis and follow up of VAP varies considerably with the clinical, radiological and microbiological criteria employed.

**Methods:** This study was aimed to correlate clinico-radiological findings with microscopy and quantitative culture of consecutive ETA from 50 mechanically ventilated patients along with the antibiogram and risk factor assessment.

**Results:** The prospective observational study in MICU revealed the incidence of VAP to be 42% with a rate of 116/1000 ventilator days. 8 had early onset and 13 late onset VAP, with no age or sex significant preponderance. The attributable mortality rate was 61.9% which rose with duration of stay. The important independent risk factors were multi-organ failure, re-intubation and pleural effusion. The most commonly isolated organisms were multidrug resistant *Acinetobacter baumannii* (76%) and *Pseudomonas aeruginosa* (42%). All enterobacterial isolates were ESBL producing and all *S. aureus* isolates were methicillin resistant. In 66%, colonization on day 1 resulted in development of VAP on day 4. Gram stain findings had a significant correlation with the quantitative culture of ETA, which by itself showed a significantly progressive increase in specificity in diagnosing VAP on day 7. The strength of association between CPIS scoring, the microbiological findings and the clinical diagnosis was found to be strong.

**Conclusion:** This study emphasizes that Gram stain and quantitative culture of ETA can be considered useful for the diagnosis of VAP and a combined clinical, radiological and microbiological approach can be successful in the management and further follow up of VAP.

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**Session: Infection Control, Nosocomial Infections and Critical Care**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Virucidal efficacy of topical antiseptics versus a novel strain of Influenza H1N1

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<sup>3</sup>3M Company, Saint Paul, MN, USA

**Background:** On April 11, 2009 the WHO raised the epidemic alert to Phase 6 and declared a pandemic of novel Influenza H1N1. During the impending influenza season, the number of "swine" influenza cases is expected to increase, emphasizing the importance to public health of contact and respiratory hygiene. This study evaluated the susceptibility of Influenza H1N1 A/California/04/2009 strain to three topical sanitizing products, designed for application in a variety of public and healthcare settings.

**Methods:** ASTM E 1052-96, Standard Test Method for Efficacy of Antimicrobial Agents against Viruses in Suspension, was used to evaluate a non-medicated soap and three topical antiseptics: Avagard<sup>TM</sup> Surgical and Healthcare Personnel Hand Antiseptic (1% Chlorhexidine Gluconate, 61% Ethanol); Avagard<sup>Ô</sup> Foam Instant Hand Antiseptic (62% Ethanol); and Avagard<sup>Ô</sup> D Instant Hand Antiseptic (61% Ethanol). All products were tested at a 90% (v/v) concentration versus Influenza H1N1 A/California/04/2009 strain with exposures of 30 seconds, 1 minute, and 2 minutes. Viral titers were calculated using the Spearman-Kärber Method.

**Results:** All products significantly reduced infectivity of the epidemic strain of Influenza H1N1. Avagard<sup>TM</sup> (1% Chlorhexidine Gluconate, 61% Ethanol) and Avagard<sup>TM</sup> D (61% Ethanol) produced 5.25 log<sub>10</sub> (>99.999%) reduction after 30 second exposure, as well as after 1 and 2 minute exposures. Avagard<sup>Ô</sup> Foam (62% Ethanol) reduced the viral population by 4.25 log<sub>10</sub> (>99.99%) after 30 second, 1, and 2 minute exposures. Testing performed on non-medicated soap revealed low virucidal efficacy after short-term exposures: 2.50 log<sub>10</sub> (99.68%) reduction after 30 second and 1 minute exposures.

**Conclusion:** Significant reduction (>99.99%) in the population of A/California/04/2009 strain was achieved after 30 second exposures of the virus to the hand sanitizers. In a pandemic situation, the rapid virucidal activity provides a decided advantage in reducing Influenza transmission via hand contact.

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**Session: Infection Control, Nosocomial Infections and Critical Care**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Risk factors and clinical outcomes of multidrug-resistant *Acinetobacter baumannii* bacteremia in a University Hospital, Thailand

**S. Anunnatsiri, P. Tonsawan**

Khon Kaen University, Khon Kaen, Thailand

**Background:** Multidrug-resistant (MDR) *Acinetobacter baumannii* has become a major threat of nosocomial infection worldwide. The study aimed to assess the incidence of bacteremia due to MDR *A. baumannii*, factors associated with the infection, and clinical outcomes.

**Methods:** A retrospective study was conducted for evaluating 49 episodes of *A. baumannii* bacteremia in adult patients admitted to a university hospital in Northeast Thailand between 2005 and 2007. Comparison of the data between patients with susceptible *A. baumannii* bacteremia and those with MDR *A. baumannii* bacteremia was performed.

**Results:** The incidence of MDR *A. baumannii* bacteremia was 3.6 episodes per 10 000 hospital admission. The mean (SD) age of the patients was comparable between the 2 study groups [56.9 (17.3) years in susceptible group and 59.4 (16.8) years in drug-resistant group]. Most of the patients had pre-existing diseases; cancer, chronic kidney disease, and diabetes mellitus were the 3 most common. The most common source of bacteremia was pneumonia. The significantly independent factors associated with MDR *A. baumannii* bacteremia were prior ICU admission (odds ratio (OR) 10.01, 95% confidence interval (CI) 1.39-72.20), prior beta-lactam/beta-lactamase inhibitor use (OR 8.06, 95%CI 1.39-46.64), and prior carbapenems use (OR 11.40, 95%CI 1.44-89.98). Overall mortality rate was significantly higher in MDR group (48% vs. 91.7% in susceptible and MDR group, respectively,  $p=0.001$ ). The significantly independent factors related to mortality were APACHE II score (OR 1.25, 95%CI 1.03-1.52) and secondary bacteremia (OR 14.86, 95%CI 1.37-161.90).

**Conclusion:** This study revealed that the significantly independent factors associated with MDR *A. baumannii* bacteremia were prior ICU admission and prior use of broad spectrum antibiotics. This infection caused high mortality rate. Emphasize on prevention, strict application of infection control and appropriate use of antibiotic could reduce the risk and control this infection.

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**Session: Infection Control, Nosocomial Infections and Critical Care**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Assessment of health care workers occupational exposure to HIV and post-exposure prophylaxis (PEP) in health centers and hospitals of Addis Ababa, Ethiopia

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**Background:** Occupational exposure that places a worker at risk of HIV infection is a percutaneous injury, contact of mucous membrane or skin with blood or other body fluids. Exploring the extent of exposures, knowledge and practices of health care workers on occupational HIV risks is important. The study was done to assess occurrence of occupational exposures and knowledge and practice regarding HIV post-exposure prophylaxis among health care workers in health centers and hospitals of Addis Ababa.

**Methods:** A facility based cross-sectional study, involving 372 health care workers, was conducted in Addis Ababa from March to April 2008. A pre-tested, interviewer administered, structured questionnaire was applied for data collection. Odds ratio with 95% confidence interval and logistic regression analysis were employed to measure the degree of association between factors and identify the predictors for occurrence of needle stick injuries

**Results:** The study revealed that 38.2% of health care workers experienced at least one needle stick injury in their life time and 19% of respondents experienced injury within the last one year. Rate of needle stick injury in the previous one year was estimated at 1.34 injuries per person. Factors associated with occurrence of injuries were being a nurse (AOR=15.39, 95%CI=3.70-18.05), having work experience for more than 10 years (AOR=2.68, 95%CI=1.30-5.54), working long hours (AOR=1.90, 95%CI=1.10-3.31), attending fewer patients per day (AOR=2.21, 95%CI=1.32-3.58), self perception of high risk HIV (AOR=2.05, 95%CI=1.10-3.82) and non-consistent use of personal protective equipments (AOR=1.67, 95%CI=1.01-2.76). Two hundred sixty four (71.0%) respondents had knowledge about HIV post-exposure prophylaxis

**Conclusion:** The findings of this study indicated that occupational exposures were common among health care workers. Health facilities should make available to their system that includes a standardized written protocol and reporting unit for management of occupational exposures. Improvement of work environment and appropriate management of exposed cases, including addressing the psychosocial burden health workers face after exposure is also imperative.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Healthcare workers compliance to infection control practices in the haemodialysis unit in Sungai Buloh Hospital Malaysia

G. Chua<sup>1</sup>, **A. Adnan**<sup>2</sup>, Z. Abdul Wahab<sup>1</sup>

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**Background:** The number of patients with end-stage renal failure treated with haemodialysis in Malaysia has been increasing in recent years. Haemodialysis patients are at a higher risk of developing infections either directly or indirectly via contaminated devices, equipment, environmental surfaces or hands of healthcare workers (HCWs). Thus compliance to infection control practices by HCWs is very crucial in the prevention of the transmission of infections among haemodialysis patients. The Haemodialysis Unit in our hospital has been in existence only for the past two years and we would like to determine the compliance of the HCWs to the infection control practices.

**Methods:** We carried out a survey on the compliance to infection control practices among HCWs in the haemodialysis unit of our hospital over a two-week period. Two infection control nurses were stationed at the unit and a standard checklist was prepared. The CDC recommendations for preventing transmission of infections among chronic haemodialysis patients were used as a guide (MMWR 2001;50(No. RR-5).

**Results:** The unit has 25 dialysis machines with an average number of 46 patients per month. Four medical assistants, 4 nurses and 4 healthcare attendants were observed during the study period. Hepatitis B seropositive patients were managed in a separate area with dedicated dialysis machine from Hepatitis B seronegative patients. The HCWs were most compliance to wearing gloves, provided dedicated items, medications or supplies for single patient use (100% compliance). The HCWs were least compliance to hand washing and removal of gloves in between patients (9% compliance rate). The staff failed to clean or disinfect the prime buckets (0%).

**Conclusion:** The results of the study provided a baseline information on the compliance of the HCWs to infection control practices in the care of haemodialysis patients in our hospital. Continuous education and training of the HCWs in infection control practices which are unique to the haemodialysis centre are required in order to create the awareness of the importance of adherence to these practices and thus will help reduce infections in the patients.

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**Final Abstract Number: 56.006**

**Session: Infection Control, Nosocomial Infections and Critical Care**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Prevalence of nosocomial sinusitis in ICUs admitted patients in Rasool Hospital, Tehran, Iran

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**Background:** We studied the incidence rate of sinusitis in patients with fever of unknown origin (FUO) in ICUs.

**Methods:** A prospective, cross sectional study done in PICU and ICU in Rasool hospital; Tehran Iran (2007-2008 ).

Paranasal sinus computed tomography was performed in all (adult )patients admitted in ICU within 48h of admission and repeat thereafter ( 4-7 days) in adult cases with fever of unknown origin (FUO) after initial clinical and diagnostic screening. Infectious sinusitis was diagnosed by microbiological analysis of sinus fluid aspirates.

**Results:** 63 cases had full criteria and followed for nosocomial sinusitis (age=1-86 years; mean 17 + 25). Acute bacterial nosocomial sinusitis proved in 82% (51/ 63), Allergic sinusitis 18% (n=12). Head trauma was the most common cause of admission in 45% (n=22) of cases. Positive cultures seen in 82% (45/51) of cases included: Staphylococcus Aureus, Streptococcus spp in %22 (n=9), gram negative organisms predominantly *Klebsiella* , *Pseudomonas* and acinetobacter species detected in 41% (n=19), mixed aerobic/anaerobic in 37% (n=17) of cases. 7 patients had negative culture but positive smear or rapid antigen detection in sinus material included: S. Pneumonia in 5 children , Hemophilus influenza detected in 2 cases. Mean age of cases for nosocomial infection included: Gram negative organisms 7 years; Staph 14 year and mixed infection 27 years. We did not observe correlation between type of organisms and GCS ; (  $P=0.3$ ).

**Conclusion:** Physicians treating critically ill patients should be aware of the high risk of nosocomial sinusitis especially in cases with head trauma. Appropriate preventive measures, including the removal of nasogastric tubes in patients requiring long-term mechanical ventilation and routine investigation of FUO should include sinus CT scan should be linked to naso tracheal intubation, but its occurrence after orotracheal intubation is less clear.

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**Final Abstract Number: 56.007**

**Session: Infection Control, Nosocomial Infections and Critical Care**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Aspects of needlestick injuries among medical students: Reported or not?

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University of Graz, Graz, Austria, <sup>5</sup>Internal Medicine, Graz, Austria

**Background:** Medical students (MS) may be at high risk for needlestick injuries (NSI) and hence of transmission of blood borne pathogens including Hepatitis B (HBV), Hepatitis C (HCV) and HIV. Information is limited regarding the frequency of NSI's, the associated factors and the barriers to reporting them.

**Methods:** A self-administered anonymous questionnaire was distributed to 81 MS during an international students meeting on public health (ISMOPH). The questionnaire consisted of 21 questions covering topics including the frequency and reporting of NSI's as well as policy and post-exposure experiences. Completion of the survey was considered implied consent for study participation. The study served as a pre-test for an international cross-sectional multi-centre study on NSI's among MS to evaluate the validity and feasibility of the developed survey questionnaire.

**Results:** A total of 32 MS completed the questionnaire giving a response rate of 40 %. 25% (8/32) reported that they had at least one NSI during their studies and for 38% of respondents, that injury had involved a high-risk patient (defined as history of HBV, HCV and/ or HIV). 7 of the students reported that the injury was "self-induced" and described a feeling of being in "hurry" as the underlying cause. 50% (4/8) did not report the most recent NSI. The main reasons for underreporting were due to an injury with a clean needle, little or no perception of risk as well as shame of having a NSI. 69% (22/32) reported that their facility has a clear policy regarding post-exposure follow-up of NSI's, whereas more than a third were not familiar with this policy. When 81 MS were asked which blood-borne pathogen they feared most, 45% identified HIV, 36% identified HCV, and 18% identified HBV.

**Conclusion:** The experience of NSI's and underreporting seems to be frequent among MS. Therefore improved education and reporting strategies are needed to encourage the awareness of MS for an effective prevention of NSI's.

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**Final Abstract Number: 56.008**

**Session: Infection Control, Nosocomial Infections and Critical Care**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Factors associated with septic shock in patients with hematological malignancies and *Pseudomonas* infections

**R. Jeddi**, H. Ghédira, R. Ben Amor, A. Turki, L. Aissaoui, K. Kacem, W. Bouteraa, Y. Ben Abdennebi, R. Ben Lakhal, H. Ben Abid, Z. Bel Hadjali, B. Meddeb  
Aziza Othmana University Hospital, Tunis, Tunisia

**Background:** *Pseudomonas* is a leading cause of nosocomial infections usually associated with high mortality. The aim of this study was to determine predictive factors of septic shock in patients with hematological malignancies and *Pseudomonas* infections.

**Methods:** This study was conducted in a teaching hospital (Aziza Othmana University hospital, Tunis, Tunisia) to evaluate the clinical profile of infection due to *Pseudomonas* species and to determine risk factors for septic shock defined according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference. Statistical analysis was performed with Pearson test. Level of significance was at  $p=0.05$ .

**Results:** Between 2001 and 2009 a total of 80 *Pseudomonas* isolates (77 *P. aeruginosa*) was collected in 66 patients: 52 with acute leukemia (79%), 7 with lymphoma (10.5%), and 7 with other hematological disorders (10.5%). The median age was 30 years (range, 2-64 years). Most common sites of the isolates were from bloodstream (45%), and skin lesions (31.5%). Median time for microbiological documentation was 8 days (range, 0-35 days) from onset of neutropenia. At least 12 patients (18.1%) had recurrent ( $\geq 2$ ) infections due to *Pseudomonas*. The most common clinical signs observed were: skin lesions (34%), diarrhea (20%), isolated fever (18%), and respiratory symptoms (14%). Susceptibility to major anti-*Pseudomonas* antibiotics revealed that isolates tested were resistant to: piperacillin/tazobactam (43%), ceftazidim (31%), imipenem-cilastatin (26%), ciprofloxacin (25%), and amikacin (26%). Septic shock occurred in 16.2% of episodes (13/80). Crude mortality was (19.6%, 13 of the 66 patients) all caused by septic shock. For the remaining 53 patients (79.4%) median time for response to antibiotherapy was 2.5 days. In univariate analysis, factors associated with septic shock were: fever lasting for more than 3 days in patients on antibiotherapy ( $p=0.019$ ), C-reactive protein  $> 150\text{mg/l}$  ( $p=0.065$ ), serum lactate  $> 5\text{mmol}$  ( $p=0.05$ ), hemoglobin level  $< 50\text{g/l}$  ( $p=0.042$ ), hypoproteinemia  $< 50\text{g/l}$  ( $p=0.01$ ), and procalcitonin  $> 10\text{ng/ml}$  ( $p=0.031$ ).

**Conclusion:** This study revealed that several factors such as high procalcitonin level, low hemoglobin level, severe hypoproteinemia, high lactate level, and antibiotherapy  $> 72\text{h}$  before microbiological documentation are significant factors associated with septic shock and increased mortality in patients with hematological malignancies and *Pseudomonas* infections.

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**Final Abstract Number: 56.009**

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**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Initiatives to decrease the incidence and transmission of Clostridium difficile (C. difficile)

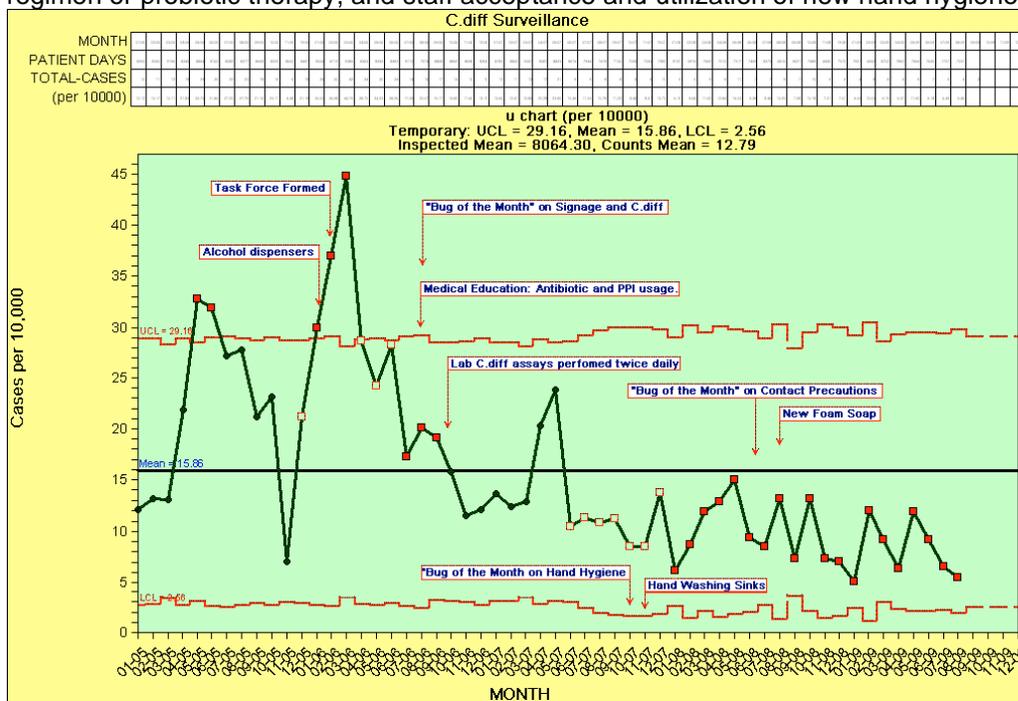
J. Murillo<sup>1</sup>, V. Heitzer<sup>2</sup>, M. Ruiz<sup>3</sup>, F. Ordieres<sup>2</sup>

<sup>1</sup>Miami, USA, <sup>2</sup>South Miami hospital, 33143, FI, USA, <sup>3</sup>South Miami Hospital, Miami, FI, USA

**Background:** Due to the increased incidence and transmission of Clostridium difficile (C. diff) in healthcare facilities in our community, surveillance and a retrospective study for 2005 was conducted. We noted in our 2006 ongoing surveillance a gradual increase in the incidence of C. diff. in our facility.

**Methods:** We convened a multidisciplinary Task Force to address the increased incidence of C. diff in 2006. The Task Force recommended the following actions: Contact isolation/precautions for all individuals with diarrhea, physician designed signage implemented to encourage hand antisepsis with soap and water followed by alcohol hand rinse for all patients with diarrhea, Fluoroquinolone evaluation was conducted and judicious prescribing practices for Fluoroquinolones was disseminated, yogurt twice a day was incorporated into patient's dietary regimen for those receiving antibiotic(s) or physicians whose patients disliked or were intolerant of yogurt were contacted by a dietitian to prescribe a prophylactic probiotic.

**Results:** Our data has demonstrated a sustained decrease in the incidence of C.diff. This was accomplished through heightened awareness of judicious prescribing of fluoroquinolones, institution of proper isolation/precautions of patients, by the addition of yogurt to patient dietary regimen or probiotic therapy, and staff acceptance and utilization of new hand hygiene products.



**Effective initiatives in decreasing the incidence of C.diff**

**Conclusion:** A sustained decrease in the incidence of C.diff over the last 3 years is attributed to the implementation of all the Task Force recommendations.

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**Final Abstract Number: 56.010**

**Session: Infection Control, Nosocomial Infections and Critical Care**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Initiatives to decrease the incidence of methicillin resistant Staphylococcus aureus (MRSA)

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<sup>1</sup>Miami, USA, <sup>2</sup>South Miami hospital, 33143, FI, USA, <sup>3</sup>South Miami Hospital, Miami, FI, USA

**Background:** Nationally MRSA has been identified as both a hospital and community concern. The CDC and some national healthcare organization have implemented on admission screening cultures and contact isolation in an effort to prevent transmission of MRSA in the healthcare environment. The system infection preventionists addressed this issue of universal screening cultures in 2006. Retrospective surveillance of our facility demonstrated we that we exceeded our 7.1 mean per 10,000 patient days. The Infection Control committee after reviewing our data determined that screening cultures and contact isolation on admission was not warranted. We acknowledge the seriousness of this rapidly escalating healthcare infection in our community and elected to implement a conservative approach based on clinical symptomatology, in conjunction with laboratory findings.

**Methods:** The Infection preventionist(s) review the daily microbiology findings, and note MRSA identification. The physical condition of the affected patient(s) is assessed for temperature, erythema, cellulitis, and drainage. Positive findings requires that the patient be placed in a private room and contact isolation precautions be instituted.

Based on the recommendation of the Infection Control Committee, the practice of hand antisepsis among the medical staff has increased due to the installation of hand washing sinks in close proximity to the physicians charting area on each medical surgical unit.

Hand hygiene compliance data was collected, reviewed and communicated to staff. Education was provided through general orientation, annual mandatory education, and the infection control newsletter "Bug of the Month". The Hand washing Team and Infection Control Week activities promoted a heightened awareness of the need for continued hand hygiene compliance.

Implementing changes in hand antisepsis (new dispenser for alcohol hand rinse and foam hand soap) for increased acceptance and usage of Hand hygiene products by all healthcare workers.

**Results:** Through heightened awareness, proper isolation precaution implementation and increase in hand hygiene compliance we have decreased the incidence of MRSA.

**Conclusion:** Increased hand antisepsis compliance had a significant effect on the decreasing rate of MRSA infection in our facility. Infection control and New Product committees need to continue to investigate new products that will increase hand hygiene in healthcare workers, patients, and visitors.

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**Final Abstract Number: 56.011**

**Session: Infection Control, Nosocomial Infections and Critical Care**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Failure of dry mist of hydrogen peroxide 5% to kill *Mycobacterium tuberculosis*

**B. M. Andersen**<sup>1</sup>, G. Syversen<sup>1</sup>, H. Thoresen<sup>1</sup>, M. Rasch<sup>1</sup>, K. Hochlin<sup>1</sup>, B. Seljordslia<sup>1</sup>, I. Snevold<sup>1</sup>, J. Berg<sup>2</sup>

<sup>1</sup>Ullevål University Hospital, Oslo, Norway, <sup>2</sup>Bakteriefritt, Oslo, Norway

**Background:** Multi-drug-resistant (MDR), or extensive drug-resistant (XDR) strains of *M tuberculosis* are increasing, resulting in spread of infections, extremely difficult to treat. *M tuberculosis* may survive in the environment for up to a year if not exposed to sunshine (or ultraviolet light). Enhanced environmental cleaning and disinfection is therefore important in the hospital environment to control the spread of infection. Disinfectants like chlorine and 5% chloramine, have been used for surface disinfection purposes after discharge of patients with contagious tuberculosis. The last few years, a dry mist of hydrogen peroxide disinfectant (DMHP) has also been used for decontamination of rooms, medical equipment and ambulances. DMHP has also been tried against *M tuberculosis* as a routine decontamination. The effect of DMHP on *M tuberculosis* is studied at Oslo University Hospital - Ullevål, Norway.

**Methods:** *Mycobacterium tuberculosis* strain CCUG 37357 was subcultured in Løwenstein Jensen (LJ) medium. A final dilution of 3 x 10<sup>5</sup> CFU/ml was added to each of 20 plastic agglutination wells and dried at room temperature for 2 h. 10 samples were placed in room temperature in laboratory A and the other 10 were placed in a separate decontamination room B and treated with 3- 6 cycles with dry mist hydrogen peroxide (H<sub>2</sub>O<sub>2</sub> 5%, Sterinis, Gloster Sante) during the night. Ppm of H<sub>2</sub>O<sub>2</sub> and spore tests were monitored during the experimental period. Controls and treated samples were eluted and grown for 4 weeks in parallel LJ and Bactec media. Four separate, repeated experiments were performed during a period of 60 days, using three decontamination cycles in two experiments and six cycles in two. The results were only read as growth or no growth.

**Results:** Growth of *M tuberculosis* was observed in all broth media (Bactec) and was present on Løwenstein Jensen media, both in controls and in treated samples. All spore control tests were negative. The ppm level reached during each cycle was high. No significant decontamination effect could be observed on bacteria grown on LJ media, neither after 3 nor 6 decontamination cycles with DMHP (5%).

**Conclusion:** A dry mist of hydrogen peroxide; DMHP (Sterinis) had no significant decontamination effect on air-dried samples of *Mycobacterium tuberculosis*.

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**Session: Infection Control, Nosocomial Infections and Critical Care**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Outbreak of methicillin-sensitive *Staphylococcus aureus* joint infections associated with magnetic resonance arthrograms performed at a single outpatient radiology center-Los Angeles County, California, USA, 2009

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County of Los Angeles, Los Angeles, CA, USA

**Background:** Septic arthritis after arthrography is rare. In May 2009, the Los Angeles County Department of Public Health investigated an outbreak of joint infections after patients received magnetic resonance arthrograms (MRAs) from one outpatient radiology center, Facility A, during a 5-day period.

**Methods:** Prospective and retrospective outbreak investigations were done, including interviewing radiology staff, observing procedures, and reviewing medical records from 27 April through 1 May 2009. We defined confirmed cases as patients from Facility A who had MRAs and septic arthritis with positive synovial fluid cultures and possible cases as patients from Facility A with acute onset of new joint pain after MRA requiring further medical evaluation.

**Results:** Seven case-patients were identified (5 confirmed, 2 possible) out of 15 who received MRAs (attack rate = 47%). Average onset time of new acute joint pain symptoms following the MRA was 1.1 days (range 1-2 days). Five knees and two shoulder joints were affected. Both possible case-patients required emergency department (ED) evaluation. Synovial fluid cultures on confirmed case-patients grew methicillin-sensitive *Staphylococcus aureus* (MSSA), requiring hospitalization and surgical arthroscopic incision and drainage, peripherally inserted catheter placement, and 6 weeks of intravenous antibiotics. Average length and cost of hospitalization was 10.8 days (range 5-16 days) and \$140,000. Two radiologic technologists routinely prepared contrast media for intra-articular injection. Contrast media and MSSA synovial fluid isolates were not available for further testing. We observed multiple breaches in aseptic technique during preparation of contrast media and improper multidose use of single-dose contrast media vials on multiple patients for cost-savings purposes.

**Conclusion:** One third of patients developed septic arthritis during a 5-day period following MRAs at a single outpatient radiology center and 47% required hospitalization or ED evaluation. This outbreak was likely caused by lapses in infection control, providing opportunity for extrinsic contamination of a single-dose contrast media vial which was misused as a multidose vial and resulted in significant morbidity and costs. The case-patients were clustered temporally with MSSA joint infection, making a localized point source most likely. No new case-patients were identified after corrective actions were instituted. This outbreak highlights the need for infection control monitoring particularly in outpatient settings which lack formal oversight.

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**Final Abstract Number: 56.013**

**Session: Infection Control, Nosocomial Infections and Critical Care**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Predictors of mortality in neutropenic patients with septic shock

R. Jeddi, H. Ghédira, **R. Ben Amor**, L. Aissaoui, W. Bouteraa, Y. Ben Abdennebi, K. Kacem, R. Ben Lakhal, A. Turki, H. Ben Abid, Z. belhadjali, B. Meddeb  
Aziza Othmana University Hospital, Tunis, Tunisia

**Background:** Chemotherapy-induced neutropenia is a risk for serious infection. Septic shock still causes high mortality among cancer patients. We aimed at identifying predictive factors of mortality in neutropenic patients ( $ANC < 0.5 \times 10^9/l$ ) who developed septic shock.

**Methods:** All cases of septic shock defined according to the ACCP/SCCM criteria observed in our department (Hematology, Aziza Othmana University Hospital) between 2005 and 2009 were included in this study. All clinical, biological and microbiological data were collected at the onset of septic shock and during outcome. Initial score on the Sequential Organ Failure Assessment (SOFA score) was performed for each patient. Statistical analysis was performed using Pearson test

**Results:** Thirty three septic shock were observed in patients with hematological malignancies: Acute leukemia (31), lymphoma (2). Median age was 27 years (range, 3-67). 58% of septic shock were observed during chemotherapy induction phase. Median time for occurrence of septic shock was 15 days from onset of neutropenia (range, 2-32). Microbiological documentation was obtained in 23 cases (69.6%). The bacteria involved were: Klebsiella (9), Pseudomonas (7), Stenotrophomonas (4), E. coli (1), Octhrobacter anthropi (1) and Acinetobacter (1). 9 (39.1 %) isolates were resistant to broad spectrum antibiotics. 20 patients (60.6%) developed ARDS during the evolution. Initial SOFA score was  $> 11$  (i.e. a risk of mortality of 95%) in only 6 patients (18.1%). Only 8 patients (24.2%) were admitted in ICU with a median time of 1.8 day (range, 1-3 days). Day 30 mortality was 85%. By univariate analysis predictors of mortality were: Disease status ( $p=0.009$ ), neutropenia lasting more than 15 days ( $p=0.012$ ), fever for more than 3 days in patient on antibiotherapy ( $p=0.009$ ), hemoglobin level  $< 50$  g/l ( $p=0.038$ ), isolate resistant to piperacillin/tazobactam ( $p=0.025$ ), presence of clinical symptoms from more than 1 site ( $p=0.008$ ), patients not on imipenem antibiotherapy at the onset of septic shock ( $p=0.019$ ), occurrence of ARDS during evolution of septic shock ( $p=0.003$ ) and non-admission in ICU ( $p=0.043$ ). No independent predictor found in multivariate analysis.

**Conclusion:** This study revealed that several factors play a significant role in mortality during septic shock. Despite low proportion of patients with baseline high SOFA score, the mortality rate in our study was very high highlighting the need for appropriate management and early admission in ICU to improve outcome.

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**Final Abstract Number: 56.014**

**Session: Infection Control, Nosocomial Infections and Critical Care**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Incidence of nosocomial respiratory tract infection in pediatric intensive care unit of University Hospital Center (UHC) of Tirana

**I. Kasmi**, G. Kasmi, S. sallabanda, E. kola, F. Zavalani, R. Llukaj, I. bakalli, A. koja, E. celaj, K. Marku

University Hospital Centre "Mother Theresa", Tirana, AL, Albania

**Background:** Nosocomial Respiratory Tract Infection (NRTI), have high morbidity, random mortality, increase hospital stay and the patient cost.

**Methods:** This is a prospective study, including all patients during the period February 2007-January 2008. NRTI was defined according to CDC criteria:

- new production of sputum
- new pulmonary infiltrates appearing in the chest x-rays associated with respiratory failure.
- lab evidence of infection

Correlation between extrinsic factors and the appearance of NRTI identified by Kendall tau b test for two nonparametric variables turned out to be significant.

**Results:** The number of NRTI was 15 from 484 patients treated in PICU.

213 patients were exposed to nasogastric tubes, 97 to endotracheal tubes, 39 to antacids usage. Out of 15 patients with NRTI in 13 were used ET, in 6 antacids, in 13 were used nasogastric tubes.

Average NRTI appearance day was 5.38.

Average stay in hospital for patients without NRTI was 3.6 days. Average stay for patients with NRTI was 18.5 days. Comparison of above mentioned averages revealed to be significant ( $p < 0.01$ ).

Etiological pattern of NRTI was as follow:

*Pseudomonas aeruginosa* 40%, *Klebsiella* sp 13.3%, *Acinetobacter* sp 13.3%, others gram-negative 19.9%, gram-positive 13.3%.

The antibiogram indicates presence of *Pseudomonas aeruginosa* resistant to quinolone and aminoglycoside (gentamycin) – in 30% .

**Conclusion:** Incidence of NRTI was 3%. Multiresistant strains pose major difficulties in managing NI.

This study show the correlation between the extrinsic factors and the appearance of NRTI. This research highlights the need to assess the preventive measures and the identification of monitoring actions.

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**Final Abstract Number: 56.015**

**Session: Infection Control, Nosocomial Infections and Critical Care**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Clinical characteristics and prognostic factors in patients with *Stenotrophomonas maltophilia* bacteremia

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University of Tokyo Hospital, Tokyo, Japan

**Background:** *Stenotrophomonas maltophilia* is a multi-resistant, non-fermentative gram-negative bacillus, which is increasingly being recognized as an important nosocomial pathogen affecting debilitated patients. *S. maltophilia* infections are associated with high morbidity and mortality. This study aimed to clarify the clinical characteristics, antibiotic susceptibility, antibiotic treatment, outcome, and prognostic factors in patients with *S. maltophilia* bacteremia.

**Methods:** A retrospective cohort study was conducted at the University of Tokyo Hospital, comprising 1,150 beds, with specialty services including intensive care and transplantation in Japan, from January 2003 to September 2009.

**Results:** During this study period, 7,216 positive blood cultures were identified. Of these, 87 (1.2%) blood cultures from 54 patients showed *S. maltophilia* growth. The mean age of the patients was  $53.3 \pm 3.0$  years. In 52 patients (96%), *S. maltophilia* bacteremia was hospital-acquired, in particular, in 49 patients (91%) it developed after prolonged hospitalization of >2 weeks. Forty-one patients (76%) had an indwelling central venous catheter (CVC), 50 (93%) had received antibiotic therapy, 48 (89%) had underlying malignancy, 11 (20%) had diabetes mellitus, and 11 (20%) were receiving corticosteroid therapy. In 19% of all the cases of bacteremia, polymicrobial isolates were confirmed. The overall and bacteremia-related mortality rates were 39% and 26%, respectively. The most common sources of bacteremia were CVC (33%) and pneumonia (15%); the source was unknown in 20% cases. Tests for antibiotic susceptibility revealed that the isolates were most sensitive to trimethoprim-sulfamethoxazole (79%). Only 47% and 43% of the isolates were susceptible for ceftazidime and ciprofloxacin, respectively. Univariate analysis revealed that bacteremia originating from the pneumonia, patients treated with inappropriate antibiotics, and patients with a persistent indwelling CVC had a significant higher mortality rate ( $P < 0.0001$ ,  $P < 0.0001$ ,  $P = 0.0027$ , respectively).

**Conclusion:** *S. maltophilia* is an important pathogen, particularly, in immunocompromised hosts. Isolation of this organism from a blood culture should prompt a careful review of the patient, with particular emphasis on removal of an indwelling CVC and commencement of appropriate antibiotic therapy. In this study, we noticed an increasing trend of resistance to ceftazidime, cefepime, and ciprofloxacin. Therefore, we believe that proper usage of antibiotics is important for infection control.

When citing these abstracts please use the following reference:  
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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Prevalence of *mecA*, *aap* genes and slime layer formation and its association with antibiotic resistance in isolated *Staphylococcus epidermidis* of TUMS hospitals health care staffs

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**Background:** *Staphylococcus epidermidis* strains are frequently associated with catheter-related infection, acute bacteremia and hospital-acquired infection. Some of the isolates have extracellular matrix called slime. The recent studies show that slime-forming bacteria are more resistant to antibiotics than planktonic bacteria. In *S.epidermidis*, the accumulation associated protein (*aap*) is essential to biofilm development and is involved in the accumulation phase of biofilm formation. methicillin resistance is mediated by the *mecA* gene. However in contrast to MRSA, very little attention is paid to MRSE. The aim of this study was to determine the prevalence of *mecA*, *aap* gene and slime production in *S.epidermidis* isolates and antibiotic resistance in nasopharynx isolates of health care personnel.

**Methods:** A descriptive cross sectional study was performed on 163 isolates. These were collected from July to December 2008. *S. epidermidis* isolates tested for slime production onto CRA and antibiotic resistance. The extracted DNA of *S. epidermidis* isolates were examined by PCR involving specific primers for *mecA* and *aap* genes.

**Results:** Among 163 collected nasal swabs 99 (60.7) were *S. epidermidis*. Of these 35.3 isolates produced slime. Significant relation between slime production and resistance to Penicillin, oxacillin ( $p < 0.0001$ ), tetracycline ( $P = 0.0005$ ), erythromycin ( $P = 0.001$ ) and clindamycin (0.003) was found. 95.8% and 94.8% isolates were PCR-positive for *mecA* and *aap*, respectively.

**Conclusion:** Surveillance of nasal colonization with slime-forming MRSE in health care workers might provide useful information for the establishment of infection control procedures toward these bacteria.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Central line associated bloodstream infection rates in 166 intensive care units of 54 cities in 23 developing countries: findings of the International Nosocomial Infection Control Consortium (INICC)

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**Background:** To determine rate, microorganism profile, bacterial resistance, extra length of stay (LOS) and extra mortality of Central Line associated Bloodstream Infection (CLAB) in intensive care units (ICUs) of 23 countries in INICC hospitals in Argentina, Brazil, China, Colombia, Costa Rica, Cuba, Ecuador, El Salvador, India, Jordan, Kosovo, Lebanon, Macedonia, Mexico, Morocco, , Pakistan, Panama, Peru, Philippines, Serbia, Thailand, Tunisia, and Turkey.

**Methods:** An open label, prospective cohort, active device-associated infection (DAI) surveillance study was conducted on adult, pediatric and neonatal patients admitted to tertiary-care ICUs. DAI rates were collected from 166 ICUs, and were recorded by using CDC-NNIS definitions. Microorganism profile, bacterial resistance, LOS and mortality data were collected in 128 out of the 166 ICUs.

Data were collected from patients with and without DAI using the INICC protocol, forms and methods, which provided researchers with a general view of patients' outcomes, allowing researchers to suspect DAI and avoid possible DAI omissions if no cultures were done. Patients with and without DAI can be matched to calculate LOS, costs, and extra mortality. Data were uploaded and analyzed at INICC office. Statistical analysis was performed using Chi-square test. P <0.05 was considered significant.

**Results:** We collected data from 01/02 to 11/08, representing 563,322 CL days. The pooled CLAB rate was 8.06 per 1000 CL days; data stratified by ICU type are shown in Table.

Overall 17.1% of all CLAB were caused by *Staphylococcus aureus* (83.1% were MRSA); 17.1% by *Coagulase-negative-staphylococci* (80.1% were methicilin resistant); 13.9% by *Acinetobacter* sp (83.3% were Piperaciline-Tazobactam resistant); and 11.8% by *Pseudomonas* sp (42.2% were Imipenem resistant). The LOS of patients without DAI was 5.3 days; and of patients with CLAB, 16.7 days (RR, 3.14), representing 11.4 extra days. 7,464 out of 52,549 (14.2%) patients without any DAI died; 397 out of 1,305 patients (30.4%) with CLAB died, the extra mortality being 16.2% (RR, 2.14, 95% CI, 1.94 – 2.37, P, 0.0001).

**Conclusion:** Pooled CLAB rate of 8.02 per 1000 CL days was higher than the 2.0 rate (medical surgical ICUs) per 1000 CL days published by CDC-NNIS. Patients with CLAB had a significantly higher LOS, with 11.4 extra days, and extra mortality of 16.2%.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Central line associated bloodstream infection rates, extra length of stay, extra mortality and microbiological profile in a German tertiary intensive care unit: findings of the International Nosocomial Infection Control Consortium.

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**Background:** To determine rate and consequences of central line associated bloodstream infection (CLAB) in 1 German surgical intensive care unit (ICU) cooperating with the International Nosocomial Infection Control Consortium (INICC).

**Methods:** We performed an open label, prospective cohort, active surveillance study in adult ICU patients of a German tertiary-care hospital. At the ICU, for all patients semi-rigid and/or glass infusion containers and stopcocks were used. INICC ([www.inicc.org](http://www.inicc.org)) protocols, forms and methods were used. According to these methods, data is collected from patients with and without healthcare associated infections (HAIs) enabling the match for variables such as age, gender, underlying diseases, diagnosis, severity-of-illness, invasive device use in order to calculate risk factors and outcome parameters. In this study, length of stay (LOS) and extra mortality are presented. Statistical analysis was performed using Chi-square test. P <0.05 was considered significant.

**Results:** 447 patients were enrolled from 02/08 to 10/08, representing 2,844 bed days. The overall CLAB rate was 7.86 per 1000 central line (CL) days. Overall, 33.3% of CLABs were caused by *Staphylococcus aureus* (n=4), 0% were methicillin resistant; 33.3% by coagulase-negative staphylococci (n= 4), 100% were methicillin resistant; and 33.3% by *Enterococcus* spp. (n=4), 0% were vancomycin resistant. LOS of patients without HAI was 4.5 days; LOS of patients with CLAB was 18.9 days (RR, 4.19; 95% CI, 3.68 - 4.77; P<0.001), representing 14.4 extra days. 20 of 406 (5%) patients without HAI died, whereas the course of 2 of 14 patients (14%) with CLAB was fatal. Extra mortality was 9% (RR, 2.90; 95% CI, 0.68 - 12.41, P = 0.13).

**Conclusion:** In this study, the CLAB rate of 7.86 per 1000 CL days is lower than the published rate of 9.2 per 1000 CL days observed in other hospitals cooperating in INICC. INICC methods allow for the comparison between patients with and without HAIs; LOS in patients with CLAB was significantly higher, mortality rate was not significantly affected and no MRSA bacteraemia was observed.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Impact of outcome surveillance on ventilator associated pneumonia rates in 3 intensive care units from 2 Mexican cities: findings of the International Nosocomial Infection Control Consortium (INICC)

M. Sobreyra Oropeza<sup>1</sup>, V. Rosenthal<sup>2</sup>, H. Torres Hernández<sup>3</sup>, A. Chávez Gómez<sup>3</sup>, J. Rivera Morales<sup>3</sup>, J. E. Valero Rodríguez<sup>3</sup>

<sup>1</sup>Hospital de la Mujer, Mexico, Mexico, <sup>2</sup>International Nosocomial Infection Control Consortium (INICC), Buenos Aires, Argentina, <sup>3</sup>Irapuato General Hospital, Irapuato, Mexico

**Background:** To determine the effect of outcome and process surveillance (intervention) on the rate of Ventilator Associated Pneumonia (VAP) infection in three intensive care units (ICU) from 2 cities of Mexico.

**Methods:** An open label, prospective cohort, active VAP surveillance, sequential study was conducted on adult and neonatal patients admitted to tertiary-care ICUs. Rates of VAP were recorded by applying the definitions provided by CDC-NNIS. The protocol, forms, and outcome and process surveillance methodology used were developed by the INICC. Data were collected from patients with and without device associated infection (DAI). Forms were designed to continuously prompt surveillance officer to suspect DAI by providing a panoramic view of outcomes for each patient (eg, vital signs, invasive device use, cultures, antibiotic use, etc); this is useful when no cultures have been done and thus DAI could be wrongly omitted. Data were collected in ICU. Data uploading and analysis were done at INICC office analyzing DAI rates, microbiological profile of isolates, bacterial resistance, LOS, extra mortality. The VAP rates during baseline were compared to the rates during an intervention period. Statistical analysis was performed using Chi-square test. P <0.05 was considered significant

**Results:** The baseline period included the first eight months of the medical centers' participation, and the intervention period lasted a mean of 8 months (range 7-9 months). During the baseline period, 584 ICU patients were enrolled, and 454 patients were enrolled during the intervention period.

Patients' characteristics were similar over the two periods (Patient gender, P: 0.3394; Diabetes, P: 0.1715; Renal Failure, P: 0.3951; Stroke, P: 0.2929).

The rate of VAP per 1,000 ventilator days during the intervention period was significantly lower than during the baseline period, 17.6 (19/1,078) vs 8.3 (15/1,797) VAP per 1000 ventilator days (RR, 0.47; 95% CI, 0.24-0.93; P 0.0267).

**Conclusion:** Outcome and process surveillance resulted in a significant reduction in the VAP rate.

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**Session: Infection Control, Nosocomial Infections and Critical Care**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Expectations and perceptions of Nigerian patients regarding infectious diseases in dentistry

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**Background:** Dental treatment procedures frequently involve blood and saliva that may be contaminated with infectious diseases. There has been no recent assessment of Nigerian patients' concern on the risk of infectious diseases transmission during dental care.

**Objective:** To assess the expectations and perception of Nigerian patients regarding infectious diseases in dentistry.

**Methods:** A questionnaire based cross-sectional survey of patients attending dental centre of University of Benin Teaching Hospitals, Nigeria was conducted in 2009.

**Results:** The majority of patients (76.5%) expressed worry about contracting infection during the dental treatment and 47.4% of them identified HIV as a risk. One hundred and three (25.4%) would avoid dental treatment because of fear of contracting HIV. Ninety-two (22.7%) would switch from HIV infected dentists and 37.8% would not allow HIV-infected dentist to treat them. Only 10.4% of the respondents would expect the dentist to refuse treat to HIV-infected patients.

**Conclusion:** This survey revealed that a high proportion of Nigerian patients are worried of infectious diseases during dental treatment which also influenced their dental care seeking behaviour. Improved public education and risk communication on dental care associated infectious diseases is needed to allay the fear of Nigerian patients.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Nosocomial infections in a pediatric hospital in Santo Domingo, Dominican Republic

**C. S. Mota**

Hospital Dr. Robert Reid Cabral , Santo Domingo, Dominican Republic

**Background:** Nosocomial infection or "healthcare associated infection" lead to significant morbidity, mortality and economy burden beyond those expected for patients' underlying diseases alone, patients with nosocomial stay extra 10-14 days. In the developing world the nosocomial infection rate can be 25%.

**Methods:** This is a descriptive, observational, cross sectional study to determine the characteristics of nosocomial infection in Hospital Infantil Dr. Robert Reid Cabral, in the period of January-June 2009; we looked through the reports from the hospital's Nosocomial Infections Surveillance Committee.

**Results:** The period between January and June 2009, 3200 patients were admitted to the hospital, from those 127 (3.96%) developed a nosocomial infection. 74.8% (N95) were admitted in areas considered high risk areas - Neonatal Intensive Care Unit (NICU) 16.5% [21/127]; Pediatric Intensive Care Unit (PICU) 9.4% [12/127]; Newborn unit 13.4% [17/127]; Burns Unit 8.6% [11/127]; Oncology 6.2% [8/127]; Surgery 14.4% [18/127]; Nephrology 6.2% [8/127]. The nosocomial infection was determinate as the direct cause of the dead in 49 (38.5%) patients. The highest mortality rate belonged to the PICU with 83.3% (10/12), follow by the NICU 71.4% (15/21); the mortality rate in the Newborn Unit was 70.5% (12/17); in contrast in the Surgery department the mortality rate was 0. In 65 of the cases at least one microorganism was isolated, being K. pneumonia Extended Spectrum Beta Lactamases (ESBLs), the most frequently isolated, 21.5% (14/65), it was also responsible of mostly of the mortal cases, and by area the most isolated from the NICU. No MRSA was isolated in this revision.

**Conclusion:** Although the creation of the Nosocomial Infections Surveillances Committee, the nosocomial infections, its burden in costs, morbidity and mortality continuing being a big concern, especially in the NICU and Newborn Unit. Moreover, the high costs of the treatment for those kinds of infections sometimes are not affordable for patients in a weak healthcare system.

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**Type: Poster Presentation**

Predominance of diarrhoeagenic *E.coli* in hospital acquired diarrhoea in a case-controlled study in a tertiary care hospital in North India

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**Background:** In developing countries nosocomial diarrhea is an important cause of mortality and morbidity in paediatric patients. Many bacterial agents such as *Salmonella*, *Shigella*, different pathotypes of Diarrhoeagenic *E.coli*, *Clostridium difficile* etc. have been reported to cause outbreaks. We wanted to establish an etiological and epidemiological profile of nosocomial diarrhoea among children admitted to the APC at PGIMER, India so that appropriate preventive measures can be formulated.

**Methods:** From January 2008 to June 2009, we systematically enrolled 100 children between the age of 2 months and 14 years as having nosocomial diarrhoea and 50 patients as control subjects. Stool samples were processed for bacterial agents by culture. Diarrhoeagenic *E.coli* were screened by using a multiplex PCR for elt-322 bp, est-147bp, bfpA-367 bp, aatA-630 bp and eae-881bp. *Rotavirus* antigen and *C.difficile* toxin A&B detection were performed by ELISA. Parasitic agents were studied by doing a direct stool examination. Patient's clinical presentation, severity score, outcome, type and courses of antibiotics received were analysed between cases and controls.

**Results:** In this study Diarrhoeagenic *E. Coli* such as E.T.E.C., E.A.E.C. and E.P.E.C. were isolated in 25%, 17% and 7% respectively. The diarrhoeagenic *E.coli* showed high levels of multidrug resistance. Maximum resistance was seen against nalidixic acid(97.5%). *C.difficile* toxin was seen in 9% of cases, while *Rotavirus* was found in 8% of the cases. Mean duration of diarrhoea and hospitalisation were 3.28 days and 27.45 days respectively.

**Conclusion:** Rotavirus and *C.difficile* are the major causative agents of hospital acquired diarrhoea in the developed world but in our country Diarrhoeagenic *E.coli* are responsible for majority of cases of hospital acquired diarrhoea. Exact reasons for this finding are not known but it could be related to lack of stringent quality control while preparing and storing formula milk preparation. The statistically significant factors for the development of nosocomial diarrhoea were hospital stay, usage of antibiotics (vancomycin, imipenem and metronidazole) and nasogastric feeding. Nosocomial diarrhoea is also most common in children less than 5 years.

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**Type: Poster Presentation**

Nosocomial infections – 5 years of prevalence studies in the Czech Republic

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**Background:** As an important part of the nosocomial infections (NI) surveillance, hospital epidemiologists from the faculty type hospitals in the Czech Republic perform the one-day prevalence studies in 10 University Hospitals since the year 2005.

Up to now, the point-prevalence studies included surgery, urology, neurology, cardiology, neurosurgery, otorhinolaryngology, traumatology, gynecology and intensive care units departments.

The aim is to obtain the descriptive and clear data in the time line, using the simple questionnaire method, easy to perform, easy to repeat and cheap.

**Methods:** The methodic approach is close to the HELICs (Hospital in Europe Link for Infection Control through Surveillance) protocol, as the current consensus for prevalence studies in european countries.

In each of the involved hospital, the medical files of all patients hospitalised in the given department at the date of prevalence study are checked.

From each medical file, the specific information for the unique questionnaire are obtained:

- I. Descriptive data (patients' identification, diagnosis)
- II. Information related to the NI and risk factors (diagnosis of NI respects CDC criteria)
- III. Laboratory and microbiology findings
- IV. Informations about ATB treatment and/or prophylaxis

Data from the questionnaires are statistically analysed using MS Excel and Epi Info.

**Results:** Up to now, 2225 hospitalised patients were checked and 112 NI was found, what represents prevalence 4,7 NI on 100 hospitalised patients.

Based on the studies performed during this 5 years in the faculty hospitals, the Czech Republic decided to perform the national pilot prevalence study on the intensive care units in autumn 2009.

All hospitals in the Czech Republic with the given departments were invited to the study on voluntary basis. 93% of hospitals (109 units) confirmed their involvement, what represents 1947 beds. The epidemiologists from the national public health institutes network are involved in the organization and data recovery.

*(ongoing at the time of abstract submission, results expected in January 2010).*

**Conclusion:** We can recommend the similar approach to the countries looking for the optimal tool to determine the NI and define NI control priorities, when resources are limited.

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**Type: Poster Presentation**

Predicting health care associated infections (HAI) in a high incidence low cost intensive care unit

**G. George**, S. Kumar, K. Sivagnanam, P. Srikanth  
Sri Ramachandra University, Chennai, India

**Background:** All across the world it has been documented that Health care associated infections increase the costs of medical care, morbidity and mortality and are a challenge in health care delivery.

Our objective was to to predict the possibility, properties and antibiotic susceptibility of HAI in patients in an ICU.

**Methods:** All patients admitted to an adult multi disciplinary ICU between July 1-August 15 2009 were monitored prospectively in our pilot study. Information on clinical parameters, risk factors, co-morbid conditions, culture source, organisms isolated and antibiotic susceptibility was documented and analysed using appropriate statistical software. The probability of a HAI in the presence of risk factor(s) was calculated. The probability of involvement of specific systems or organisms, given a HAI was calculated.

**Results:** In all 108 patients were studied. 104 cultures were performed on 51 of the 108 patients. The only significant risk factors ( $p < 0.01$ ) for HAI were found to be duration of stay (eg. independent probabilities of 0.25 and 1 at 5 and 20 days respectively), duration of IV lines and urinary catheterization (irrespective of duration). Probability of pulmonary and urinary infections respectively increased independently with duration of hospital stay and catheterization (eg. probability of a pulmonary infection at 20 days-0.55). Most of the isolates were gram negative. The probability of a urine culture growing *Klebsiella* or *E.coli* was 0.5. The probability of de novo sepsis was 0.13 while the probability of sepsis occurring in the presence of a preceding pulmonary or urinary infection was 0.3 irrespective of duration of stay. The independent probability of being infected with an MDR organism within 20 days of stay was 0.11. The probability of *E.Coli*, *Klebsiella*, *Acinetobacter* and *Pseudomonas* being MDR were 0.78, 0.36, 1 and 1 respectively. Most of the *Acinetobacter* were found to be sensitive to Imipinem(75%), *Ecoli* to Imipinem(85.7%) and Amikacin(71.4%), *Enterococci* to Linezolid(100%) and Vancomycin(100%). Sensitivity of *Pseudomonas* was high to Amikacin(75%) and Ciprofloxacin(71.4%) but low to Imipinem(25%).

**Conclusion:** Dynamic monitoring of HAI can help predict their occurrence and most likely susceptibility pattern of the most likely organism(s) for a given set of risk factors prior to culture. This can help choose better empirical antibiotics and decrease morbidity associated with HAI.

When citing these abstracts please use the following reference:  
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**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Gram negative bacillary catheter related blood stream infection in the era of increased resistance

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**Background:** Gram negative bacilli (GN) are recognized as an important cause of catheter related blood stream infections (CRBSI). CRBSI caused by resistant GN (RGN) is less well described. Our objective was to 1) evaluate whether cases with discharge diagnosis of GN-CRBSI were diagnosed appropriately based on predetermined criteria, and whether correct diagnosis had an effect on outcome 2) describe the epidemiology of GN-CRBSI and its impact on patient outcomes

**Methods:** A retrospective chart review in a 670 bed tertiary care hospital from 1/2002-11/2008. Patients with discharge diagnosis of CRBSI and GN bacteremia were included. Definite GN-CRBSI was defined as positive cultures (PC) from peripheral blood and either positive central venous catheter (CVC) or CVC tip cultures without another source of GN bacteremia. Probable GN-CRBSI was defined as positive CVC blood cultures or CVC tip cultures and no other source of infection. Data was collected for demographics, diagnoses, type and site of CVC, bacterial isolates, length of stay, and outcomes.

**Results:** Ninety two patients were included: 25 met criteria for definite GN-CRBSI and 24 met criteria for probable GN-CRBSI. 43(47%) patients did not meet our criteria for CRBSI: 23 had another source of bacteremia, 19 had PC from peripheral blood only. Comparison between patients with definite and probable GN CRBSI and patients without these diagnoses did not show a difference in mortality (12% vs 7%,  $p=0.39$ ) or median time to discharge (13 days vs 10days:  $p=0.92$ ). Of all the GN isolated, *Klebsiella* spp. (59%) and *Enterobacter* spp. (25%) predominated. Twenty percent (10/49) of all GN isolates from definite and probable CRBSI were RGN including extended spectrum  $\beta$  lactamase producing isolates and multidrug resistant isolates. Twenty-four percent (12/49) of patients received inappropriate initial antibiotics and 83% (10/12) of these were patients who had RGN. Mortality trended higher in patients with RGN infection compared to other GN bacilli (3/10, 30% vs. 3/39, 7.7%;  $p=0.06$ ).

**Conclusion:** Most patients diagnosed with GN-CRBSI did not meet our strict criteria for diagnosis, a fact that suggests that GN-CRBSI is misdiagnosed. However, the true diagnosis of GN-CRBSI does not clearly impact mortality or median length of stay. There was a non-significant trend towards increased mortality in patients with RGN infection. Antibiotics active against RGN should be considered as part of initial empiric coverage.

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**Type: Poster Presentation**

Device-associated infection rates, extra length of stay, extra mortality, microorganism profile, and bacterial resistance in two ICUs from Cuba: Findings of the international nosocomial infection control consortium (INICC)

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**Background:** To determine the rates and consequences of device-associated infections (DAI) in two intensive care units (ICU) of two INICC members hospitals in Cuba.

**Methods:** An open label, prospective cohort, active DAI surveillance study was conducted on adults admitted to two tertiary-care ICUs of Cuba. The protocol and methodology implemented were developed by INICC. Data collection was performed in the participating ICUs. Data uploading and analyses were conducted at INICC headquarters on proprietary software. DAI Rates were recorded by applying the definitions of the CDC NNIS. We analyzed the DAI, mechanical ventilator-associated pneumonia (VAP), central line-associated bloodstream infection (CLAB), and catheter-associated urinary tract infection (CAUTI) rates, microorganism profile, bacterial resistance, extra length of stay (ELOS) and extra mortality.

**Results:** From 5/06 to 9/09, we enrolled 1,914 patients, representing 14,157 bed days. The overall DAI rate was 22.6% (95% CI, 20.7 – 24.5) and 30.6 per 1000 bed days (95% CI, 27.8 – 33.5). The VAP rate was 43.67 per 1000 device days (95% CI, 39.17 – 48.5), CLAB rate was 1.25 per 1000 CL days (95% CI, 0.72 – 2.00), and CAUTI rate was 6.37 (95% CI, 5.09 – 7.86). Overall 28.0% of all DAI were caused by *E.Coli*—100% were resistant to Ceftazidime and 100% Ceftriaxone—; 20% by *Klebsiella* sp.—100.0% of which were resistant to ceftazidime, 100% to ceftriaxone and 100% to imipenem—; 20% by *Coagulasa Negative Staphylococci*; and 16.0% by *Pseudomonas* sp—100.0% were resistant to ceftazidime, and 100% were resistant to ceftriaxone. The LOS of patients without DAI was 5.0 days; the LOS of patients with CLAB was 10.0 days (RR, 2.00; 95% CI, 1.29-3.10; P, 0.0016), representing 5.0 extra days; the LOS of patients with VAP was 17.0 days (RR, 3.41; 95% CI, 3.08-3.77; P, 0.0001), representing 12.0 extra days; and the LOS of patients with CAUTI was 15.1 (RR, 3.03; 95% CI, 2.50 – 3.67, P 0.0001), representing 10.1 extra days.

A total of 245 out of 721 (34.0%) patients without DAI died; 1 out of 2 patients with CLAB died (50.7%), the extra mortality being 16% (RR, 1.47, 95% CI 0.21 – 10.49, P, 0.6981); 20 out of 24 patients with VAP died (83.3%), the extra mortality being 49% (RR, 2.45, 95% CI 1.55 – 3.87, P, 0.0001); 4 out of 7 patients with CAUTI died (57%), the extra mortality being 23% (RR 1.68, 95% CI 0.63 – 4.52, P 0.2970).

**Conclusion:** The VAP rate is higher than NHSN's rates and lower than INICC's rates, CLAB rates are lower than INICC's and NHSN's rates, and CAUTI rates are similar to NHSN's rates and lower than INICC's rates. VAP increased significantly the LOS and mortality rate.

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**Session: Infection Control, Nosocomial Infections and Critical Care**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Characterization of hospital-acquired infections in a University Hospital in Colombia: January 2005 - July 2009

J. G. Jiménez, **J. K. Balparda**, D. M. Castrillón, S. Y. Díaz, J. A. Echeverri, C. Estrada, C. Lopera, M. M. Raigosa, L. V. Vásquez  
Universidad Pontificia Bolivariana, Medellín, Colombia

**Background:** Hospital-Acquired Infections represent a growing concern in Colombia's healthcare system, due to its relationship with an extended length of hospital stay, a higher morbidity and mortality and an increase in overall costs. The purpose of the following study was to perform an epidemiological characterization of Hospital-Acquired Infections reported during a four and a half years period in a University Hospital in Colombia.

**Methods:** Information regarding the occurrence of Hospital-Acquired Infections was initially obtained from the Hospital's Epidemiologic Surveillance Committee Databases comprising the sought period. In cases in which all the information was not readily available, additional details of every patient were obtained from their respective clinical records. All data was condensed into a single database developed for the present study, and analyzed using statistical software.

**Results:** A total of 1136 Hospital-Acquired Infections occurring in 953 patients were included, for an overall risk of 2,2 infections per 100 hospital discharges. Most of the (63,7%) were female, with an average age of 39,6±25,8 years and a mean length of stay of 17,7 days. The most common infections, along with their relative frequencies, were: surgical site infections (25,9%), urinary tract infections (16,3%) and pneumonia (12,4%). The most commonly isolated microorganisms were: *Escherichia coli* (27,9%), *Staphylococcus aureus* (17,9%), *Klebsiella pneumoniae* (11,1%) and *Pseudomonas aeruginosa* (10,2%). After the start of intensive educational programs regarding techniques for the reduction of Hospital-Acquired Infections, these have dropped significantly, specially surgical site infections (p=0,0213) and nosocomial sepsis (p=0,0078); nevertheless, urinary tract infections have risen 104,8% during the study years.

**Conclusion:** Hospital-Acquired Infections are relatively low in the studied hospital, when compared to similar Colombian and international institutions. The most frequent nosocomial infection was that of the surgical site, with the most common microorganism being *E. coli*, which showed a medium-to-low antibiotic resistance to first- and second-line antibiotics. In the studied hospital, intensive educational programs have showed a marked success in diminishing Hospital-Acquired Infection rates.

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**Final Abstract Number: 56.028**

**Session: Infection Control, Nosocomial Infections and Critical Care**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

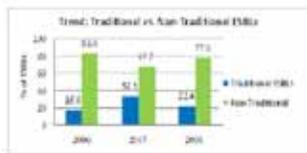
“Non Traditional” Extended Spectrum Beta-lactamase-producing Isolates at a Community Hospital

**R. Nog**, K. Singaravelu, P. Dhaubhadel, A. Guddati, S. Upadhyay, S. Mannheimer, C. Badshah, V. Sivapalan

Columbia University College Of Physicians and Surgeons affiliation at Harlem Hospital Center, New York, NY, USA

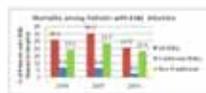
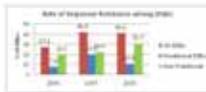
**Background:** Extended spectrum  $\beta$ -lactamase (ESBL)-producing gram-negative bacteria (GNB) represent the most common multidrug-resistant organism infections worldwide, with limited treatment options and frequent adverse outcomes. Two decades since their initial recognition, ESBL producing organisms continue to pose a challenge to infectious disease and infection control practitioners. ESBL activity is most frequently described in *Klebsiella* spp. and *Escherichia coli*. The prevalence and mortality associated with other gram-negative bacteria with ESBL activity has not been documented as well.

**Objective:** To describe trends of ESBL activity in culture isolates obtained at a community hospital in New York.



#### Trend : Traditional vs Non-Traditional ESBLs

**Methods:** A retrospective chart- review of inpatients over a three-year period (1/1/06 to 12/31/08) was conducted to identify cultures positive for GNB. ESBL activity was defined based on antibiogram resistance to cephalosporins, monobactams and penicillin. Data on demographics, imipenem/cilastatin (IMP) resistance and discharge status was collected. *E. coli* and *Klebsiella* were defined as Traditional ESBL's (T-ESBL) and other GNB (*Pseudomonas*, *Serratia*, *Citrobacter*, *Proteus* etc.) were grouped as Non-Traditional ESBL (NT-ESBL). The mortality rate was calculated by discharge status.



#### ESBLs Rate of Imipenem resistance and All Cause Mortality

**Results:** Among a total of 3032 isolates evaluated during study period, 19.1% (n=579) were defined as ESBL. Of these 579 isolates, 23.3% (n=135) were T-ESBL and 76.7% (n=444) were NT-ESBL. The proportion of NT-ESBL remained consistently high during this period (68 to 83%). Among ESBL isolates, 211 (36.44%) exhibited IMP resistance of which 70 (33.18%) were T-ESBL and 141 (66.82%) were NT-ESBL. This greater proportion of IMP resistant NT-ESBL organisms was consistently represented (rates of 72.58%, 52.86% and 74.68% in 2006, 07 and 08 respectively). Of the 269 unique patients with ESBL infection identified during the study 25.65% (n=69) expired. Two thirds of these patients (78.03%, n=54) had NT-ESBL organisms.

**Conclusion:** The majority of the ESBL-producing isolates examined over a three year period at our institution, were non-traditional (other than *E.coli* and *Klebsiella* spp) ESBL-producing organisms. These were more likely to be imipenem-resistant and associated with fatal outcomes. Therefore, monitoring ESBL activity in *Klebsiella* spp. and *Escherichia coli* isolates alone may underestimate the real extent of problem, precluding timely initiation of appropriate antibiotics and infection control practices.

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**Session: Infection Control, Nosocomial Infections and Critical Care**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Ventilator-associated pneumonia in HIV-infected ICU patients

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**Background:** Ventilator-associated pneumonia (VAP) is the main complication of mechanical ventilation (MV), it occurs in 27% of the mechanical ventilated (MV) patients in our Unit. VAP in HIV infected patients has the same incidence and etiology than in HIV-non infected. The objective of the study is to determine the incidence, etiology and outcome of VAP in HIV infected patients admitted in an ICU.

**Methods:** We performed a retrospective analysis of all the HIV infected patients admitted to ICU of Hospital Maciel from June 1990 to December 2008 who were on MV.

Categorical variables were expressed in median and interquartile range. Chi square was used when applied. P value <0.05 was considered significant.

**Results:** 351 HIV patients were assisted during the period. Median age was 32 YO (27-39). 73.5% male and 26.5% female. Median SAPS II 35 (29-43). 186 (54%) were on MV. Length of stay was 6 days (3-11). 162 patients (46.2%) died.

Considering patients on MV, SAPS II was 41 (34-51), length of stay in ICU was 6 days (2-10) and 143 died (76.9%)

VAP occurred as a complication in 43 (23%) patients on MV, median SAPS II was 36 (31-42), length of stay 12 days (9-17), 39/43 died (90.7%) 35 (89.7%) of whom died in septic shock. Etiology: Acinetobacter 17, Pseudomonas aeruginosa 14, Methicillin-sensitive Staphylococcus aureus 4, Methicillin-resistant Staphylococcus aureus 4 and Klebsiella sp 4.

143 patients (77%) on MV did not develop VAP, SAPS II was 43 (36-54), length of stay in ICU 4 days (2-8), 104 (72.7%) died. Patients on MV who developed VAP were less ill at admission (SAPS II 36 vs 43 p<0.001) length of stay was longer (12 vs 4 p< 0.001 and mortality was higher (39/43 vs 104/143 OR 3.66 [CI 1.15-12.9])

**Conclusion:** Although HIV-infected patients VAP incidence and etiology is similar to that of the HIV-non infected patients, its overwhelming evolution to multiorgan failure and much higher mortality almost always represents the final event in the clinical evolution of this group of ICU patients.

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**Session: Infection Control, Nosocomial Infections and Critical Care**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Implementation of a central line bundle to reduce central line associated bacteremia at the intensive care unit

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**Background:** Central line associated bacteremia (CLAB) is associated with an increased length of stay of up to 6 days at the intensive care unit (ICU), with an attributable mortality of 35 % approximately. The reduction in the incidence of Methicillin-resistant staphylococcus aureus (MRSA) central line associated bloodstream Infections continues being a challenge according to the last tendencies published by the CDC in the (ICU) of the United States. *S. aureus* is the most frequent pathogen causing blood stream infections in Latin America (SENTRY 1996-2006). The prevalence of nosocomial *S. aureus* oxacillin resistant is 46% in Argentina. The purpose of this study was to decrease CLAB at the ICU, particularly MRSA (most prevalent pathogen of CLAB in our institution)

**Methods:** Data was collected from September 1, 2007 to July 1, 2009, on all ICU patients who had central venous catheter placed at the Clínica y Maternidad Suizo Argentina. After one year of baseline data collection the infection control team implemented a set of measures for the insertion of CVC (bundle): antiseptic handwashing, maximal barrier precaution, skin preparation with chlorhexidine 2% instead of iodopovidone solution, use of gown mask and cap for the assistant and avoidance of femoral lines. The staff of physician and nurses was educated on bloodstream-infection-control practices before the new elements were added to the protocol. A check list form was filled out by an ICU nurse manager after each central line placement. The NHSN criteria for CLAB were met. The data were collected by an infection control nurse. The rates of CLAB were calculated with Excel and Epi 5 Control Argentina program.

**Results:** During the pre-intervention period the rate of CLAB was 9,47 ‰ (1900 CVC line-day) and decreased to 3,62 ‰ post-intervention (1934 CVC line-day). The rate of gram positive CLAB also decreased from 6,84 ‰ (IC. 0.31%-0.15%) to 1,55 ‰ (IC. 0,02%--0,33%). P:<0,022. This decreasing tendency was also observed in relation to the MRSA(5 ‰ versus 1,09 ‰)

**Conclusion:** The use of central line bundle and to monitor its adherence with a central line insertion check list, significantly reduced the rate of CLAB.

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**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Phenotypic and molecular characteristics of *Clostridium difficile* infections in a tertiary-care center

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**Background:** An increased number of cases of *C. difficile* associated disease (CDAD) has been observed, associated with the emergence of a particular strain, NAP1/BI/027. The objective of this prospective study was to characterise *C. difficile* isolates in a referral hospital.

**Methods:** From 6/1/2008 to 9/30/2009 all stool samples sent to the clinical microbiology laboratory for *C. difficile* toxin A/B detection were processed by a commercial EIA and culture. A further RT-PCR for detection of TcdC was done in all positive samples for either toxin or culture; in each isolate multiplex-PCR was performed to detect TcdA/TcdB and CdtA/CdtB genes, and all isolates were ribotyped. Clinical data was recorded from patients with a positive result. CDAD was diagnosed when the microbiology data supported the clinical findings.

**Results:** A total of 1093 samples were screened for toxin A/B and culture; 6.2% were positive by toxin A/B detection (68/1093), 8.32% (91/1093) by culture, and 10.52% (115/1093) by any of them. Out of the 115 cases, 13 were excluded. From the 102 remaining patients, only 85 (83%) had CDAD; 25 (29.5%) had severe CDAD; 55 received oral metronidazole: 89% cured, 7.3% relapsed, and 11% failed. From 64 isolates from CDAD patients: 45.3% had a negative toxin A/B, 89% a positive TcdA/TcdB, and 87.5% had a positive TcdC; 35.7% revealed the characteristic 18 pb deletion in TcdC, and 10 patients had severe disease (40% vs 16%,  $p < 0.02$ ). From 55 ribotyped isolates, 49 (89%) were grouped in 11 clusters, none similar to the 027 ribotype. From 20 severe CDAD isolates analysed, 3 showed the 18 bp deletion, positive CdtA/CdtB genes and quinolone resistance.

**Conclusion:** Our data shows the presence of a diversity of *C. difficile* strains, however 89% of the isolates were clustered, and among them some hypervirulent strains have emerged.

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**Session: Infection Control, Nosocomial Infections and Critical Care**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Epidemiology of diarrhea due to *Clostridium difficile* in a tertiary general hospital in San José, Costa Rica, from January to October, 2009

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<sup>1</sup>Hospital San Juan de Dios, San José, Costa Rica, <sup>2</sup>Hospital San Juan de Dios, San Jose, Costa Rica

**Background:** Description of the epidemiological behavior of diarrhoea associated to *Clostridium difficile* in patients admitted to Hospital San Juan de Dios (San José, Costa Rica) from January 1st to October 31st. This is the largest hospital of Costa Rica, a tertiary level general medical center, managed by the Costa Rican Bureau of Social Security. It has 730 beds and had a mean of 30,467 discharges per year in the 2004-2008 period.

**Methods:** Collection of all positive immunoassays for *C. difficile*, reported by the Clinical Laboratory of the Hospital, from stool samples of hospitalized patients who were diagnosed with diarrhoea during the 10 month period previously described.

**Results:** A total of 442 positive results of *C. difficile* from 427 hospitalized patients with diarrhoea were obtained. Out of these patients 413 had one diarrheal episode and 14 two or more. During this period of time there were 27040 recorded discharges from the Hospital, which makes for a rate of 16,42 cases/1000 discharges. The 442 episodes include 11 recurrences and 3 reinfections. There were 44 deaths recorded in this group, for a mortality rate of 10,30%.

**Conclusion:** There are no hospital incidence reports of diarrhoea due to *Clostridium difficile* in Costa Rica. Epidemiological reports of nosocomial diarrhoea from this agent are scarce in the literature, thus making it difficult to establish endemic channels for this disease.

Diarrhea from *Clostridium difficile* is endemic at Hospital San Juan de Dios and due to its frequency, pathogenicity, virulence and communicability it is important to improve and maintain an active epidemiological surveillance system. This work provides the first epidemiological description of diarrhoea from *C. difficile* at a general hospital in Costa Rica.

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**Session: Infection Control, Nosocomial Infections and Critical Care**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Impact of pretransplant nutritional status and supplement on postoperative sepsis in living donor liver transplantation

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Kyoto University School of Medicine, Kyoto, Japan

**Background:** Protein-energy malnutrition is common in patients with end-stage liver disease requiring liver transplantation (LT) and is a risk factor for posttransplant morbidity including sepsis. Especially, infectious complications including sepsis often occur after LT and are the most frequent causes of in-hospital death despite recent advances in surgical procedures and perioperative management. Therefore, it is important to accurately estimate preoperative nutritional status and provide adequate perioperative nutritional support in order to improve the results of LT. We examined the efficacy of preoperative nutritional assessment by bioelectric impedance analysis and prognostic factors of posttransplant sepsis in patients undergoing living donor LT (LDLT). Moreover, we investigated the effects of individually tailored preoperative aggressive nutritional therapy.

**Methods:** 1) We prospectively measured body cell mass (BCM) using a body composition analyzer and various nutritional parameters including prealbumin, branched-chain amino acids (BCAA)/tyrosine ratio, and zinc as well as common nutritional parameters such as albumin, cholinesterase, and total lymphocyte count in 50 consecutive recipients undergoing LDLT between February 2008 and February 2009. Multivariate analysis was performed to determine the prognostic factors of posttransplant sepsis, focusing on nutritional parameters. 2) We developed an individually tailored preoperative aggressive nutritional treatment based on the nutritional status of each patient especially in poorly nourished recipients in April 2009; the treatment consists of BCAA-enriched nutrient mixture followed by immunonutrition for 7 days prior to surgery and synbiotics. The usefulness of the new nutritional treatment was examined.

**Results:** The incidence of severe perioperative infection and in-hospital death was significantly higher in patients with low BCM than in patients with normal or high BCM. Multivariate analysis showed that preoperative low BCM, absence of preoperative supplementation with BCAA-enriched nutrient mixture, and a Model for End-stage Liver Disease (MELD) score of 20 or above, but not common nutritional parameters, were of prognostic significance. Pretransplant aggressive nutritional treatment reduced the incidence of postoperative sepsis.

**Conclusion:** Pretransplant nutritional assessment with a body composition analyzer could predict the postoperative clinical course. Malnutrition before LT was closely related to the incidence of posttransplant sepsis and pretransplant aggressive nutritional therapy was useful in preventing postoperative sepsis.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Multidrug-resistant *Acinetobacter* spp. bloodstream infections in a University Hospital of Buenos Aires, Argentina: frequency and antimicrobial susceptibility

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**Background:** Bloodstream infections caused by multidrug-resistant *Acinetobacter* spp. (MR-A) have increased worldwide, particularly in Latin America. The limited therapeutic options against this nosocomial microorganism constitute a major problem for establishing an adequate antimicrobial treatment. Therefore, early identification of this microorganism and surveillance of its resistance patterns are important tools to reduce morbidity and mortality.

Our objective was to determine the: 1) frequency of bloodstream infection by MR-A; 2) time to blood culture positivization; 3) primary sources of bloodstream infection; 4) overall mortality rate; 4) antimicrobial susceptibility profile.

**Methods:** Retrospective analysis of episodes of MR-A bloodstream infections in a tertiary university hospital in Buenos Aires, Argentina (period: January 2006 - October 2009). Blood samples were routinely cultured in Bact / Alert bottles (Biomérieux) and incubated for 5 days. Biochemical identification was performed according to Murray *et al.* (2008) and automated VITEK. Antimicrobial susceptibility was determined by disk diffusion method according to CLSI, 2008 and automated VITEK (Biomérieux). The Minimum Inhibitory Concentration (MIC) to colistin was determined by E-test methodology according to manufacturer's recommendations (AB biodisk). Multidrug resistance was defined as resistance to two or more antimicrobial drug families

**Results:** One hundred and three episodes of MR-A bloodstream infection occurred in 3346 inpatients (3.0%). Median time to blood culture positivization was 10 hours (range: 2.5-44.1). Median age of patients was 61 years (range: 21-91). The documented clinical sources were catheter: 39%; nosocomial pneumonia: 29%; skin and soft tissue infection: 5%; abdominal: 4%; and urinary tract: 1%. In 22% of the cases, no primary source could be documented (primary bacteremia). Overall mortality was 22%. Antimicrobial susceptibility was: ampicillin-sulbactam:7%, ceftazidime:1%, cefepime:2%, piperacillin-tazobactam:1%, imipenem:5%, meropenem:4%, amikacin:3%, gentamicin:26%, ciprofloxacin:1%, minocycline:100%, colistin:100% and tigecycline:100%.

**Conclusion:** The frequency of MR-A bloodstream infections in our institution is higher than the reported in international literature. Overall mortality is comparable to previous reports. In this scenery, early identification of this microorganism and periodic surveillance of its antimicrobial resistance are of utmost importance to guide the physician in the selection of an adequate initial empirical therapy in order to reduce morbidity and mortality by this pathogen.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Recovery rate of NTM from AFB smear-positive sputum specimens during the pseudo-outbreak of mycobacterium species at a long-term care facility

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**Background:** Sputum smears for acid-fast bacilli (AFB) examined microscopically is the most important diagnostic test for pulmonary tuberculosis. However, the AFB observed on the smear may represent either *M. tuberculosis* or nontuberculous mycobacteria (NTM). This study examined the recovery rate of NTM from the AFB smear-positive sputum specimens during the pseudo-outbreak of mycobacterium species at a 300 bed long-term care facility.

**Methods:** We detected a significant increase the positive results of AFB smear during January 2009 that led us to suspect of an outbreak. we performed an epidemiological investigation of the outbreak including acid-fast stain and culture for *Mycobacterium tuberculosis* between January 2008 and July 2008.

**Results:** A total of 49 patients were enrolled in the study. 42 sputum specimens collected from 28 patients were positive AFB smear or culture. NTM were recovered from 52.4% (22/42) of the sputum specimens, and from 50.0% (14/28) of the patients. NTM were recovered from 13.0% (3/23) of the smear-positive sputum specimens, and from 18.75% (3/16) of patients with smear-positive sputum. We think the reservoir for this pseudo-outbreak could be associated with a contaminated suction lines and bottles.

**Conclusion:** These results suggest that a substantial proportion of patients with AFB smear-positive sputum specimens may have NTM during the pseudo-outbreak of mycobacterium species.

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**Type: Poster Presentation**

Clinical impact of Systemic Inflammatory Response Syndrome (SIRS) in patients with bloodstream infection

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**Background:** To evaluate the clinical impact of systemic inflammatory response syndrome (SIRS) in patients with Bloodstream infection

**Methods:** We prospectively collected medical records of adult patients with bloodstream infection who visited to all departments of Jeju National University Hospital for 2 years (from March 2007 through March 2009), including clinical data to meet the criteria of SIRS when blood culture was done.

**Results:** A total of 309 patients with bloodstream infection were enrolled in this study. We compared 191 (61.8%) patients who met criteria for SIRS at the time of blood collection (SIRS group) with 118 patients (38.2%) who did not meet criteria for SIRS (non-SIRS group). Underlying diseases of bloodstream infection were malignancy (21.7%), diabetes mellitus (14.9%) and liver disease (13.3%). The probable sources of bloodstream infections were urinary tract infection (30.1%) intraabdominal infection (28.5%), respiratory tract infection (12.6%) and catheter-related infection (4.5%). There were no significantly statistic differences between SIRS group and non-SIRS group on probable infection sources and underlying condition. But community-acquired infections were more common in non-SIRS group ( $p=0.01$ ). 30-days of overall and infection-related mortality were significantly higher in SIRS group ( $p=0.001$  and  $p=0.004$ , respectively).

**Conclusion:** The presence of SIRS when blood culture was done could be a prognostic marker in patients with bloodstream infection.

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**Type: Poster Presentation**

Risk factors for central venous catheter-related infections in cardiac unit of tertiary care hospital in northern India

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**Background:** The need for therapy and nutrition of severely ill patients has led to ever increasing use of indwelling central intravascular catheters. Intravascular catheters are indispensable in modern-day medical practice, particularly in intensive care units (ICUs). This study estimated the incidence and risk factors of central venous catheter related infections.

**Methods:** During a 18-month period, a total of 150 patients who underwent central venous catheterization were enrolled. Catheters (n=150) were cultured semiquantitatively and blood cultures done simultaneously. Isolates obtained were identified and the risk factors were analyzed statistically.

**Results:** The rate of catheter related bloodstream infection was 3/1000 catheter days. On multivariate analysis, fever was found to be independent predictor of CRBSI. The rate of CVC colonization was 54.01/1000 catheter days. On univariate analysis, statistically significant correlation was found with duration of catheterization, raised total leukocyte count and fever on day of removal of catheter. On multivariate analysis co-morbid state such as Diabetes mellitus and Hypertension, raised TLC and fever on the day of removal of catheter were found to be independent predictors of CVC colonization. The organisms isolated were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Non Haemolytic Streptococci*, *E.coli*, *Acinetobacter baumannii*, *Candida spp.*, *Staphylococcus epidermidis* and *Stenotrophomonas maltophilia*.

**Conclusion:** Despite their potential preventability, catheter-related infections still impose a substantial burden on critically ill patients. Recent studies have brought better understanding of the risk factors for intravascular catheter infections and have clarified preventive infection control strategies.

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**Type: Poster Presentation**

Success in stopping transmission of enterococci in a Brazilian public teaching hospital

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**Background:** Vancomycin-resistant enterococci (VRE) are a major problem in many hospitals mainly because of their capability to colonize or cause disease among high risk patients in addition to their ability to contaminate the environment.

**Objective:** To describe an outbreak of VRE in a Brazilian teaching hospital and evaluate the impact of educational and engineering measures in its control.

**Methods:** We conducted a retrospective study from February 2008 to January 2009. Medical reports were reviewed in respect of demographic data, underlying diseases, comorbidities, risk factors, wards, and length of stay. Primary outcomes were if the patient was colonized or infected and death. Differences between the variables were considered significant when  $p < 0.05$

**Results:** There were 150 patients with isolation of VRE, 139 (93%) of them in rectal swab. The 11 (7%) left patients were infected in blood ( $n=4$ ), ascitic liquid ( $n=2$ ), central venous catheter ( $n=2$ ), and in pleural effusion, urine and wound infection ( $n=1$  each). There were 94 (63%) men and the median age was 50 yrs-old. The main wards were Medical, Onco-Hematology, Trauma, Clinical Emergency and Gastroenterology, corresponding of 73% of patients. There were no differences between patients in respect of being colonized or infected according sex, age, underlying disease and comorbidities. Patients with infection were observed more frequently among those in mechanical ventilation ( $p=0.013$ ), with central venous catheter ( $p=0.043$ ), indwelling urinary catheter ( $p=0.049$ ) or surgical drains ( $p=0.049$ ). Death was more frequent among infected (73%) than in colonized (17%) patients ( $p < 0.001$ ). An informative campaign was carried out, comprising of lectures for the healthcare professionals and distribution of leaflets for patients. Environmental cleaning was reinforced, and gel alcohol dispensers were widely distributed in all hospital areas. Isolation precautions for VRE and restriction to visits were implemented. The ongoing monitoring of the outbreak shows a significant decrease in the number of cases, with 25 new cases in 8 months.

**Conclusion:** Educational measures and reinforcement of environmental cleaning were effective for deterring the dissemination of VRE.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Pseudooutbreak of *Cedecea lapagei* bacteremia in emergency room

**W. S. Choi**, Y. K. Youn, Y. M. Jo, J. Y. Kim, M. J. Kim, W. J. Kim, H. J. Cheong, J.-W. Sohn, J. Y. Song, D. W. Park

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**Background:** *Cedecea lapagei* is a very rare pathogen for human infection. There was only one report of prior isolation of *C. lapagei* from blood. We experienced a pseudooutbreak of *C. lapagei* bacteremia in emergency room (ER). We described the outbreak and outbreak investigation, along with performing genetic analysis of the isolated bacteria.

**Methods:** From September 28 to November 5, 2009, 19 blood samples, which were collected from 11 patients in ER, were positive for *C. lapagei*. The 19 isolates showed similar antibiogram. The isolates were genotyped by pulsed-field gel electrophoresis (PFGE). We performed case-control study to identify risk factors of the outbreak. Environmental surveillance culture was performed to determine the source of the outbreak.

**Results:** The case patients had been diagnosed with various different types of diseases at the time of the admission to ER. There was no influence in the clinical course of the patients even though proper antibiotics were not administered for *C. lapagei*. The PFGE results showed an indistinguishable pattern. In the results of case-control study, the case patients were older than the control patients ( $51.27 \pm 23.31$  years vs.  $27.22 \pm 28.54$  years,  $P=0.007$ ). The case patients had more drugs injected intravenously during staying in the ER than control patients ( $1.64 \pm 1.57$  vs.  $0.38 \pm 0.81$ ,  $p<0.001$ ). The blood sampling of the case patients were performed by seven different interns. There were no significant differences in sex, chronic underlying diseases, use of a nebulizer, O2 supply, wound dressing, total parenteral nutrition, urinary catheter, central catheter, nasogastric tube, length of stay in ER before performing blood culture, and the area of the patients' staying in the ER. Among 73 environmental samples, there was no sample positive for *C. lapagei*. After the outbreak, the infection control unit instituted infection control measures including aseptic techniques, and no more outbreak of *C. lapagei* has been documented for one month in the ER.

**Conclusion:** We suggested that multiple manipulations such as intravenous injection might be a risk factor of a pseudooutbreak and aseptic technique should be emphasized for preventing contamination.

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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Development of subsequent bloodstream infections in patients with positive catheter-drawn blood culture results and negative peripheral blood culture results  
 K.-H. Park, S.-H. Kim, O.-H. Cho, Y. P. Chong, S.-O. Lee, S.-H. Choi, Y. S. Kim, **J. H. WOO**  
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**Background:** There are limited data on incidence of development of subsequent bloodstream infection (BSI) and effect of systemic antibiotic therapy in patients who positive catheter-drawn blood culture results and negative peripheral blood culture results.

**Methods:** We retrospectively evaluated all paired blood cultures from adult patients with indwelling Hickman catheters (HC) on the hematology-oncology ward of a tertiary care hospital over a 12-year period. A positive HC-drawn blood culture as a case in which a paired blood culture was done and HC-drawn blood culture revealed any organism and all peripheral blood culture revealed no organism. Subsequent BSI was defined as a case with development of bacteremia or fungemia caused by same organism between 2 and 28 days after initial positive blood culture.

**Results:** One hundred and twelve episodes from 103 patients with HC met the criteria of isolated positive HC-drawn blood culture. Nine (8.0%, 95% CI 3.0% to 13.1%) of the 112 episodes developed subsequent BSI within 28 days. The detailed data are shown in Table. There was more frequent occurrence of subsequent candidemia (50%, 2 of 4) than that of subsequent bacteremia (6%, 7 of 108) ( $P = .03$ ). Subsequent BSI developed in 6 (20%) of 30 episodes in which empiric antibiotic treatment were inappropriate while but in 3 (4%) of 82 episodes in which empiric antibiotic treatment were appropriate ( $P = .01$ ).

**Table. Development of bloodstream infections subsequent to isolated positive catheter-drawn blood cultures in Hematologic cancer patients with indwelling Hickman Catheters.**

Microorganisms	Subsequent BSI/ isolated positive HC-drawn blood culture (%)	Subsequent BSI/Isolated positive HC-drawn blood culture (%)		<i>P</i>
		Appropriate empiric therapy	Inappropriate empiric therapy	
Gram-positive organisms	2/58 (3)	1/39 (3)	1/19 (0)	1.00
CNS	0/29 (0)	0/18 (0)	0/11 (0)	...
<i>Enterococcus</i>	1/10 (10)	0/5 (0)	1/5 (25)	...
<i>Corynebacterium</i>	0/5 (0)	0/4 (0)	0/1 (0)	...
<i>Bacillus</i>	0/4 (0)	0/3 (0)	0/1 (0)	...
Viridans strep	0/3 (0)	0/2 (0)	0/1 (0)	...
<i>S aureus</i>	1/2 (50)	1/2 (50)	0/0	...
<i>Leuconostoc</i>	0/2 (0)	0/2 (0)	0/0	...
Other g(+) cocci	0/3 (0)	0/3 (0)	0/0	...
Gram-negative organisms	4/42 (10)	1/33 (3)	3/9 (33)	0.03
<i>E coli</i>	0/10 (0)	0/10 (0)	0/0	...
<i>Klebsiella</i> spp	0/8 (0)	0/8 (0)	0/0	...
<i>P aeruginosa</i>	0/6 (0)	0/5 (0)	0/1 (0)	...
<i>Acinetobacter</i>	2/5 (40)	1/4 (25)	1/1 (100)	...
<i>Enterobacter</i>	1/5 (20)	0/4 (0)	1/1 (100)	...
<i>S maltophilia</i>	1/4 (25)	0/1 (0)	1/3 (33)	...
<i>B cepacia</i>	0/2 (0)	0/0	0/2 (0)	...
Other g(-) rods	0/2 (0)	0/1 (0)	0/1 (0)	...
<i>Candida</i>	2/4 (50)	0/3 (0)	2/2 (100)	0.33
Polymicrobial	1/8 (13)	0/6 (0)	1/2 (50)	1.00
Total	9/112 (8)	3/82 (4)	6/30 (21)	0.01

**Conclusion:** In patients with isolated positive HC-drawn blood cultures, the overall incidence of subsequent BSI was 8.0%. Isolation of *Candida* species and inappropriate empiric treatment were associated with development of subsequent BSI.

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**Type: Poster Presentation**

Prevalence and factors associated with hospital acquired infections in Kayunga district hospital, central Uganda

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**Background:** Globally about 2 million people annually suffer Hospital Acquired Infections (HAIs), mainly patients hospitalized for other conditions leading to prolonged stay, increased morbidity and mortality. The magnitude of HAIs in Kayunga district hospital (KDH) was unknown in 2009; however, it is acknowledged that the lack of basic equipment and infrastructure compromises infection control practice making transmission of HAIs favorable. This study set out to estimate the prevalence and assess factors associated with HAIs in Kayunga district hospital (KDH) to enable the hospital management design interventions to address HAIs.

**Methods:** We conducted a cross sectional survey in the first week of July 2009 in Kayunga district hospital; - with 100 bed capacity. One hundred in-patients who had spent at least 72 hours were recruited consecutively into the study. Face-to-face interviews with study participants were conducted using a structured questionnaire and an observational check list filled on each ward. Ministry of Health, Epidemiological and Surveillance case definitions were used to identify new infections categorized as HAIs based on the disease-specific incubation period. Data was analyzed using SPSS version 12.0; the primary outcome was having a HAI.

**Results:** The prevalence of HAIs was 34.0% (34/100). Majority cases of HAIs had multiple infections (26.5%), followed by single infections; Respiratory tract infections (23.5%), Diarrhoea (17.6%), Urinary tract infections (14.7%) and others. Infection control practice on the wards was compromised by lack of hand washing facilities, lack of an isolation ward and lack of supplies for disinfection. HAIs in KDH were associated with having a surgical procedure at the current admission (OR 2.68, 95% CI: 1.10-6.53).

**Conclusion:** The prevalence of HAIs in KDH is high necessitating enforcement and monitoring of infection control practices in the hospital. Creation of an isolation unit for highly communicable diseases in the KDH should be offered priority. The District Health Team should lobby to ensure that flowing piped water is available in KDH. Active surveillance of HAIs among Post operative patients should be instituted. There is need for a study to investigate the relationship between having surgery and developing a HAI.

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**Type: Poster Presentation**

Vancomycin-resistant enterococci cross-transmission among patients admitted to public and private hospitals in central Brazil

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<sup>1</sup>Federal University of Goias, Goiania, Brazil, <sup>2</sup>INGOH, Goiania, Brazil, <sup>3</sup>Goias Health State Secretariat, Goiania, Brazil

**Background:** Enterococci harbouring a vancomycin resistance gene have emerged as an important nosocomial pathogen associated with high mortality. In Brazil, vancomycin-resistant enterococci (VRE) infection was first reported in the late 90s, in the South region. In August 2007 the first case of VRE was identified in the city of Goiania (~1 million inhabitants), Central Brazil. We herein present the molecular characteristics of VRE isolated two years apart from its first isolation in our municipality.

**Methods:** Screening for VRE was performed in the two major public hospitals and one private hospital. Microbiological samples (rectal swabs, blood, urine, surgical specimens) were collected from adult patients admitted to clinical and surgical wards and Intensive Care Units (ICU). Suspected VRE colonies were submitted to identification using conventional biochemical tests and antimicrobial susceptibility (CLSI, 2008). Detection of vancomycin-resistance genes was performed by PCR. The genetic relatedness of bacterial strains was evaluated by PFGE and the results were analyzed using Tenover criteria and BioNumerics (v. 5.0.)

**Results:** A total of 58 VRE (41 *E. faecium* and 17 *E. faecalis*) were isolated from 58 patients. Patients' age ranged from 13 to 84 years (median of 54 years). 76.5% of *E. faecalis* represented colonization (rectal swab) in contrast, almost half of *E. faecium* were associated with infection (blood, urine or surgical wound secretion). All VRE strains carried the *vanA* gene. 27 out of 41 (65.8%) strains of *E. faecium* belonged to the same cluster (genetic similarity ≥80%) and to 5 lineages highly related. Strains from private hospital were identical and not correlated to the ones from public hospitals. *E. faecalis* strains were classified in 5 clusters. 10.3% of the samples were susceptible to ampicillin and 86.2% to linezolid.

**Conclusion:** The study discloses the presence of VRE genetically related strains in distinct hospitals, suggesting cross-transmission intra and inter-hospitals. Genotyping surveillance is an important tool for understanding transmission patterns and for guiding infection control interventions.

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**Type: Poster Presentation**

Determinants of good adherence to hand hygiene among surgical health care workers

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**Background:** Hand hygiene is the main pillar on standard precautions (hospital infection prevention), and of increasing concern. Nevertheless few is known about cognitive, attitude, perception and motivational determinants of hand hygiene's health care workers in a surgical arena where they have never been exposed to a multimodal hand hygiene campaign.

**Methods:** Cross-sectional study in a 900-bed university teaching hospital in Barcelona (Spain). A confidential self-administered and validated questionnaire survey was conducted in a sample of 176 surgical units' healthcare workers (medical and nursing) before a multimodal Hand hygiene (HH) campaign started.

**Results:**

A total of 172 questionnaires were returned (response rate 97.7%), 54 (30.7%) were physicians, 75 (42.6%) were nurses and 47 (26.7%) nursing assistants. The median years of institution experience was 13.00 years (IQR 20.0), 68.8% were women, 21% were from the General Surgery ward, 21.6% were from the Urology, 39.8% from the Orthopedics and 17.6% from the Vascular Surgery ward. The self HH compliance estimated was 77.47(DE17.74), being in 107 participants (63.7%) higher than 80%.

Knowledge determinants were poorly ranked as determinants of hand hygiene (OR 1.08, CI95% 1,02-1.15), while motivation to improve the hand hygiene compliance (OR 0.59, CI95% 0,39-0.88) and medical staff (OR 0.11, CI95% 0.02-0.55) or nurses (OR 0.15, CI95% 0.04-0.66) versus nursing assitants were higly ranked as determinants, independently of positive perception about hand hygiene, gender, attitude toward HH and years of institutional experience.

**Conclusion:** In a setting of absent exposure to hand hygiene campaign, knowledge about hand hygiene, motivation and professional status are strongly in favor of hand hygiene, rather than behavioral beliefs, independently of gender, more than 10 years of institution experience, and professional category. These results are quite different from that observed in an extensive exposure to hand hygiene campaigns where behavioral beliefs and the perception of high self-efficacy are related to hand hygiene compliance rather than reasoning. This additional insight should be considered in settings that have never been exposed to promotion activities about standard precautions.

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Effectiveness of copper contact surfaces in reducing the microbial burden (MB) in the intensive care unit (ICU) of hospital del Cobre, Calama, Chile

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**Background:** The environment plays a role as reservoir and transmission of microbes in hospital settings. In vitro studies have demonstrated the microbicidal activity of copper (Cu). We studied the ability of Cu to reduce the MB associated with commonly contact surfaces within an ICU of Hospital del Cobre de Calama, located in a semi-desert area in the north of Chile.

**Methods:** Copperized (Cu) surfaces (bed rails, bed lever, tray tables, chair arms, touch screen monitor stylus, and IV poles) were placed into 3 randomly selected ICU rooms. Three paired rooms were compared by measuring the MB during 18 weeks. The mean(m) MB for each Cu object, determined as colony forming units (cfu)/100cm<sup>2</sup> was compared to non-copper rooms. Antimicrobial efficacy of Cu was calculated as the difference in mMB between the Cu and non-Cu objects. The environmental cleaning procedures were equivalent for Cu and non-Cu rooms. The Kruskal-Wallis test for two groups was used.

**Results:** The mMB associated with 594 Cu surfaces from 54 rooms containing Cu was compared against the mMB of equivalent surfaces from 54 non-Cu rooms from an ICU with humidity between 7.2 to 19.7%. Cu was effective in reducing mMB on all 6 surfaces tested (bed rails by 86%, bed lever by 84%, tray tables by 93%, chair arms by 88%, monitor pen by 49%, and IV poles by 89%). Total mMB count in rooms with Cu (2,142 cfu/100cm<sup>2</sup>) was significantly lower than in rooms without Cu (13,215 cfu/100cm<sup>2</sup>) p< 0.00001. Staphylococci were the most predominant microorganism isolated. Cu was equivalently effective and significant in reducing the staphylococcal burden associated with the objects, p<0.00001. MRSA and VRE were never isolated, regardless of the presence of Cu. Gram negatives were infrequently isolated from some surfaces, and Cu reduced the Gram negative burden by 74% - 88%.

**Conclusion:** The antimicrobial effect of Cu was evident and significant in this arid region of Chile; reduced the total mMB, mMB of staphylococcal and Gram negative in ICU rooms containing Cu. Further studies regarding the clinical impact of reducing the mMB by Cu objects within the environment of hospitals is warranted.

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Cytomegalovirus antibodies amongst immunocompromised (HIV) patients at Lagos University Teaching Hospital (LUTH) Idi-Araba, Lagos

**A. Akinbami**  
LASUCOM, LAGOS, LAGOS, Nigeria

**Background:** CMV infection constitutes a real risk of pathogenicity in immunocompromised patient. HIV- infected patients who require transfusion are at high risk of developing symptomatic CMV infection when they are transfused with CMV-infected donor blood. This study intends to determine the risk that blood transfusion poses in CMV infection in a population of HIV- infected patients

**Methods:** A study was carried out amongst clients attending HIV clinic at Lagos University Teaching Hospital (LUTH), they were recruited consecutively. Replacement blood donors of the hospitals were recruited as controls A total of 10mls of blood sample was collected per consenting participant. The serum obtained from 5ml of blood was assayed for CMV IgG/IgM using an enzyme- linked immunosorbent assay (ELISA) based Kit whilst full blood count and CD4 cell counts were also performed on the remaining 5mls. The descriptive data was given as mean  $\pm$  standard deviation (SD). Chi-squared test was used for the analytic assessment. The differences were considered to be statistically significant when the p value obtained was less than 0.05.

**Results:** A total of 129 HIV-infected were studied. All (100%) were IgG anti-CMV positive. 8 (6.6%) were IgM anti-CMV positive. This difference was found to be statistically significant  $P=0.004$ , ( $P<0.05$ ). Of 122 replacement blood donor controls, 118 (96%) of them were IgG anti-CMV positive whilst 26 (19.5%) of 121 were IgM anti-CMV positive. The mean CD4+ cell count of HIV-infected patients was ( $234 \pm 173.181$ ). Previous history of blood transfusion did not affect CMV antibody positivity directly.

**Conclusion:** The seroprevalence of CMV is very high in HIV-infected patients, which is comparable to the seroprevalence amongst the general population The use of leukoreduced blood units for anaemic HIV infected patients, is recommended.

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**Type: Poster Presentation**

Evaluation of Tanzania National Measles Surveillance System with laboratory component, for 2006 and 2007

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**Background:** Measles is among the seven vaccine preventable diseases of priority under the Expanded Program on Immunization in Tanzania. The objectives of the measles surveillance system which was established in 1975 are to establish disease early detection, prevention, control of its outbreak, monitor disease pattern and integrate enhanced surveillance for polio and measles cases. The objective of this evaluation was to determine whether the surveillance system is achieving its objectives, purposes and to assess its attributes.

**Methods:** Used the Centres for Disease Control and Prevention (CDC) guideline document, discussions and interviews with different stakeholders, reviewing National measles data for 2006 and 2007 with reference to WHO indicators of quality measles surveillance system.

**Results:** There was a total of 7656 suspected measles cases with 2871 (37.5%) of cases confirmed by IgM laboratory testing which is above WHO target of 10%.

The system is highly sensitive with low Predictive Value Positive, flexible and acceptable. Not simple as it requires laboratory confirmation. Quality of data in terms of completeness and timeliness is good with description of age and vaccination status. There is poor data feedback communication with low levels and between Expanded Programme in Immunization headquarters and Epidemiology Section.

**Conclusion:** The System mainly achieves its objectives and purposes, but should strengthen the timely distribution of feedback to lower levels, and strengthens data communication with Epidemiology section, for better preparedness in case of any outbreak.

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The first needle and syringe exchange program in Iranian prisons

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**Background:** Needle and syringe exchange programs within prisons have been reported in international literature to be associated with reduced blood-borne pathogens among intravenous (IV) drug users. Due to lack of previous evaluations for its effect on prevalence of IV drug using among Iranian prisoners, herein we decided to perform the first evaluation of this program in the Iranian prisons.

**Methods:** In a period of nine months, from July 2008 to March 2009, 347 volunteer prisoners enrolled and followed up. All the volunteers were IV drug abusers. The program had been performed in three major prison centers in Iran, including Tehran, Isfahan and Hamadan. The prisoners were given sterile needles and syringes weekly, and the used ones were also collected regularly. Data including number of syringes and shared ones used by each person was collected at the beginning of program; and this data was also documented on a weekly basis as the program proceeded. Information regarding blood-borne diseases was also given to the enrolled prisoners continuously.

**Results:** Among 347 volunteer prisoners enrolled in this program, an average of 17 syringes were reported to be used weekly for IV drug injection before starting the program. Moreover, the volunteers reported to use an average of 3.7 shared syringes during a week. Documented infection with blood-borne diseases (including Human Immunodeficiency Virus, Hepatitis B or C) was also found in 44 prisoners. At the end of the program, prevalence of using shared syringes among volunteers was decline to zero.

**Conclusion:** Execution of needle and syringe exchange programs within prisons can play an important role in decreasing the number of prisoners using shared syringes and also number of shared syringes used by each prisoner. Therefore, it can greatly help medical staff with controlling blood-borne diseases.

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Prevalence of urinary schistosomiasis in Offinso-South Municipality in Ashanti Region-Ghana  
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**Background:** Of the 200 million people infected worldwide, 85% of the burden is concentrated in Africa. Ghana, a sub-Sahara Africa nation has records of Schistosomiasis and other Soil Transmitted Helminthes infection. The pathology of SH infection has known public health effects on reproduction and productivity in affected persons

**Methods:** The exploratory survey was undertaken in the Offinso South Municipality, in the Ashanti Region of Ghana West Africa for the period of January 2007 to August 2009 involving 10759 subjects within the ages of 9 to 25 years. The selection of the site was influenced by the presence of river Ofin which has adjoining tributaries that pass through the main town. The observation which was hospital-based looked at results of all diagnostic laboratory requests for samples of out-patients for the stated period. Urine of at least 10ml was routinely collected. Urine chemistry was measured for all samples using urine test strip and analyzer by Roche™ following all standard procedures. Schistosoma haematobium (SH) ova identification and quantification was done at low power magnification (x10 objective). Quantification of SH ova was done using the routine diagnostic plus (+) system.

**Results:** 1.69% of the samples were positive for SH ova. The intensity of ova seen on each positive slide corresponded to the level of hematuria reported per urine appearance and proteinuria. The concentrations of leucocytes recorded were normal and did not suggest any parasitic infection.

**Conclusion:** A prevalence of 1.69% is low when compared with values from other studies. Hunter et al, 2003, saw prevalence from 17% to 51% in a farming community that lived by a Dam in Upper East Region of Ghana. The adequate treatment schedule realized from the hospital could also contribute to the probable reduce transmission rate of SH infection. It also shows probable reduced risk associated with water-contact pattern in its infection. Even though urinary schistosomiasis infection is not a public health concern at this moment in the municipality, proper surveillance and effective intervention should be in place to eradicate the infection in the municipality and other parts of the country. This could also help to reduce co-morbidity that results from SH infections.

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Influenza-like illness among US pilgrims returning from the Hajj in the Kingdom of Saudi Arabia compared to other US-bound Travelers: Data from the CDC quarantine activity reporting system (QARS), 2006-2008

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<sup>3</sup>Centers for Disease Control and Prevention, Atlanta, GA, USA

**Background:** Every year, approximately two million Muslim pilgrims (Hajjis) from almost 140 countries gather in the Kingdom of Saudi Arabia (KSA) for 2-3 weeks to perform Hajj religious rites in a limited geographic area. Crowded conditions have historically played a role in the transmission of infectious diseases among Hajjis. Influenza-like illness (ILI) was defined as fever  $\geq 100^{\circ}\text{F}$  with cough and/or sore throat, in the absence of a known cause other than influenza. The objective of this study was to describe the occurrence of ILI among Hajjis returning to the United States from KSA

**Methods:** Data for all ill Hajjis traveling from KSA to the United States reported into QARS during three consecutive annual Hajj seasons were analyzed to identify the relative risk (RR) of reported ILI among Hajjis versus other US-bound travelers during January 2006, 2007 and 2008. US Bureau of Transportation Statistics data were used to identify the number of direct US-bound travelers from KSA and countries with ILI cases reported in the QARS during each year. The numbers of US-bound travelers from both the Middle East countries and other countries reporting ILI cases in QARS were used as denominators to calculate the incidence of ILI among returning US Hajjis versus its incidence among US-bound travelers returning from other countries. The RR was then calculated.

**Results:** The RR of having ILI was significantly higher among US-bound Hajjis than other US-bound travelers in January 2006 (RR=9.73, P=0.006) but was insignificantly higher in January 2008 (RR=2.62, P=0.2). No ILI case was reported among hajjis in January 2007 compared to 11 cases reported among travelers from other countries.

**Conclusion:** A potential reporting bias of ILI among hajjis versus other travelers may represent a limitation to the study results. However, travelers to mass gatherings such as the Hajj may be at significant risk of acquiring ILI. QARS data could permit early detection of infectious diseases among US-bound travelers. QARS data can also be a useful adjunct to other surveillance systems to determine the need for enhanced health surveillance at the US ports of entry for travelers returning from mass gatherings.

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Assessing burden and depth of hepatitis B infection within Asian Pacific islander families in San Francisco

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**Background:** Community-based data suggest that the prevalence of chronic Hepatitis B (HBV) infection among Asian and Pacific Islanders (API) is 6-14%. At least 25% of persons of API descent with chronic HBV will develop complications from liver disease and/or hepatocellular carcinoma during their lifetime. Many family members of patients with known HBV have not been screened. For each HBV-infected person of API descent, we estimated the number of family contacts that had not been screened for HBV.

**Methods:** From May-September 2009, 31 HBV-infected persons of API descent from a UCSF Liver Clinic who agreed to participate in the study completed a detailed questionnaire about their family history and knowledge of their disease. Each index case subsequently was given coupons to give to family members whose kinship was defined by descent and marriage and whose HBV status remained unknown. These coupons contained information to attend a free screening clinic as well as an identification number linking our index cases to each potential family member. A chart review for each index case was also performed; laboratory values recorded were taken at the time of visit.

**Results:** Study participants had a median age of 48 and were predominantly men born outside the US (table 1). For every one index case, 7.5 family members who were at risk were identified; in total, we identified 233 family members whose HBV status was unknown including 26 parents, 20 siblings and 10 children. Fifty-three percent of index cases stated that they had acquired HBV from their parents, while 33% stated that they did not know how they had acquired it. Alternative transmission modes included from dirty water, "homegrown" anti-malarial medication, dentists, and street vendors. Ninety-three percent of index cases stated that they had informed their family members of their status; reasons for not informing include stigma, shame and unclear understanding of transmission methods.

Table 1. Index Case Characteristics (n=31)

Mean Age	48 (22-87)
Male	63%
Ethnicity	39% Chinese 13% Vietnamese 13% Filipino 7% Korean 9% South/Southeast Asian
Born outside of US	83%
Mean Albumin	4.0 (2.7-4.7)
Mean ALT	41 (11-182)
Detectable HBV viral load	82%

**Table 1. Index Case Characteristics**

**Conclusion:** Though nationwide vaccination campaigns are beginning to target this underrepresented immigrant population, screening Asian and Pacific Islander families for HBV remains a challenge.

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Serological typing improves the epidemiological surveillance of gonorrhoea among men who has sex with men

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**Background:** Gonorrhoea remains a major sexually transmitted infection (STI) worldwide. *Neisseria gonorrhoea* has nowadays a high prevalence of resistance to several antibiotics traditionally used for treatment. During April 2004 to February 2007, we characterized all isolates from cases of gonorrhoea reported to the County Medical Officer of Uppsala county serologically with monoclonal antibodies and for antibiotic susceptibility.

**Methods:** Isolates were characterized by using two panels of different monoclonal antibodies commercially available (Gentec Systems and Pharmacia). Susceptibility testing was performed according to standardized methodology using Etest for determination of MIC for ampicillin, cefixime, ceftriaxone, azithromycin, ciprofloxacin and spectinomycin. Production of beta-lactamase was examined by using nitrocefin disks.

**Results:** Of 57 isolates studied, 7 strains were producers of beta-lactamase (13%). Decreased susceptibility to ampicillin and ciprofloxacin was observed in 81 and 56%, respectively. Eight isolates (14%) exhibited reduced susceptibility to azithromycin. All isolates were susceptible to cefixim, ceftriaxon and spectinomycin. The majority of the isolates belonged to serogroup WII/III (98%). Bropyst (49%) and Brpyust (11%) was the most common Ph serovar. The epidemiological study demonstrated that most ciprofloxacin resistant isolates were isolated from men who had sex with men and that the majority of these isolates belonged to the same serovar. A difference in gender was obvious (4 women, 7%). The mean age was for women 36 years and for men 24 years.

**Conclusion:** A clonal outbreak of gonorrhoea among men who has sex with men was observed during 2005 to 2006 in Uppsala county. This clone is resistant to ciprofloxacin and has reduced susceptibility to ampicillin. Preventive measures and intensive epidemiological control of gonorrhoea among men who has sex with men is warranted.

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Surveillance of human infections with Avian influenza in Guangzhou during 1997–2006

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**Background:** The special geographic location of Guangzhou, as well as certain practices of poultry breeding and marketing, and the habits of food handling in Guangdong, have made this region a hot spot of influenza infection. Therefore, since 1997, disease surveillance has been conducted in Guangzhou.

**Methods:** Surveillance was conducted in Guangzhou from 1997 among a group of workers involved in the breeding, selling and slaughtering of poultry and other animals which may potentially carry avian influenza viruses. Serum samples were collected from this high risk population to detect specific antibodies via the microhemagglutination inhibition test. An epidemiological survey was conducted on people infected with H9N2 or H5N1.

**Results:** Among the total of 2546 samples collected within the period 1997–2005, the positive rate for H9 was 4.56%, while the testing for anti-H5 antibody resulted in no positive response. Among serum samples collected in 2006 from 226 workers in the live poultry food markets, where the first H5N1 case was found, one person working in poultry slaughtering was positive for anti-H5 antibody, while another positive was found among the 432 samples from persons without contact with poultry. The H9 infection rate among the occupational group that handled poultry in Guangzhou was 4.56% (0.51%–10.00%), There was no significant difference among different age groups and different gender but the rate is higher than that in the urban group that practiced scattered home-breeding of poultry.

One characterized human case of avian influenza A (H5N1) in Guangzhou in 2006 was reported to have visited many live poultry food markets before the onset of the disease. A poultry slaughterer working in one of these live poultry food markets had a positive anti-H5N1 antibody response, while a person with anti-H5N1 antibody and a patient with anti-H9N2 antibody had no history of direct contact with poultry.

**Conclusion:** H5N1 and H9N2 may cause an asymptomatic or low symptomatic infection in humans. In addition to people working in poultry farms, those involved in avian breeding, selling and slaughtering of poultry are also at high risk for avian influenza viral infection.

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Rapid analysis of known and unknown pathogens using a pan-microbial detection microarray  
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**Background:** Rapid analysis of known, emerging and unknown viral and bacterial infections is critical for public health. Assays that can only identify a small handful of pathogens are not sufficient to deal with complex microbial infections. In this study, we report our development of a high-throughput microarray designed to detect known and discover unknown pathogens.

**Methods:** We designed a pan-Microbial Detection Array (MDA) to detect all viruses, bacteria, and plasmids with 2195 viral species and 924 bacterial species represented. We took an approach balancing the goals of conservation and uniqueness for probe design, aiming for uniqueness relative to other families and kingdoms, and for conservation, to the extent possible, within a family. Long probes were selected to tolerate some sequence variation to enable detection of novel, divergent species with homology to sequenced organisms. We have used our arrays to identify pathogen infections from various clinical samples. We developed a novel statistical analysis method, maximum likelihood analysis method that enabled quantifiable predictions of likelihood for the presence of multiple organisms in a complex sample.

**Results:** The MDA correctly identified multiple viral and bacterial infections in unknown fecal, sputum and nasopharyngeal samples that were later confirmed through independent high-throughput sequencing or PCR assays. Up to strain level identification can be achieved for fully sequenced pathogens in as little as a few hours. Family level identification was achieved for highly divergent viruses. The array is highly sensitive and able to achieve low-copy viral genome detection of spiked samples when coupled with whole genome random amplification.

**Conclusion:** Our Microbial Detection Array is a fast, efficient and cost-effective tool to rapidly characterize known, emerging and unknown pathogens from clinical and environmental samples. The MDA has higher probe density and larger phylogenetic representation of viral and bacterial sequenced genomes than other available array designs. It can be applied to problems in viral and bacterial detection from pure or complex environmental or clinical samples. It will enable informed responses to novel biological threats and infectious agents and provide a complement to high-throughput sequencing data.

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Indexing infectious disease information into a relational database for useful queries on the World Wide Web

**J. Brown**

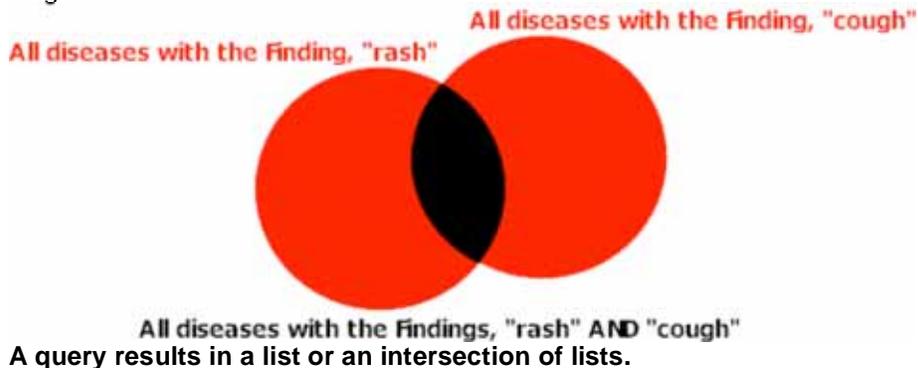
National Library of Medicine, Tacoma, WA, USA

**Background:** In the twenty-first century, we now have new tools for indexing infectious disease information (the relational database) and for disseminating that information worldwide (the Internet). A book such as the "Control of Communicable Diseases Manual" has a helpful index, but it does not have an electronic index that can provide the powerful queries of a relational database, and it cannot be accessed by the millions of computer-users around the world.

**Methods:** All pertinent infectious disease information was envisioned as a knowledge domain that could be mapped into a relational database using indexes that are structured and unambiguous. The database would be designed to provide queries that return useful differential diagnoses, e.g., all diseases matching the syndrome "Rash and Fever." The database would contain a table of Diseases and related tables of Findings (signs and symptoms), Jobs, Risk Factors, and Syndromes.

**Results:** The author has designed three different databases that use queries to find all infectious diseases that match one or more search criteria. One of the databases covers 275 communicable diseases and contains 119 signs & symptoms, 39 epidemiological factors, and 16 regions of the world. An example of a query is to search for all diseases that match the job "Veterinarian" and the syndrome "Acute Neurological and Fever." The search criteria are the indexes that were built into the tables at the beginning of the project, not the ambiguous and unsystematized indexes that are added at the end of a book. Each disease profile shows initial symptoms, incubation period, common findings, endemic areas, laboratory diagnostics, and epidemiological factors. Epidemiological factors include entry (e.g., ingestion), source (e.g., soil), vector (e.g., ticks), and reservoir (e.g., cats). These factors are displayed for each disease, and they are also used as search criteria. Haz-Map is the earliest demonstration of this tool, and it is available for free searching on the National Library of Medicine website.

**Conclusion:** All useful infectious disease information can be collected and indexed into a web-based relational database to help practitioners quickly build accurate, up-to-date differential diagnoses and find detailed information.



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Respiratory disease surveillance aboard Peruvian Navy Ships: Detection of a pandemic influenza A (pH1N1) outbreak

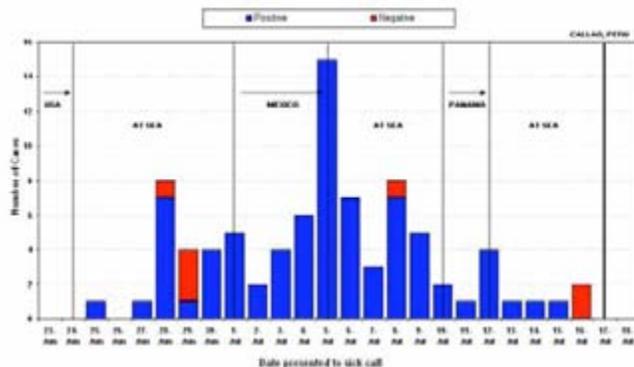
**D. Vera**<sup>1</sup>, R. A. Hora<sup>1</sup>, V. Gonzaga<sup>1</sup>, J. Quispe<sup>1</sup>, J. M. Neyra<sup>1</sup>, M. Ramos<sup>1</sup>, C. Loret de Mola<sup>1</sup>, P. Mote<sup>2</sup>, M. Fernández<sup>3</sup>, J. Montgomery<sup>1</sup>

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**Background:** Living conditions aboard navy ships provide an ideal environment for respiratory disease transmission and place military personnel in these settings at higher risk for outbreaks of viral respiratory diseases. We sought to determine the incidence rates and etiologies of Febrile Acute Respiratory Infections (FARI) on deployed Peruvian Navy ships by implementing an onboard respiratory disease surveillance system. Notably, the system allowed for detection of an outbreak of pH1N1 in the first study ship.

**Methods:** The onboard surveillance system was designed to include all Peruvian navy ships deployed on sea missions from May 2009-2011. Prior to deployment, onboard health personnel were trained to maintain daily counts of crewmembers who presented to sick call with FARI symptoms (fever >100.5°F and either cough or sore throat). Trained personnel completed a case report form for each person meeting the case definition and obtained respiratory samples for both rapid Quick View® Influenza and real-time reverse transcriptase polymerase chain reaction (rRT-PCR) testing.

**Results:** From June 25 to July 17, 2009, 85 of 355 crewmembers from the first Peruvian navy study ship presented to sick call with FARI symptoms. Of those, 52 tested positive for influenza A with the rapid test (attack rate 14.6%), and 78 tested positive for pH1N1 with rRT-PCR (attack rate 21.7%) [See Figure]. Descriptive analysis of cases showed that 9% were female; mean age was 25.5 ± 8.4 years; mean temperature was 38.6 ± 0.5°C; and that the mean number of days between onset of symptoms and presentation to sick call was 1.6 ± 0.8 days. The most frequent symptoms, other than fever, included cough and headache (both 75%), malaise (74%), nasal congestion (73%), and sore throat (55%). A majority (99%) of the cases had been vaccinated against seasonal Influenza (Agrimipal® S-1) before deployment. Approximately 8% were asthmatic and 43.6% had past history of tobacco use. No complications or fatal outcomes were observed among confirmed cases.



**Presentation of FARI cases to sick call aboard a Peruvian Navy Ship, June – July, 2009.**

**Conclusion:** Our findings highlight the importance of the implementation of robust respiratory surveillance systems aboard military ships for timely detection and control of respiratory disease outbreaks, ultimately preventing potential dissemination back to their country of origin.

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Population-based seroprevalence of hepatitis C virus infection in the South and Southeast regions of Brazil

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**Background:** In Brazil, few studies describe HCV prevalence in general population and it is estimated from 0.4 to 3.0% in distinct regions of the country. The objective of this study was to estimate the seroprevalence of hepatitis C in the South and Southeast regions of Brazil

**Methods:** A population-based survey was carried out in the South and Southeast regions of Brazil (2007/2008), as part of a national survey conducted in all five regions of Brazil. The sample was representative of the 7 state capitals in these regions and people from 10 to 69 years old were considered. In each capital census tracts and, then, blocks were drawn with probability proportional to size; subsequently a systematic sample of households was drawn and their residents selected. The study was approved by regional and national ethical committees and signed informed consent was obtained. Hepatitis C antibodies (anti-HCV) was screened by automated ELISA ( Axsym™ – ABBOTT Laboratories) and confirmed by manual test (DiaSorin™). It was considered as positive serum samples with reagent results by both tests.

**Results:** A total of 7,835 individuals were investigated from both regions. Anti-HCV prevalence was 0.056% (95%-CI: 0.000-0.167) in the age group 10-19 and 1.1% (95%-CI:0.581-1.633) in the age group 20 to 69 from South region. From Southeast region Anti-HCV prevalence was 0.112% (95%-CI:0.000-0.268) in the age group 10-19 and 0.76% (95%-CI:0.389-1.130) in the group 20-69.

**Conclusion:** Our data show low HCV prevalence in these Brazilian regions. The occurrence of HCV in children, despite the fact of low proportion, means that HCV prevention measures must be improved.

Sponsors: Brazilian Ministry of Health, Pan-American Health Organization

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The European Centre for Disease Prevention and Control strengthening MedISys as a tool to accelerate detection of threats to human health from communicable diseases in the EU

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**Background:** MedISys is an automated, web-based news aggregator developed at the European Commission Joint Research Center (EC-JRC) that uses weighted keywords and keywords combinations to collect articles of public health interest in 42 languages. The European Center for Disease Prevention and Control (ECDC) is mandated to detect and assess health threats from communicable diseases and to strengthen Epidemic Intelligence (EI) within the EU. In 2008, an internal analysis indicated that although MedISys was rarely used to detect threats, it issued alerts faster than human-mediated sources. Considering that real-time screening is crucial for early threat detection, ECDC is exploiting Medisys potential as early detection tool.

**Methods:** ECDC performed a descriptive study of MedISys, identifying search strategies, sources, web interface and user capacity as targets for improvement. To reduce the detection of irrelevant articles, alternative English search strategies for 15 diseases were tested against existing alerts. Precision was measured using Positive Predictive Value (PPV), defined as the probability of detected article to have public health relevance, where relevance was determined by EI experts (human filter) who evaluated 13,679 English articles scanned within 24 hours. EU national public health experts supported sources and interface revision.

**Results:** Alternative search strategies successfully reduced false positives without reducing sensitivity (PPV from 15% to 30%). Best refinement of search strategies requires using fewer keywords (diseases basic definitions), adjusting their weights with an extensive use of negative values and using epidemiological terms in the keywords combinations. New alerts have been set up for English and translation to other EU languages is ongoing with the support of public health experts. Expansion of the sources and restructuring of the interface are underway based on EU Member States feedback.

**Conclusion:** Results indicate that optimization of search strategies and revision of sources with the support of national public health experts can help MedISys reaching its potential. Plans for 2010 include extension of the analysis to all diseases and languages and capacity building within the EU to encourage knowledge and use of the revised system. ECDC plans to create customized versions for EU Member States (country editions) and, on request, special versions for mass gathering surveillance.

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Changing trend of measles and rubella in Nepal: Is it time to introduce rubella immunization?

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**Background:** Measles and rubella are serious public health problems in many developing countries, though the incidence is significantly reduced in developed countries. Measles is under national immunization activity in Nepal while there is no such program for rubella. The lack of information on the burden of rubella is one of the obstacles for such initiatives.

**Methods:** The present study was conducted from January 2003 through December 2007 under national measles surveillance program. Blood specimens from measles like cases (based on case definition) during any outbreak throughout the country were collected, serum separated, transported to National Measles Referral Laboratory and tested for detection of anti-measles IgM by ELISA technique. Measles negative specimens were further tested for anti- rubella IgM to analyze the trend.

**Results:** Of the total cases, 43.65%(805/1844) and 42.95%(424/987) were found to be positive for measles and rubella respectively. Distribution of measles cases was observed as 82.7%(158/191), 72.6%(600/827), 6.4%(19/295), 2.8%(6/214) and 6.9%(22/317) in the year 2003, 2004, 2005, 2006 and 2007 respectively. In contrast, rubella cases were reported as 0%(0/33), 28.5%(57/200), 67.1%(169/252), 50.75%(105/207) and 31.5%(93/295) in the year 2003, 2004, 2005, 2006 and 2007 respectively. We found that the number of measles cases significantly decreased ( $P<0.001$ ) with the increasing rubella cases ( $P<0.0001$ ) till 2005 and rubella remained at high burden level in the following years as well. In the recent years, most of the clinical measles cases were turned out to be rubella by results of laboratory investigation.

**Conclusion:** The incidence of measles has been rapidly reduced to a minimal after the measles immunization campaign (started at late 2004) and the routine immunization activity; however the emerging and increasing trend of rubella infection is a serious concern in Nepal. Continuation of measles immunization with the introduction of rubella immunization (both routine and supplementary) into national immunization strategy is the key measure to address this emerging problem.

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**Type: Poster Presentation**

Influenza A activities in Shanghai, China during 2004 to 2009

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**Background:** Influenza has been a considerable public health problem in China as well as the global for it remains inadequately controlled. The knowledge about the epidemiological features is valuable for surveillance strategy, and molecular characteristics of viruses are useful for selection of variant strains.

**Methods:** The seasonal influenza was intensively monitored for the entire year in Shanghai area during 2004 to 2009. The clinical activities of the Outpatient Department of Minhang Centre Hospital were documented. The respiratory specimens from patients with influenza-like illness were collected and then identified by viral isolation and RT-PCR. The molecular characteristics of A/H1 and A/H3 viruses were also analyzed by sequencing and comparing the genetic diversity of HA segment respectively.

**Results:** Two separated high peaks of influenza appeared between February to March and between July to August, which were consistently correlated with the waves of ILI consultant proportion. Obviously succession of predominant influenza A viruses was observed while influenza B virus kept in a mild level: A/H3 predominated in 2004 and 2005, and then was subrogated by A/H1 in 2006. A/H3 increased again in 2007 till the winter of 2008, but A/H1 became dominated in the summer of 2008, and maintained dominating in 2009. All the isolates from both subtypes were similar to the strains of the same period in other countries and regions. Phylogenetic analysis of HA1 subunit revealed a linear trunk with succession of A/H3 isolates in different influenza seasons, and a multi-directional tree with cluster of A/H1 isolates in same influenza season.

**Conclusion:** The influenza activities in Shanghai revealed typical winter and summer seasonal feature. The predominant influenza A virus changed almost every season. The ever-changing spectrum of circulating influenza strains and their molecular characteristics support the need for seasonal surveillance of influenza viruses in Shanghai, for more precise information about the circulating strains may have implications for predicting the following season strains and selecting vaccine composition.

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Serological survey on influenza A (H1, H3, H5 and H9) antibodies in human populations in Shanghai, China in 2008 and 2009

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**Background:** Influenza A is a common respiratory disease in human, and A/H1 and A/H3 are currently co-circulating in the world. Meanwhile, influenza A/H5N1 virus has been spreading throughout South-East Asia and extended to Russia, Europe, Africa, the Indian subcontinent and the Middle East with associated human infections as well as influenza A/H9N2 virus has also caused repeated human infections in Asia since 1998. The periodic serological survey is useful to know the current seroprevalence of human A/H1 and A/H3 influenza antibodies in human population and to provide seroepidemiological information of avian H5, H9 influenza A virus infections in humans in Shanghai.

**Methods:** Yearly serological surveys were carried out in the occupational exposure personnel (OEP) and general population (GP) in Shanghai from 2008 to 2009. Human A/H1, A/H3 and avian A/H5, A/H9 influenza antibodies were detected using hemagglutination inhibition (HI) assays.

**Results:** The prevalences of human A/H1 influenza antibodies were 45.3% in 2008 and 64% in 2009 respectively while the prevalences of A/H3 influenza antibodies were 37.4% in 2008 and 40.7% in 2009 respectively. Obviously increasing of anti-H9 antibodies prevalence were observed: 36.3% in 2009, significant higher than 17.7% in 2008 in OEP; 3.3% in 2009, slightly higher than 2.6% in 2008 in GP. However, anti-H5 antibodies prevalence kept relatively stable: 4.0% in 2008 and 3.8% in 2009 in OEP as well as 0.3% in 2008 and 0.3 in 2009 in GP.

**Conclusion:** The seroprevalences of human H1 and H3 influenza antibodies were consistent with the activities of influenza virus in Shanghai recent years. There were potential human infections with influenza A/H5 and A/H9 in Shanghai, especially in the occupational exposure personnel, and meanwhile the risk of human influenza A/H9 virus infection was increased.

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A survey of lymphatic filariasis using ICT test in Attapeu Province, Lao PDR

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**Background:** Baseline observations suggest lymphatic filariasis (LF) is likely to be endemic in parts of Lao PDR. To date in Lao diagnosis has relied on the use of night blood film examination, which through its inconvenient testing time has limited the ability to conduct a regional LF prevalence survey. In Lao's southernmost province of Attapeu, two of the province's five districts have been identified as endemic (>1% prevalence) using night blood film examination. In this study we have conducted a regional survey of the remaining three districts in Attapeu province using immunochromatographic (ICT) filarial test kits. All work was performed in collaboration with the Centre for Malariology, Parasitology and Entomology, Ministry of Health, Lao PDR.

**Methods:** In August 2009 ICT filariasis tests were performed on 320 adults across the districts of Sanamxai, Samakkhixai and Sanxai in Attapeu province. Sample villages were evenly distributed across each district to ensure geographical spread. Minimum village sample size was 50 persons.

**Results:** Twenty-two ICT positive cases were identified; 3 in Sanxai (n=157 tested), 5 in Samakkhixai (n=112 tested) and 14 in Sanamxai (n=51 tested). The prevalence per district sample was 1.9% in Sanxai, 4.5% in Samakkhixai and 27.5% in Sanamxai. Night blood film examination was performed on 20 ICT positive cases; of these 2 showed microfilariae on thick blood film. There were no observed cases of elephantiasis or hydrocoele. Cases without contraindications were treated with DEC and/or Albdenazole.

**Conclusion:** This is the first ICT filariasis test survey performed in Lao PDR. Given that each district showed >1% prevalence, this study's findings support a mass drug administration programme to eliminate filariasis in Attapeu province. The greater convenience and efficacy of the ICT test compared with night blood film examination confers a significant prospect of further LF surveys in Lao.

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Evaluation of bacterial meningitis Sentinel Surveillance Program (BMSSP) introduced in 2007 in Sao Paulo state, Brazil

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**Background:** Lab confirmation of bacterial meningitis (BM) is cumbersome, because of the low sensitivity of lab tests and problems associated with storage of samples and laboratorial practices. The proportion of BM of unknown etiology is high, especially after use of antibiotics.

**Methods:** We describe implementation and results of a PAHO program (BMSSP) to get better surveillance of BM, based Sentinel Hospitals, that add multiplex PCR to traditional tests (culture and antigen detection). Since 2007, 13 sentinel hospitals (10 in São Paulo and 3 in Campinas city) have been selected to monitoring BM in São Paulo state. To evaluate the results of this program, we compared 5 selected criteria (table 1) in 2006 and 2008, pre and post implementation of BMSSP. Multiplex PCR is able to identify *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae* in both serum and CSF.

**Results:** The results, summarized in table 1, showed that BMSSP improve significantly the quality of diagnosis of BM, especially when caused by *N. meningitidis*, and showed that the serogroups prevalence is changing.

Table 1. Indicators selected to evaluate the results of BMSSP, introduced in São Paulo state in 2007 – comparing the frequency of indicators between 2006 and 2008.

INDICATORS	Year Test			
	2006 (%)	2008 (%)	Chi-square test	p
Bacterial meningitis lab + *	40.7	60.7	53.7	<0.01
Non-specified bacterial meningitis	39.1	19.5	57.5	<0,01
Culture + **	31.1	36.3	4.15	0.04
Culture positive for <i>N. meningitidis</i>	33.3	50.0	36.1	<0.01
Identification of serogroup ( <i>N. meningitidis</i> )	48.0	64.1	15.7	<0.01
<i>N. meningitidis</i> serogroup				
B	24.2	14.3		
C	68.5	75.0		
W135	5.0	8.5		
Y	0.4	1.2		
Others	1.9	1.0		

Notes: \* positive for one or more laboratorial test (culture, antigen detection or multiplex PCR); \*\* culture positive for *N. meningitidis*, *S. pneumoniae* or *H. influenzae*.

**Conclusion:** The PAHO BMSSP is an excellent tool for monitoring MB. The detection of *N. meningitidis* with identification of serogroups will give support for public health decisions involving future strategies of immunization.

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The proportion of influenza infections from severe acute respiratory infection (SARI) cases in Indonesia 2008-2009

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**Background:** Severe acute respiratory infections (SARI) is an acute infections occur in respiratory systems especially in alveoli or interstitial tissue in human lung. The symptoms which lead to SARI are fever, cough, sore throat, difficulty of breathing and requiring hospitalization. Bacterial and viruses can be responsible for acute respiratory infections in human. Since Indonesia is a country with the highest number of fatal cases caused by Avian Influenza from July 2005 until recently, therefore Influenza was one of major concern in solving health problems in Indonesia.

**Methods:** National Institute of Health Research and Development, MOH of Indonesia has been conducting SARI surveillance and collected 549 samples of nasal and throat swabs from SARI patients during April 2008 until March 2009 from eight hospitals in eight provinces in Indonesia. The samples were analyzed for Influenza by using real-time RT-PCR. The RT-PCR test used several primers. These primers were specific for influenza A (A/H1N1, A/H3N2 and A/H5N1) and Influenza B. The sequence of these primers was provided by CDC, Atlanta.

**Results:** About 516 (94%) of the specimens testing results were not influenza A or B viruses. We found 33 (6%) of SARI cases caused by Influenza A and B viruses, which are 21 (4%) cases caused by influenza A and 12 (2%) caused by influenza B. From the influenza A cases we found one case of SARI was caused by A/H1N1, two cases were A/H5N1, 17 cases were A/H3N2 and one case was unidentified Influenza A.

**Conclusion:** The majority of SARI cases were not caused by influenza viruses. From this surveillance the most common influenza A related to severe acute respiratory infection in Indonesia is A/H3N2. Since the avian influenza virus A/H5N1 cases were still found in Indonesia and the spread of novel virus influenza A/H1N1 in 2009 have raised our concern about the importance of SARI surveillance.

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Ranking the burden of infectious diseases in Ontario, Canada

**S. Ratnasingham**<sup>1</sup>, J. Kwong<sup>1</sup>, N. Daneman<sup>2</sup>, M. Campitelli<sup>1</sup>, N. Crowcroft<sup>3</sup>

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**Background:** Evidence-based priority-setting requires knowledge about the relative contributions of diseases to the loss of healthy life. The Global Burden of Disease method uses the Disability-Adjusted Life Year (DALY), a measure that incorporates the population burden of mortality and morbidity, to compare the relative contributions of diseases. We calculated DALYs for infectious pathogens in Ontario to inform the policies of the province's new public health agency.

**Methods:** The DALY is a health gap measure comprising two components: 1) years of life lost due to premature mortality (YLL); and 2) years of "healthy" life lost due to disability (YLD). Health gap measures quantify the amount of "healthy" life lost by estimating the difference between actual population health and a specified ideal. We used an incidence-based approach and incorporated modeling techniques where possible to estimate the disease burden of various infectious disease agents. Data sources included census estimates, vital statistics, public health reportable disease data, health care utilization data, cancer registries, and epidemiological studies.

**Results:** For most infectious diseases, mortality (YLLs) contributed more to DALYs than morbidity (YLDs). Overall, the infectious agents accounting for the greatest burden were: influenza, *Streptococcus pneumoniae*, hepatitis C virus (HCV), *Escherichia coli*, Gram negative bacteria excluding *E. coli*, HIV/AIDS, *Staphylococcus aureus*, human papilloma virus (HPV), hepatitis B virus (HBV), and other Gram positive bacteria. The DALYs for HPV and urinary tract infections were greater in females while the DALYs for HIV/AIDS, HCV, and HBV were greater in males.

**Conclusion:** Strategic priorities for specific infectious diseases in Ontario should focus on those identified here to have the greatest burden. Further work is also required to improve the timeliness of data access and the quality of information available. While the prevention of some infectious diseases will require the development of novel interventions, much of Ontario's infectious disease burden could be reduced through better implementation of existing interventions.

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Burden of new swine-origin H1N1 influenza virus in a subtropical region of Mexico

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**Background:** The current influenza pandemic caused by the novel H1N1 virus was first reported in Mexico City on April 18, by the end of May the number of confirmed H1N1 cases was of 5, 563 nationwide. Through strict self protection measures and the closure of all school and non essential activities, the epidemic was controlled and the number of infected people at least in central and North Mexico diminished down to undetectable cases in some Federal Entities.

Surprisingly, the epidemic of influenza virus H1N1 had a geographical shift and by mid June the number of suspected and confirmed H1N1 cases exponentially increase in those federal entities of the South, mainly Yucatan and Chiapas. The burden and impact of influenza virus in tropical and subtropical regions has been poorly analyzed and recent reports have underline the importance of influenza virus circulation in tropical regions associated to persistence of the virus in temperate countries or reintroduction of influenza from the tropics every year.

**Methods:** Using retrospective influenza surveillance data from Yucatan and the epidemiology of the actual influenza H1N1 and concurrent meteorological information we described in here the burden of the novel swine H1N1 human influenza virus in a subtropical region of Mexico.

**Results:** Restrospective analysis of influenza surveillance in Yucatan, Mexico has showed us a seasonal trend on influenza circulation typical of tropical countries. In general, it occurs twice a year, with the more intense activity during the rainy season. The novel H1N1 virus was first detected in Yucatan on April 21st, however despite the national contingence, the actual outbreak in Yucatan occurred until the end of May when the numbers of infected people increase to such level that Chiapas and Yucatan were mostly the only two Mexican states reporting cases of infection with the novel H1N1v.

**Conclusion:** Now, at 6 months after the first cases were reported, Yucatan still is the third Mexican state reporting cases of infection with the novel H1N1v. It would be interesting to assess the contribution of sustained transmission of the virus in this tropical region of Mexico.

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Measles, neonatal tetanus (NNT), pertussis, cerebro-spinal meningitis (CSM) and polio trends in Nigeria, 1994 to 2007

**M. O. Akhimien**

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**Background:** There has been concerted effort by the Nigerian public health system both at the National and State levels to tackle the problem of childhood vaccine-preventable diseases. This study was done to monitor the trend of these vaccine-preventable diseases and to assess the effects of the various interventions on these disease burden.

**Methods:** Data on some childhood vaccine-preventable diseases from 1994 to 2007 were obtained from the Federal Ministry of Health, Nigeria. These data were reviewed, summarized and graphically analyzed.

**Results:** Measles cases were highest (217,151) in 1999 and dropped steadily to 12,925 in 2007. CSM cases recorded their highest number (17,028) in 2003 and their lowest (3,547) in 2007. Pertussis cases dropped steadily from 50,175 in 1999 to 17,141 in 2006. NNT and polio cases maintained low values with peak values of 2,175 NNT cases in 2005 and 2,860 Polio cases in 2002. The case fatality rate (CFR) for NNT increased steadily from 3.5% in 1999 to 19.1% in 2007; CSM dropped from 18.8% in 1995 to 2.5% in 1997 and spiked again to 13.3% in 2003. Measles CFR dropped from 15.5% in 1997 to 1.8% in 2007. Pertussis had an annual steady CFR of 0.7% while Polio had a steady drop from 0.7% in 2001 to 0% in 2007.

**Conclusion:** The steady increase in the CFR of NNT, though it has low annual cases, calls for public health attention. Efforts should be intensified towards CSM control. The public health interventions for measles, polio and pertussis seem to be effective.

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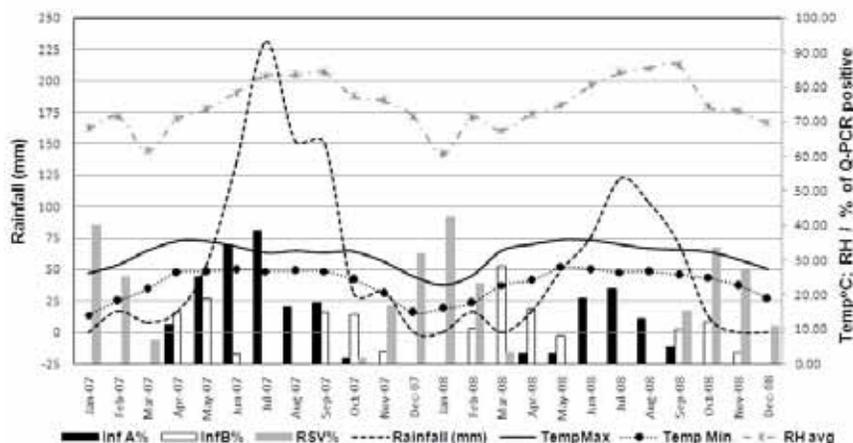
A two year surveillance for influenza and RSV using Real-time PCR among children with acute respiratory infections in Kolkata reveals distinct seasonality of infection

A. S. Agrawal, S. Chakrabarti, **M. Chawla-Sarkar**  
National Institute of Cholera and Enteric Diseases, Kolkata, India

**Background:** Respiratory tract infections are one of the most common cause of morbidity and mortality in young children all over the world. Influenza and Respiratory Syncytial viruses (RSV) are the predominant etiology during seasonal epidemics and thus rapid and sensitive molecular tests for screening & timely identification of epidemics are required. In this study, we report comparison of real time PCR (Q-PCR) with conventional RT-PCR for parallel identification of Influenza A or -B and RSV.

**Methods:** Nasal and throat swabs were collected from 1091 children <5 years of age with fever and 2 or more symptoms of ARTI (cold/cough, sore throat, myalgia, bodyache) from the B. C. Roy Memorial Hospital for children, Kolkata, India for 2 years (Jan 2007-Dec 2008). RNA was extracted from the clinical samples using RNeasy Mini Kit (Qiagen). Two one step multiplex Q-PCR assays were standardized. First assay was for matrix (M) genes of Inf-A and Inf-B {S. Lindstrom, CDC, USA}. Second assay was specific to the polymerase gene of RSV and the RNase P.

**Results:** Of 1091 samples, 275 (25.21%) were positive for either Influenza or RSV by Q-PCR compared to 262 (24%) positives by RT-PCR. Overall Inf-A, -B and RSV were detected in a total of 121 (11.075%), 59 (5.38%) and 95 (8.68%) samples respectively. Age distribution of virus infection in infants (< 1 year), toddlers (1-2 years) or pre-school going children (2-5 years), the positive samples was analyzed. We observed increased prevalence of RSV in 0-1.0 (13.9%) and 1.0-2.0 years (12.1%) age group compared to only 3.8% positivity among 2-5 year old children. Influenza (A+B) was predominant in 1.0-2.0 and 2.0-5.0 yearr (19.6% and 17.2%) age group in Kolkata. In spite of overlapping clinical symptoms, RSV and Influenza showed distinct seasonal peaks. Inf-A positively and RSV, negatively correlated with rainfall and temperature (Fig1). No distinct seasonality was observed in Inf-B infections.



**Fig. 1 Correlation of meteorological variations with prevalence of Influenza-A, -B and RSV during 2007-08 in Kolkata.**

**Conclusion:** This is the first report of a systemic surveillance of respiratory viruses with seasonal correlation and prevalence rate from Eastern India. The two year study confirmed feasibility of using Q-PCR in developing countries, which will not only improve scope for prevention of epidemics but also provide crucial epidemiological data from the tropical regions.

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Sampling considerations for Flavivirus arthropod vectors in Western Canada – Making sense at a data collection level

**R. Waeckerlin**, S. Cork  
University of Calgary, Calgary, AB, Canada

**Background:** West Nile virus has displayed a remarkable geographical expansion in North America during the last ten years, with peaking numbers of diagnosed cases in 2003 and 2007, entailing numerous arthropod and vertebrate host monitoring programs throughout Canada. *Culex tarsalis* has been identified as the main vector for West Nile Virus transmission in Western Canada, however, other arthropod species are also discussed as potential vectors.

**Methods:** The presented work outlines factors that influence the spread of West Nile virus and epidemiological and ecological considerations to be taken into account to ensure appropriate data collection for the assessment of West Nile arthropod vector dynamics in the province of Alberta. The main factors that result in a change in vector composition and abundance are likely to be climate and habitat changes. The dynamics of successful viral transmission are subject to the suitable arthropod vector and vertebrate host communities, introduction of previously non-indigenous insect species, changes in mosquito habitats, genetic adaptations of the virus, and changes in insect biting behavior.

**Results:** Considering previously collected data in Canada, we propose weekly CO2 trap sampling for a minimum period from June to August in five of the six distinct geographic regions of Alberta (Boreal Forest, Canadian Shield, Foothills, Parkland and Grassland). The boundaries between Grassland and Parkland as well as Parkland and Boreal Forest are to be especially emphasized in the sampling. Vector speciation in conjunction with assessment of biting behavior of individual arthropod species on a molecular level is necessary for examination of variations in vector and vertebrate host interaction between these regions. Screening, characterization and phylogenetic analysis of variable regions (NS5 protein gene) of mosquito borne Flaviviruses from insects collected allows implementation of molecular epidemiologic relationships with preexisting data and assessment of possible routes of virus spread in relation to vector and host ecology and distribution.

**Conclusion:** Taking into account the complex ecology of West Nile virus spread, the importance of appropriate sampling is evident. The outlined experimental design covers virological, epidemiological ecological and entomological aspects in the context of the unique landscape of Alberta.

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Crossborder infectious disease surveillance in the Netherlands, Germany and Belgium

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<sup>1</sup>inFact UK, Bradford, United Kingdom, <sup>2</sup>GGD Zuid Limburg, Heerlen, Netherlands

**Background:** The Euregion Meuse-Rhine (EMR) covers an area of 10,478 square kilometres with 3.9 million people and includes the Provinces of Limburg-South (Netherlands), Limburg (Belgium) and Liege (Belgium), Region Aachen (Germany) and the German-speaking community in Belgium. The heavy daily traffic of goods, services and people across the borders poses a serious threat in respect of infectious disease control. The recent pandemic influenza experience has re-emphasised the urgent need for improved collaboration and integration of surveillance and response systems especially in crossborder countries where political systems, medical infrastructures, economies, and cultures are varied. Challenges include the handling of differences in legislation for sharing data and resources, surveillance algorithms, communication with the public and response protocols.

**Methods:** This paper discusses an EU funded project by the Euregion Meuse-Rhine (EMR) to enhance crossborder cooperation on infectious diseases surveillance and response. The project included the customization and subsequent implementation of two secure web-based decision support applications for the management of pandemic flu (FluZone) and general infectious disease control (HPZone). FluZone and HPZone were developed and successfully deployed nationally in England.

**Results:** The implementation of FluZone and HPZone in the EMR has yielded the following outcomes:

- Formalised approach to synthesising and exchanging experiences on pandemic flu preparedness and securing a consolidated plan allowing local nuances and including:
  - Preparing for an emergency
  - Surveillance, case investigation and treatment
  - Preventing spread of disease
- Real-time surveillance dashboard on sources, cases, contacts, enquiries and outbreaks
- Consolidation of continuously changing case definitions, protocols and triage methods.
- Rapid exchange of epidemiology and laboratory data
- Geographical mapping of cases, contacts, and contextual locations such as schools and care homes.
- Secure communications between health protection professionals and the crossborder community.

**Conclusion:** Cross-border collaboration on infectious disease surveillance is complex but essential. Using a common, realtime and secure web-based system has significantly streamlined the management of cases and outbreaks, facilitated realtime crossborder surveillance and improved communication and cooperation.

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Realtime intelligence for infectious disease prevention and control

**C. Kara-Zaïtri**, R. Hamilton  
inFact UK, Bradford, United Kingdom

**Background:** Infectious disease surveillance is complex, multi-disciplinary and high profile. The emergence of new diseases and their speed of onset, coupled with current bio-terrorism threats have re-emphasised the urgent need for novel methods for harvesting and acting upon appropriate and timely surveillance data using intelligent information systems based on enhanced communication technologies.

**Methods:** The methodology developed is based on two secure web-based applications - HPZone and HP-Intelligence.

HPZone is a decision support tool for infectious disease control at the local Health protection Unit level or Zone and has been deployed nationally in England and six regions in the Netherlands. HPZone captures a rich data set to support all the key business processes from the handling of routine Enquiries, through Case management and Contact tracing, right up to the management of Outbreaks and Incidents.

HP-Intelligence is a data mining application which harvests surveillance data from each of the HP-Zones and persistently analyses all entries to provide alerts given a protocol of rules. Data mining functions include the detection of coincidences of events (time, place and scenario), associations (infection versus seasonal parameters), sequential patterns (behavioural trends), and ad-hoc queries. Rules can be entered on an individual zone, region, national or even international levels with individual users being able to subscribe to specific alerts.

**Results:** HP-Intelligence yielded positive results including the discovery of relationships, which were not obvious. Immediate outcomes include:

- Coincidence, incidence and threshold alerts at National, Regional and Locals levels.
- Rich data sets for scientific evaluation to forecast projections and prioritise options for control strategies.
- Timely assessment of the descriptive epidemiology of a major public health incident including progress and outcome.
- Capture of key contextual data including environmental information such as contact with animals, travel history and possible transmission mode.
- National Incident Board for viewing aggregate summary Situation, Case, Contact and laboratory data nationally using various means including GIS.

**Conclusion:** Immediate gains from HPZone and HP-Intelligence include an efficient early warning function for problems that are “bubbling away”, and the capture of individual, regional and national wisdom using easily sharable rules and protocols for enhanced intelligence in health protection.

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The implement of airport fever screening for dengue sentinel in Taiwan, 2003-2008

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**Background:** This study aimed to examine the epidemiological trends of dengue as well as the impacts of the imported cases and airport fever screening on community transmission in Taiwan, a dengue non-endemic island.

**Methods:** All the data of dengue cases obtained from the surveillance system in Taiwan CDC were analyzed to present the relationship among cases or population factors by Pearson correlations, linear regression and/or GIS-based mapping. The comparison of the impact on whether implementing airport fever screening or not was evaluated the Student's and the two-way ANOVA.

**Results:** Being a dengue non-endemic region, 44.6% (244/542; 99% CI: 33.1-57.8%) imported dengue cases with fever symptomatic was at airport rapid screened by non-contact infrared thermometer and subsequently confirmed by laboratory. A positive association of the imported dengue cases screened by airport fever screening with the total imported dengue cases was found i.e. number of total imported cases =  $3.459 + 0.418 \times$  number of imported cases detected by fever screening ( $n=5$ ,  $R=0.890$ ,  $R^2=0.792$ ) during 2003-2007. Moreover, by viewing of geographic heterogeneity, the southern Taiwan, supposed to be as the competent region for dengue endemics with tropic climate and existence of *Aedes aegypti*, where the imported dengue cases was positively associated with indigenous cases in 2003-2007 with the implementing airport fever screening i.e. the number of indigenous dengue cases =  $53.398 \times$  the number of imported dengue cases - 169.283 ( $n=25$ ,  $R=0.778$ ,  $R^2=0.605$ ) whereas it was lesser association ( $n=25$ ,  $R=0.312$ ,  $R^2=0.017$ ) during 1998-2002 without this intervention in Southern Taiwan. On the other hand, in Northern (Subtropical) Taiwan, less association between the number of imported cases and the number of indigenous cases was shown.

**Conclusion:** It implicates first time that upon conducting of this border screening by airport fever screening followed by laboratory's confirmation, the numbers of imported dengue cases could provide for early alert and sentinel of dengue to predict the the numbers of indigenous dengue cases in non-endemic but dengue competent (risk) region and therefore may help in targeting the controlling of local dengue transmission, while those undetected cases due to latent or asymptomatic infection would be the source of new dengue outbreaks each year.

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Comparison of three time series models for predicting campylobacteriosis risk in Georgia, Minnesota and Oregon

**J. Weisent**

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**Background:** Three time series models (regression, decomposition, and Box-Jenkins Autoregressive Integrated Moving Averages) were applied to national surveillance data for campylobacteriosis with the goal of disease forecasting in three U.S. states.

**Methods:** Data sets spanned 1998 to 2007 for Minnesota and Oregon, 1999 to 2007 for Georgia. Year 2008 was used to validate model results. Mean absolute percent error, mean square error and coefficient of determination (R<sup>2</sup>) were the evaluation fit statistics.

**Results:** Results showed that decomposition best captured the temporal patterns in disease risk. Training dataset R<sup>2</sup> values were 72.2, 76.3 and 89.9% and validation year R<sup>2</sup> values were 66.2, 52.6 and 79.9% respectively for Georgia, Oregon and Minnesota. All three techniques could be utilized to predict monthly risk of infection for *Campylobacter* sp. However, the decomposition model provided the fastest, most accurate, user-friendly method.

**Conclusion:** Use of this model can assist public health personnel in predicting epidemics and developing disease intervention strategies.

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Anticipating the species jump: Surveillance for emerging viral threats

**M. Flanagan**

Penn State University, State College, PA, USA

Emerging infectious diseases (EID) may pose new international security threats because of their potential to inflict harm upon humans, crops, livestock, health infrastructure, and economies. Some viruses pose unique challenges because of their high mutation rates, which enable them to evade host immunity, persist in the environment, and infect new host species. For example, influenza and human immunodeficiency viruses originally infected animals, but subsequent mutations enabled these viruses to “jump” to new human hosts. Current disease surveillance efforts rely upon data from clinical specimens – that is, from individuals who are already sick. As a result, little is known about the abundance of viruses that exist in nature but do not (yet) cause human disease. Human populations could be preemptively protected if researchers can learn what events lead to viral species jumping. If researchers can discover common genetic and ecological determinants that precede species jumping, then these determinants may be used to detect viruses teetering on the threshold of human pathogenicity. Knowledge of such determinants may ultimately prove useful to threat analysts and others charged with anticipating the genetically engineered viruses of the future, be they natural or man-made. This paper will report the findings of a thought leaders workshop convened to debate the feasibility of identifying determinants and hypothesize what information and technologies might be needed to make such surveillance possible.

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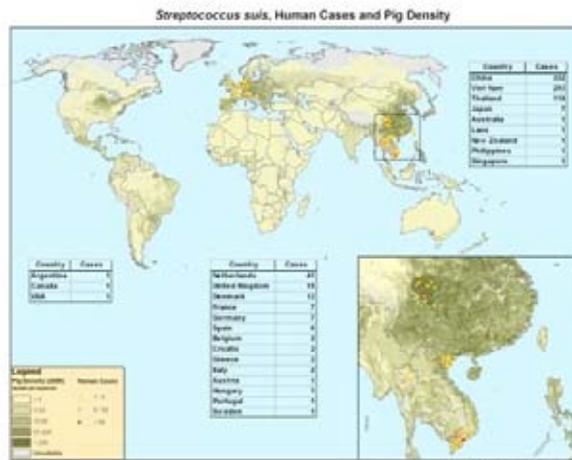
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The making of a world atlas of infectious diseases  
H. F. Wertheim<sup>1</sup>, P. Horby<sup>1</sup>, T. L. Viet<sup>1</sup>, T. N. T. Tanh<sup>1</sup>, **J. WOODALL**<sup>2</sup>  
<sup>1</sup>Oxford University, Hanoi, Viet Nam, <sup>2</sup>ProMED-mail, Brookline, MA, USA

**Background:** Many infectious diseases are restricted in their geographical range due to climatic conditions, presence of vectors or specific reservoir hosts, local food customs, hygiene, antibiotic use, among others. Currently there is no comprehensive illustrated overview of the geographic spread of infectious diseases and their determinants. Such a compendium of world infectious disease maps is crucial for teaching, research and creating awareness of risk.

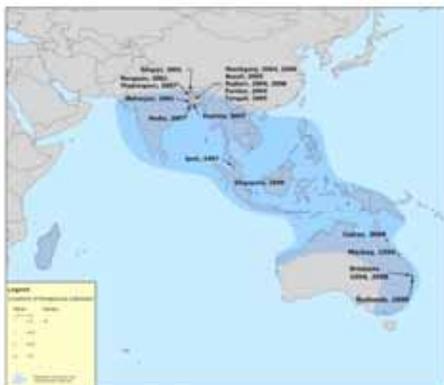
**Methods:** With the collaboration of experts from around the globe we are collecting and making updated maps of more than 100 infectious diseases and their preferred conditions. We do extensive data searches to arrive at the best geospatial data available. Each map will be accompanied by an explanatory text. All our maps are sent out for independent peer review to ensure their quality and accuracy.

**Results:** The atlas will be published by Wiley-Blackwell, and at a later stage the maps will be updated and made available online under open access.



### **Streptococcus suis, Human Cases and Pig Density**

**Conclusion:** The printed atlas will be followed up with a web-portal for global infectious disease mapping activities, where all the data will be bundled, visualised and made available to anyone interested, and updated regularly.



### **Henipaviruses and distribution of flying fox bat (Pteropus)**

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Pertussis in Latin America: Developing the tools to face the challenge ahead  
**F. Coronado**<sup>1</sup>, M. Griffith<sup>1</sup>, M. L. Tondella<sup>1</sup>, M. Bonkosky<sup>1</sup>, M. Landaverde<sup>2</sup>, T. Clark<sup>1</sup>  
<sup>1</sup>Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA, <sup>2</sup>Pan American Health Organization, Washington, DC, USA

**Background:** Pertussis surveillance worldwide is limited by reduced clinical recognition of disease, suboptimal confirmatory testing, and poor reporting, yet adequate understanding of pertussis is necessary for implementation and evaluation of prevention and control measures. We describe the current status of pertussis and vaccination coverage in Latin America (LA) and a process to achieve enhanced pertussis surveillance.

**Methods:** We reviewed publicly available pertussis surveillance and vaccination coverage data for LA and calculated incidence rates by region and country. Field evaluations of surveillance capacity are being conducted in selected LA countries, followed by development and implementation of locally-responsive capacity building at selected sentinel sites to strengthen recognition, confirmation and reporting of pertussis.

**Results:** From 1990 to 2008, >160,000 cases of pertussis were reported in LA. Cases decreased from 25,409 in 1990 to 7,827 in 2007, with the lowest number (3,595) in 2002. The rate also decreased from 1990 (5.8 cases/100,000 pop.) to 2002 (0.68 cases/100,000 pop.), but then increased to 1.4 cases/100,000 pop. in 2007. Reported three-dose childhood diphtheria-pertussis-tetanus vaccine coverage has steadily increased from 39% in 1980 to 91% in 2008. Collaborative efforts among LA Ministries of Health and international stakeholders are now focusing on evaluating current surveillance systems for pertussis, with implementation of capacity building and enhanced surveillance activities beginning 3rd quarter 2010.

**Conclusion:** Although vaccination efforts in LA have reduced pertussis morbidity, trends indicate that incidence may be increasing again. Efforts to prevent pertussis have focused on childhood vaccination; however, burden of disease does not strictly correlate with vaccine coverage. Additionally, limitations of reported data imply that the true burden of pertussis remains uncertain. Enhanced sentinel site pertussis surveillance, endorsed by the WHO's Strategic Advisory Group of Experts on immunization, may prove an efficient method to improve pertussis surveillance and support evolving vaccination policy.

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Establishing the tuberculosis monitoring system at Brazil's, Paraguay's and Argentina's frontier  
**M. Iise**<sup>1</sup>, J. R. P. D. Santos<sup>1</sup>, R. G. Abreu<sup>1</sup>, P. Colombana<sup>2</sup>, C. Martinez<sup>3</sup>, D. Barreira<sup>1</sup>  
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**Background:** Brazil shares frontiers with 10 South American countries. From 2001 to 2009, in Brazil, it was registered and treated 225 TB cases among non residents. There is no surveillance system among South American countries to detect TB cases and its contacts. Our objective was describe the proposals and activities (2008-2009) whose main goal was establishing a "Surveillance System of TB Cases at the frontier among Brazil, Paraguay and Argentina"

**Methods:** There were organized eighteen meetings with the presence of TB sector decision makers from the three countries. There was a meeting with the coordinators of the National TB Programs and the Brazilian Representation of the Pan-American Health Organization. There were two technical training courses: one about the DOT Strategy and another one about the epidemiology at the Bi-national Hydroelectric in Foz do Iguacu (Brazil).

**Results:** At the end, a total of 139 professionals were involved in the creation of the Surveillance System, and 77 technicians from different management levels of the three countries were trained. The coordinators meeting established responsibilities and identified the necessity of creating a bilingual TB notification report, a bi-national information, standards for laboratorial diagnosis, forms of identifying circulating strains, standards for the treatment at the country of origin. During the DOT Strategy course, it was introduced a methodology, activities and a proposal to standardize country actions. At the applied epidemiology course, it was discussed fundamental concepts of epidemiology surveillance and integration among countries. The municipal technicians involved have informed and have referred patients in treatment at the frontiers municipalities. It is in course the creation of a bi-national card for the identification of Tb cases and the establishing of primary care common protocols.

**Conclusion:** The Surveillance System will allow the diagnostic of the TB magnitude in the regions of frontiers, identifying the non residents' cases, widening the treatment offer at the diagnostic places and at the countries of origin, widening the contact investigation, raising the directly observed treatment rate and decreasing the TB default. Those actions will strengthen the TB surveillance system in strategic areas contributing to the better understanding of the migration flows for TB treatment in neighbor countries.

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**Type: Poster Presentation**

Trends in pediatric bacterial meningitis in Sudan 2005-2006

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**Background:** Asurveillance of epidemiological pediatric bacterial meningitis was carried out between January 2005 and January 2006 in Sudan, in National Reference Laboratory

**Methods:** 932 CSF samples were collected from children suspected having bacterial meningitis, gram stain, culture, biochemical tests and serotyping was done,

**Results:** with the following results: Bacterial meningitis cases numbered (46 boys, 32 girls), from a total of 932 children suspected having bacterial meningitis. The age distribution for the infections was the highest under 2 years of age, decreasing with increasing age. *N. meningitidis* was the most common pathogen, followed by *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus* (one isolate). The relationship between causative pathogens and age distribution was as follows: *staph aureus* was isolated from child of 6 months of age and *H. influenzae* and *S. pneumoniae* were major pathogens less than 5 years of age, where meningococci were from more than 5 years of age. Susceptibility tests demonstrated that . 60% of *H. influenzae* isolates and . 58% of *S. pneumoniae* isolates were drug-resistant.

**Conclusion:** Ceftriaxone is effective against meningococci, where chloramphenicol antibiotics are currently less effective. A combination of ampicillin and cephem antibiotics is used first line at many facilities for patients below 4 months of age. A combination of carbapenem which showed effective against PRSP and cephem which showed effective against *H. influenzae* is the first choice in childhood bacterial meningitis for patients above 4 months of age.

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Elimination of neglected diseases in Latin America and the Caribbean: A mapping of selected diseases

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**Background:** In Latin America and the Caribbean, around 195 million people live in poverty, a situation that increases the burden of some infectious diseases. Neglected diseases, in particular, are often restricted to poor, marginalized sections of the population. Most cause chronic conditions, which in turn reduce learning capabilities, productivity and income earning capacity. Cost-effective tools exist to combat these diseases, making it imperative to work towards their elimination. In 2009, the Pan American Health Organization received a mandate to support the countries in the Region in eliminating neglected diseases and other poverty-related infections.

**Methods:** This study analyzed and determined which countries in the Region exhibit evidence of selected poverty-related diseases, determine areas where particular diseases coexist, and detect "hotspots" where countries can implement interprogrammatic and intersectoral approaches. Five diseases with available information were analyzed using geo-processing techniques at the first subnational level (states), identifying the presence and overlap of these diseases.

**Results:** In the Region, there are approximately 570 states. Lymphatic filariasis is present in four countries (29 states), onchocerciasis in six countries (25 states), schistosomiasis in four countries (39 states), trachoma in three countries (29 states), and human rabies transmitted by dogs in ten countries (20 states). The information indicates that 102 of the 570 states have one or more of the selected neglected diseases, thus they may be considered "hotspots" in the Region. 33 states present two of the diseases, 2 states present three of the diseases, and only one state has evidence of four of the selected neglected diseases, classifying these areas as the major "hotspots."

**Conclusion:** The analysis suggests a majority of the selected diseases are not widespread and can be considered part of an unfinished agenda with the elimination of neglected diseases as its goal. Integrated plans and a comprehensive approach, ensuring access to existing diagnostic and treatment tools, and establishing a multi-sectoral agenda that addresses social determinants of disease, are required. More specific baselines are needed for most of the diseases included in the study and criteria to measure the presence of some diseases need to be reviewed. Future studies can include additional diseases, socio-economic and environmental variables

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warning System in Gaza Strip, post conflict 2009

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**Background:** After the Gaza war ceasefire, WHO indicated outbreaks as one of the highest public health risks in the post-conflict phase. UNRWA, the United Nations Relief and Works Agency for Palestine Refugees in collaboration with WHO set up an early warning (EWAR) system for epidemic prone infectious diseases.

**Methods:** After four and half months of activity, the system was evaluated. The EWAR relies on weekly notification of 9 syndromes from 17 PHC facilities, data is analysed in Gaza City, used to trigger outbreak investigations and transmitted as a bulletin to health stakeholders.

**Results:** Between 01 January to 16 April 2009, a total of 11 alerts were flagged, two cases of meningococcal meningitis identified and alarm thresholds breached for hepatitis and watery diarrhoea. No outbreaks were confirmed. The UNRWA EWAR is a simple system built on the Agency's existing surveillance network, did not require additional investment or massive training for implementation. The first output from the system was available less than one month after cessation of hostilities. The system includes all routine surveillance data, but due to the lack of outbreaks, it was not possible to measure the sensitivity (SE). The positive-predictive-value for outbreaks of seasonal diseases was low. Over the past years, UNRWA recorded a reporting incidence increase of seasonal diseases due to over-reporting. Thresholds using historical data were set with alarm thresholds at 3 standard deviations but they still proved to be too sensitive. Acceptability and timeliness were very high, 100% of reporting units submitted every week. It was not possible to evaluate flexibility in adapting to changes in epidemiology, however adaptation of thresholds to data quality showed flexibility in methodology. Representativeness was over 80% for the refugee community who account for over 70% of the entire Gaza Strip population.

**Conclusion:** The UNRWA EWAR system is still the only source of epidemiological information in Gaza Strip and was used regularly by WHO to monitor outbreaks in the area. It was a very useful instrument in the wake of a humanitarian crisis for monitoring the health of the affected population and proved feasible to implement even during a complex emergency.

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**Type: Poster Presentation**

Prevalent infections post hurricanes Hanna & Ike

**S. Font**

Hospital Mariano y Luciano De La Vega, Moreno, Buenos Aires, Argentina

**Background:** Commune of Gonaives, Haiti 2008

**Methods:** Surveillance of infectious pathologies at the arrival of the Voluntary physicians response team.

<b>Results:</b> Age:	Days 1st to 22nd	
All ages		
Sex:		
Both		
Pathologies	Cases	%
Acute respiratory infection	2087	15,6%
Case of Paludism	661	4,9%
Case of Typhoid	342	2,6%
Case of Dengue	0	0,0%
Acute hemorrhagic fever syndrome	10	0,1%
Acute infectious icteric syndrome	4	0,0%
Fever of undetermined origin	314	2,3%
Case of German measles (Rubella)	35	0,3%
Infectious watery acute diarrhea	1338	10,0%
Vaginal infections	1791	13,4%
Urinary tract infections	1156	8,6%
Infectious bloody acute diarrhea	0	0,0%
Acute malnutrition	9	0,1%
Tetanus	2	0,0%
Trauma - infected grazes	1453	10,9%
Infected burns	7	0,1%
Infectious cutaneous dermatitis	1120	8,4%
Arterial hypertension	438	3,3%
Intestinal parasites	432	3,2%
Others	0	0,0%
Other unspecified infections	2192	16,4%
<b>TOTAL</b>	<b>13391</b>	<b>100,0%</b>

**Conclusion:** See image

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Sentinel surveillance of pandemic influenza A H1N1 in Kenya in the period August – November 2009

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USAMRU-K, Nairobi, Kenya

**Background:** In April 2009 a new variant of Influenza virus that was later declared a pandemic strain by the World Health Organization appeared on the American continent. The first case of the pandemic Influenza A H1N1 (pH1N1) in Kenya was detected on 29th June 2009. The first sentinel surveillance case appeared in August 2009 and since then increasing numbers of pH1N1 have been detected within the regular sentinel surveillance network.

**Methods:** Eight public hospitals distributed across and representing the entire country comprise our sentinel surveillance sites. Nasopharyngeal swab specimens are collected within 72 hours of symptom onset from consenting patients presenting with influenza like illness. Specimens are transported in liquid nitrogen to the National Influenza Center. For all patient specimens, RNA is extracted followed by reverse transcription and real time PCR assays. The specific assays include screening for seasonal influenza A and B. All samples positive for Influenza A are further subtyped for Seasonal H1, Seasonal H3 and pandemic H1.

**Results:** In the four months from August to November, a total of 1329 samples were screened from the sentinel surveillance network. Out of these 160 samples representing 12% were positive for pH1N1. Of these, 83(51.8%) were from males and 120 (75%) of the patients were aged 5 years and below. The ages of the positive cases ranged from 9 months to 53 years. No deaths were recorded in the country during that period. 55% of the cases were found in the Western region, 18% in the central region and 27% in the Eastern/Coastal region. The epicenter of the outbreak was in the Western region and this is reflected in the higher percentages of cases from that region. The Eastern/Coastal region receives many tourists from outside Africa and this also is reflected in the high percentages of cases reported here.

**Conclusion:** These results indicate that within a few months of introduction into Kenya, the pH1N1 quickly spread throughout the country. Most of the cases were seen among children less than five years of age. The results show that gender was not a risk factor in contracting the virus in Kenya.

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**Type: Poster Presentation**

Cholerae in the Americas region: An eradicated problem?

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**Background:** The Pan American Health Organization (PAHO) proposed, in 1996, monitoring of resistance to antibiotics in the Americas Region. Thus organizing the *Surveillance and Monitoring Network of Antimicrobial Resistances* countries committed to compiling information on the identification of isolated species and their sensitivity to antibiotics.

The protocols of surveillance used within the Network are consistent with the recommendations of WHO for diarrheal diseases and respiratory tract infections. As pathogen responsible for severe disease and serious impact on public health, *Vibrio cholerae* from human isolates has been included among the surveyed pathogens. This study aims at describing the capacity of the Network for detecting *V. cholerae* outbreaks, by geographic distribution and the trends of resistance to antibiotics.

**Methods:** Information about the serogroups and antimicrobial susceptibility patterns of *V. cholerae* isolates were annually sent to the *Network* by the countries. Period of study: 2000-2007. *V. cholerae* were identified by standard laboratory methods. Serotype characterization was performed by serologic test (slide agglutination technique with polyvalent antisera). Susceptibility to antimicrobial agents was determined by the Kirby-Bauer disk diffusion method and interpreted according to the recommendations by the Clinical Laboratory Standards Institute.

**Results:** The total number of *V. cholerae* human isolates notified during the study period was 2095. *V. cholerae* isolates were only reported from Brazil, Cuba, El Salvador, Mexico and Peru. *V. cholerae* serogroups O1 and non-O1 constituted 179 (8.5%) and 1561 (74.5%) isolates, respectively. During 2000, 2001 and 2002, 355 (17%) isolates were notified without serotype characterization. Since 2003 all the isolates were reported with the corresponding serotype characterization. For *V. cholerae* serogroup non-O1, the resistance to ciprofloxacin and clorafenicol has remained low throughout these years. Nevertheless, the resistance to tetracycline, co-trimoxazole and erythromycin were variable during the study period.

**Conclusion:** The number of isolates reported through the *Network* was different from those published by the annual summaries of cholera cases in WHO *Weekly Epidemiological Record*. These discrepancies reflect the limitations in surveillance and reporting systems.

Data demonstrate that cholera is present in the Americas Region with potential for outbreaks. Laboratory surveillance should be integrated in the national surveillance systems for accurate information on outbreaks and burden of disease.

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**Type: Poster Presentation**

Evidence for influenza A virus infection among patients with respiratory tract infections in Nigeria  
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**Background:** Influenza viruses cause a highly contagious acute respiratory disease that has resulted in epidemic and pandemic in human populations over the years. In February 2006, poultry outbreaks of highly pathogenic avian influenza A (H5N1) virus were confirmed in Nigeria. A survey was therefore set up to assess the role of influenza A virus in several respiratory tract infections seen in some hospitals in some hospitals in Lagos, Nigeria.

**Methods:** From April, 2008 to November 2009, sterile swabs were used to swab the throat of 182 patients presenting with flu-like symptoms. These patients had no knowledge of exposure to any strain of influenza virus. Viral RNA were eluted in transport media, extracted, transcribed and subsequently, the polymerase chain reaction (PCR) was used to amplify the complementary DNA.

**Results:** Seventy-eight (42.9%) tested positive for influenza A virus: 53.8% and 46.2% were males and females respectively. Overall, sampling was done in exactly the same way for the wet (rainy) and dry seasons but 41 (52.6%) of the positive patients were seen during the wet season while 37 (47.4%) were seen in the dry season. There was no statistically significant difference between these values ( $p < 0.05$ ).

**Conclusion:** The results shows influenza A virus plays a significant role in respiratory tract infections seen all through the year. As diagnosis is hardly considered in the management of these infections, it is advised that there should be continued surveillance for influenza A infections in humans. Seroprevalence investigations are also needed to assess the risk associated with strains of influenza A virus, given that these viruses continue to circulate and evolve.

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Relevance of infectious diseases in nephrology patients: A retrospective single center Review of twenty renal biopsies

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**Background:** Previous studies have examined the specific entities of renal pathology with bacterial/ viral diseases. Few have broadly studied infectious disease relevance in patients affected by current immunosuppressive and cytotoxic regimens. This retrospective was performed on patients who had renal biopsy to assess renal dysfunctions.

**Methods:** Twenty transplant/ non-transplant renal biopsies performed between January 1, 2009 and November 30, 2009 were randomly reviewed. Concurrent infectious diseases were identified.

**Results:** The biopsies belonged to 13 males & 6 females (7 cases: native biopsies & 13 cases: transplant biopsies). Of the transplant cases, 4 cases showed concurrent infectious diseases (23%). These cases included 2 cases (15 %) from 1 patient with recurrent polyoma nephropathy, 2 cases (15 %) with chronic allograft nephropathy (including 1 case with pyelonephritis/ reflux nephropathy & 1 case with HIV having acute tubular injury). The others, with no infectious complications, included 5 cases (38 %) with delayed graft functions, 1 case (8 %) with severe transplant glomerulopathy, and 1 case (8 %) with chronic allograft nephropathy/ recurrence of focal proliferative lupus nephropathy.

Of the native renal cases, 2 cases (29 %) were identified with infectious complication. The cases include 1 case (14 %) with HIV/ HCV (& hypertension/ chronic kidney disease/ recurrent bacterial pneumonia) and 1 case with urethral stenosis and chronic pyelonephritis. The remaining cases without infections include recurrent focal segmental glomerulosclerosis (FSGS), 1 case (14 %) with membranous lupus nephropathy, 1 case (14 %) with focal proliferative nephritis & Henoch-Schonlein Purpura (HSP), 1 case with minimal change (14 %) in complete remission, and 1 hypertensive nephropathy case with thrombotic microangiopathy (TMA).

With current immunosuppressive/ cytotoxic drug regimen and careful follow up, few patients demonstrate concurrent infectious disease. The few affected patients have history of urinary tract infections associated urinary tract reflux/ obstruction. Recurrent polyoma viral nephropathy, seen in 2 cases of a pediatric patient, needed decreased immunosuppressive regimen, altered cytotoxic therapy, and antiviral medication. One native kidney case of a patient with HIV/ Hepatitis C infection showed recurrent pneumonia.

**Conclusion:** In this small review, most native & all recently transplanted specimens (within several months) did not show systemic infections despite heavy initial dose of cytotoxic/ immunosuppressive regimen. Few affected cases showed underlying chronic conditions such as HIV or obstructive/ reflux uropathy. Current regimens and clinical management of medical renal diseases/ renal transplants appear adequate & effective without risking the patient to unnecessary infections.

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The impact of socioeconomic factors on risk and frequency of hospitalization for infectious diseases among teenagers in sub-sahara Africa

**O. udorji**<sup>1</sup>, C. ude<sup>2</sup>

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**Background:** The association between socioeconomic status and illness in adults and childhood has been studied extensively but the relationship between socioeconomic status and the risk and frequency of hospitalization has not been well studied among teenagers.

The purpose of this study was to shed light on the individual socioeconomic status on the risk and frequency of hospitalization in a survey among Teenagers in sub-sahara Africa.

This study was to evaluate the influence of socioeconomic factors on the hospitalization of Teenagers with infectious diseases

**Methods:** A survey of 1250 teenagers were conducted to ascertain the impact of socioeconomic factors on the risk and frequency of hospitalization of infectious diseases.

The main exposure variables adjusted for confounding factors were parents education, parents income and residence location and type of apartment. Hence multiple linear regression analysis was applied for the analysis.

**Results:** 1250 respondents with age 15.61+3.71 yrs, male 52.5% ,female 47.5% .Teenagers with low income parents showed the greatest rate of hospitalization than for the average and high income parents.

In respect of the risk of sickness, teens with high income parents showed the highest level compared to the average and low income counterparts.

The risk of hospitalization was increased in teenagers of parents with a low level of education compared with vocational education .

Malaria was the leading infectious disease in respective of the level of income, education, residence type and location. Malaria is a major health problem and among the top leading causes of morbidity in Africa.

Low socioeconomic status showed a significant association with the rate of being sick of infectious diseases.

There was no significant relationship that exist for the risk and frequency of hospitalization with the location and type of apartment.

**Conclusion:** The results of this study provides evidence that socioeconomic factors can be incriminated as a predisposing factor for increased rate of hospitalization but could not be established for the risk of infectious diseases. Hence, support for undertaking larger epidemiological studies to ascertain objectively the impact of socioeconomic status on the risk and frequency of hospitalization in sub-sahara Africa, so that future preventive efforts can be better understood.

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**Type: Poster Presentation**

Seroprevalence of *Toxoplasma gondii* antibodies amongst pregnant women at Lagos State University Teaching Hospital

**A. Akinbami**

LASUCOM, LAGOS, LAGOS, Nigeria

**Background:** *Toxoplasma gondii* (*T.gondii*), an obligate intracellular parasite found in many species throughout the world, causes a variety of clinical syndromes in humans and animals. It is also associated with morbidity and mortality in pregnancy. Hence the need to determine the seroprevalence of antibody to toxoplasmosis gondii amongst pregnant women.

**Methods:** A cross-sectional study was carried out using patients attending the ante-natal clinic of Lagos State University Teaching Hospital Ikeja. All consenting newly registered ante-natal patients were recruited consecutively into the study within a time frame of six weeks during which a total of 179 pregnant participants were obtained. Participants filled self administered questionnaires. Five milliliters of blood was collected from each participant after obtaining patient's consent. Sera were assayed for antitoxoplasmosis IgG antibody by enzyme linked immunosorbent assay. (ELISA.)

**Results:** A total of 179 pregnant women were studied. An assessment of patients' status to anti-toxoplasmosis IgG showed 40.8% were positive while 59.2% were negative.

**Conclusion:** It appears that seroprevalence of toxoplasmosis IgG antibody amongst the pregnant women in this study population is high. Therefore, it is valuable to follow up the IgM-antibody status of their off springs which indicates recent exposure.

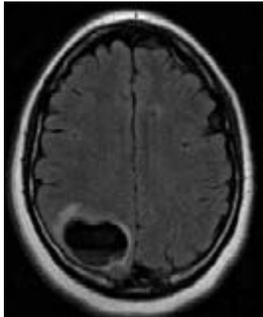
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Atypical neuroimaging of neurocystercosis  
**L. D. Wilke**, J. Farrell  
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**Background:** A 29 year-old Haitian-born woman, who moved to Chicago at the age of five, was brought to the emergency department (ED) by the local police, where she had a witnessed seizure. She was afebrile and physical exam was only remarkable for several tongue lacerations consistent with bite marks. MRI revealed three ring enhancing supratentorial cystic lesions. The largest was a 4.1 x 2.7 cm well circumscribed cyst centered within the subcortical white matter of the right posterior parietal lobe containing a fluid/fluid level with a small rim of circumferential edema.



**Right posterior parietal lobe NCC cyst on MRI**

**Methods:** CT scan of the head demonstrated multiple, bilateral supratentorial calcific nodules highly suggestive of Neurocystercosis (NCC). Abdominal ultrasound was negative for focal hepatic lesions. Anticonvulsant and steroid treatment were begun.

**Results:** Serum was sent to the parasitology lab at the CDC for NCC serology, and it was positive. The patient completed an 8-day course of albendazole as an outpatient without incident, but presented one month later with recurrent seizures refractory to IV anticonvulsant therapy.

**Conclusion:** NCC is a parasitic infection of the brain caused by the tapeworm *Taenia solium*, and is the most common cause of late-onset seizures in countries where the infection is endemic, such as Haiti (1, 2). Diagnosis can be made clinically or by serology, but neuroimaging using MRI and CT have improved diagnostic accuracy (1). Vesicular (living) cysticerci appear as isodense cystic lesions with thin walls without perilesional edema. The scolex is usually visualized as a “hole-with-dot” lesion (3). Colloidal cysts (degenerating parasites) are ill-defined ring-enhancing lesions surrounded by edema. Granular cysticerci (continued degeneration) appear as nodular hyperdense lesions surrounded by edema or a rim of gliosis. Calcified cysticerci appear on contrast enhanced images as small hyperdense nodules without perilesional edema or abnormal enhancement after parasite death.

The large fluid/fluid containing cyst noted in our patient presented a diagnostic challenge. Published reports of large central nervous system cysts with intracystic fluid/fluid levels are lacking in the NCC literature. But the CT findings, positive serology for NCC and the low likelihood of two parasitic diseases of the brain convinced us of the diagnosis of NCC

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**Session: Parasites**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Detection of *Toxoplasma gondii* antibody for diagnosis of ocular toxoplasmosis

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**Background:** Forty patients with cataracts, as well as 40 patients with proven ocular toxoplasmosis were enrolled in this prospective clinical study.

**Methods:** Serum IgG and aqueous IgG in both group were measured by enzyme linked immunosorbent assay (ELISA) and the corresponding ratios were calculated.

**Results:** Serum IgG/ Aqueous humor IgG ratio was less than 100 in the patient group and more than 100 in the control group. On the other hand in the chorioretinitis subgroup the ratio was less than 13, while in the group with uveitis, the ratio was more than 13 ( $P < 0.05$ ).

**Conclusion:** The results revealed that calculating the ratio = Serum IgG (antitoxo) / Aqueous IgG (antitoxo) may be helpful as an adjunct to diagnosis in cases with clinically atypical ocular toxoplasmosis.

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**Type: Poster Presentation**

Two-dimensional gel electrophoresis analysis of *T. solium* cysticerci lower molecular mass (10-30 kDa) antigens for the serodiagnosis of neurocysticercosis in children

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Postgraduate Institute of Medical Education and Research, 160012, Chandigarh, India

**Background:** Neurocysticercosis (NCC) caused by *T. solium* cysticerci is an increasingly important health issue in Indian children. The sensitivity and specificity of available serological techniques were low in case of single cysticercus granuloma cases which is a more common feature in Indian patients.

**Methods:** Serum samples were collected from 13 clinically and radiologically suggestive NCC children and seropositive by ELISA, 25 clinically and radiologically suggestive NCC children and seronegative by ELISA and 25 control subjects. The 10-30 kDa antigens of *T. solium* cysticerci were subjected to 2- dimensional gel electrophoresis (2D-PAGE) followed by Enzyme-linked immunoelectrotransfer blot (EITB) assay to detect antibody in serum samples. PDQuest software (Bio-RAD) was used to analyze the Isoelectric Point of the protein spots obtained in the 2D-PAGE.

**Results:** Analysis of 10-30 kDa antigenic fraction 2D-PAGE map showed 31 proteins between 10- ≤28 kDa and innumerable proteins between >28-30 kDa with the Isoelectric point of 3-10. All the 13 (100%) NCC seropositive and 15 (60%) out of 25 NCC seronegative samples were reactive with 2D fraction antigens. In the control group, none of the serum was reactive except 2 hydatid samples (92% specificity). Antigenic fractions present in between 28-30 kDa with 3-10 pI were immunoreactive with significantly higher number of samples

**Conclusion:** The sensitivity and specificity of 2D-PAGE EITB assay were higher than that reported earlier with the use of lentil lectin purified glycoprotein antigenic fractions EITB assay, which is considered the gold standard serodiagnostic method for neurocysticercosis.

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**Type: Poster Presentation**

Prevalence of intestinal parasitic infections in the Ministry of Health Hospitals in Sharjah, UAE: 2-year retrospective study

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<sup>1</sup>University of Sharjah, Sharjah, United Arab Emirates, <sup>2</sup>Higher College of Technology, Sharjah, United Arab Emirates, <sup>3</sup>Al-Qassimi Hospital, Sharjah, Sharjah, United Arab Emirates

**Background:** In United Arab Emirates (UAE) having four and a half million population, Sharjah boasts the third most populated emirate having 600,000 people [1]. Approximately 80% of the population is expatriates coming from countries where parasitic infections are endemic. Many of them find suitable jobs in various food manufacturing industries, hotels, restaurants and fast food outlets. They also work as food handlers, housemaids and baby sitters. Thus, possible transmission can occur between the immigrants workforce and native emarati population in the community. The present study investigates the prevalence of the intestinal parasitic infections in Ministry of Health hospitals in Sharjah emirate of United Arab Emirates.

**Methods:** Retrospective laboratory data analysis of ten thousand five hundred fourteen fecal specimens in five different Ministry of Health hospitals within the emirate of Sharjah, was carried out between January 2007 and December 2008. The stool specimens were examined using different microbiological analysis including direct and stool concentration techniques.

**Results:** During the study period, eight hundred fourteen stool specimens were found to be positive for intestinal parasites. Of this 814 positive specimens, five hundred ninety three (73%) were from local emarati people and rest 27% belongs to the expatriates. There was a higher rate of protozoal infections (92.2%) than the helminths infection (7.8%). *Entamoeba histolytica* (71.8%) and *Giardia lamblia* (17.5%) were the commonest intestinal parasites identified. Among the helminths, *Ancylostoma duodenale* and *Ascaris lumbricoides* were the common ones. The rate of protozoa infection in native emarati population was 71% (577). On the other hand, the helminth's infestations were more common among the expatriates (6%).

**Conclusion:** Possibly the most surprising and unanticipated finding was the far greater prevalence of intestinal parasitic infections among the native emarati population (73%) compared with the expatriates (27%), and this may be attributable to better health seeking attitude and more accessibility of native emarati people to government hospitals than the expatriates. The high prevalence rate among the local people indicates that there is a high transmission rate in the community. The existing screening methods especially for food handlers and housemaids need to be improved and strengthened.

#### **Reference**

1. *UAE Population*. (2009, April 28). Retrieved April 28, 2009, from <http://www.asiarooms.com/travel-guide/united-arab-emirates/uae-overview/uae-population.html>

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**Type: Poster Presentation**

A study of medically important fish - transmitted parasites in Alexandria  
R. M. G. Diab, **M. M. ElTemsahy**, E. D. ElKerdany, M. Gaafar, M. A. Nasr  
Faculty of Medicine - Alexandria University, Alexandria, Alexandria, Egypt

**Background:** Seafood has traditionally been a popular part of the diet in many parts of the world. However, salt and freshwater fish can serve as sources of parasitic infections to man. This study aimed to identify parasites of medical importance in the commonly consumed fish in Alexandria namely, *Tilapia zilli*, *Mugil cephalus*, *Clarias gariepinus*, *Brycinus nurse* and *Atherina boyeri*, and to study their infectivity to laboratory mice.

**Methods:** The study was applied on 300 fish during both cold and hot months throughout a year starting from November 2006 to October 2007. They were examined for the presence of parasites in their intestines and flesh, using modified Ziehl-Neelsen, safranin and modified trichrome stains. Furthermore, fish flesh was screened for metacercariae of Heterophyidae, which were then isolated by in vitro pepsin-hydrochloric acid digestion. Infection of laboratory mice with the detected parasitic protozoa and the encysted metacercariae was done to study their infectivity and their pathological outcome. The obtained heterophyids were examined after clearance in lactophenol and staining with carmine stain.

**Results:** Results showed that the following parasites were detected; *Microsporidia*, *Cryptosporidia* and *Cyclospora* in fish intestine, and metacercaria of *Pygidiopsis genata* in fish flesh. *Tilapia zilli* was the most infected fish, followed by *Clarias gariepinus*, then, *Atherina boyeri* and *Brycinus nurse*. The least infected fish was *Mugil cephalus*. All the detected parasites were found to be infective to experimental mice as proved by observation of the previously mentioned protozoa and heterophyid eggs in their stool samples, and also by the histopathological changes in their intestinal sections.

**Conclusion:** Thus, the present work proved that the studied fish harboured infective parasites in their flesh and intestine that can have great impact on the human health.

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**Type: Poster Presentation**

Study of the clinical efficiency of covariumtm10 on patients suffering from intestinal amoebiasis

**M. N. Emmanuel**

University of Yaoundé I, Yaoundé, Centre, Cameroon

**Background:** Amoebiasis is caused by a protozoan parasite, *Entamoeba histolytica* with or without clinical symptoms and is the third leading cause of death from parasitic diseases. This study evaluated the efficiency of the improved traditional medicine (CovariumTM10) in the treatment of intestinal amoebiasis.

**Methods:** Thirty patients suffering from intestinal amoebiasis and aged from 3-77 years were selected and grouped according to the stage of infection. These patients were sequentially treated with 2 zip-locked bags of CovariumTM10 for two days, by administration of one bag once per day. Six days after, stool was collected for parasitological examination. Concomitantly, blood samples were collected from all patients for the quantification of transaminases (AST, ALT) and creatinin. An additional bag of CavariumTM10 was prescribed to patients who still presented some *Entamoeba histolytica* cysts in their stool and 4 days after the administration, another parasitological examination was carried out.

**Results:** The research of amoebae in stool on day 6 of treatment showed that 25 of the 30 patients (83.33%) were cured with 2 zip-locked bags of CovariumTM10 and the rest (5 of the 30: 16.67%) were cured after taking the third bag of the drug. It was observed that, only adult patients (20% of them) needed an additional bag of the drug for complete treatment while all the children were cured with 2 zip-locked bags of CovariumTM10. Blood chemistry showed no abnormal changes in creatinin and transaminases levels. All of these parameters were maintained at their normal level 6 days after completion of the therapy.

**Conclusion:** CovariumTM10 is efficient in treating patients suffering from intestinal amoebiasis and a posology of two bags of CovariumTM10 for children and three for adults, taking one bag once per day, may efficiently cure patients suffering from this infection. This medicine can now be considered as an alternative drug in the treatment of patients suffering from intestinal amoebiasis.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

*Anisakis simplex* from the Adriatic sea

**I. ZURAK**

Hospital/University, Zagreb, Croatia

**Background:** The presence of parasites in fish is very common, between more species of helminth parasites from fish are known to cause diseases in man, more important is *Anisakis simplex*. *Anisakis simplex* is ubiquitous parasite in world sea, distributed and in the Adriatic sea. During cleaning of fish, many fail to notice the presence of the lively larva of the *Anisakis simplex*, parasite in the abdominal cavity.

**Methods:** The examine randomly collected fish, available a few fish market at the Zagreb. During cleaning of fish to field the pesence very mobile of the lively *Anisakis simplex*. The parasites can be found in the viscera, in the musculature, collected from different organs in the abdominal cavity, the prime site of infection was the liver. *Anisakis simplex* is find in: *Scomber scombrus*, *Spanish mackerel*, *Scomber japonicus colias*, *Trachurus trachurus and mediterrabeus*, *Sarda sarda* and *Merlucius merlucius caught*. During cleaning of fish to field the pesence very mobile of the lively *Anisakis simplex*. The parasites can be found in the viscera, in the musculature, collected from different organs in the abdominal cavity, the prime site of infection was the liver. *Anisakis simplex* is find in: *Scomber scombrus*, *Spanish mackerel*, *Scomber japonicus colias*, *Trachurus trachurus and mediterrabeus*, *Sarda sarda* and *Merlucius merlucius caught*.

**Results:** The most intensive infection was noticed in Horse mackerel, Spanish mackerel and then Atlantic mackerel. Do not discover in pilchard. Activity was in muscles kept at 4oC. Immersed in a saline solution in a Petri dish, swam undulating the body. In these conditions, they could survive several days at room temperature. Low (-20oC) or high temperatures (55oC during 1 minute) and high salt concentrations (NaCl) may be used to kill or inactivate nematodes in fish.

**Conclusion:** *Anisakis simplex* is of much medical importance because of the severe allergic reactions and gastrointestinal symptoms it causes in humans after eating or handling infected fish or crustaceans. Bad, inaccessible laboratory diagnostics conditions miss in the rareripe discovering of falling ill. Infection is high in the fish species from Adriatic area and it is expected to be reflected on human health.

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**Type: Poster Presentation**

Evaluation of OSOM *Trichomonas* rapid test for diagnosis of *Trichomonas vaginalis* infection in females

**F. shujatullah**<sup>1</sup>, H. M. Khan<sup>1</sup>, R. Khatoon<sup>1</sup>, T. Rabbani<sup>1</sup>, M. Shahid<sup>2</sup>, A. Raza<sup>1</sup>

<sup>1</sup>J.N.Medical college, 202001, India, <sup>2</sup>JN Medical College & Hospital, 202002, India

**Background:** *Trichomonas vaginalis* is a parasitic protozoan, sexually transmitted world wide. WHO estimates show that this infection accounts for almost half of all curable sexually transmitted infections. Diagnosis is usually done by observing motile parasite from vaginal or cervical swab specimens by direct wet mount examination. Other diagnostic test includes giemsa staining, fluorescent staining and culture..

OSOM rapid test (Genzyme Diagnostics Cambridge Massachusettes) based on immunochromatographic capillary flow dipstick is newer method available for diagnosis of sexually transmitted disease.

**Objectives:** To compare wet mount examination, giemsa staining and acridine orange staining, culture as diagnostic test for *Trichomonas Vaginalis*

To compare sensitivity, specificity of OSOM trichomonas rapid test with culture and different staining techniques available for diagnosis of this sexually transmitted disease.

**Methods:** The study was carried out on 405 aged 15-45 years patients attending gynecological out patient department with complaint of vaginal discharge. Four high vaginal swabs from posterior fornix of vagina were taken from each patient. One swab used for preparation of wet mount, one for making smear of giemsa and acridine orange staining and other for OSOM trichomonas rapid test and culture done on kupferberg culture medium.

**Results:** Out of 405 patient of vaginal discharge included in the study, Wet mount and giemsa staining showed *T.vaginalis* in 7 (1.7%) cases each. Acridine orange staining was positive in 12 cases (3.0%), while OSOM rapid test detected vaginalis infection in 15 cases. Culture on kupferberg medium was positive in 18 (4.4%) patient, sensitivity; specificity of wet mount examination was found to be 38.9%, 100%, positive and negative predictive values, 100% and 97.2%. Giemsa and acridine orange staining showed sensitivity of 38.9% and 66.67% and specificity 100 % each. OSOM rapid test was found to be most sensitive when compared to wet mount examination and staining techniques taking culture on kupferberg medium as gold standard, while staining methods and OSOM test were found to be equally specific.

**Conclusion:** OSOM rapid test can be used as adjunct to microscopy and culture. Its high sensitivity and specificity advocates its use in settings where culture is not possible as direct microscopic examination and different staining techniques fails to detect infection in large number of cases.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

*Giardia intestinalis*: DNA extraction approaches to improve PCR results

**Z. Babaei**<sup>1</sup>, E. Razmjou<sup>2</sup>, H. Oormazdi<sup>3</sup>, S. Rezaie<sup>4</sup>, M. Rezaeian<sup>1</sup>

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**Background:** Difficulty in disrupting cysts of *Giardia intestinalis*, a cosmopolitan protozoan parasite, decreases the yield of DNA extracted and reduces the effectiveness of the polymerase chain reaction (PCR). To improve the detection of the *Giardia* Glutamate Dehydrogenase (*gdh*) gene, we re-evaluated the effects of deoxyribonucleic acid (DNA) extraction methods.

**Methods:** The *Giardia* cysts were concentrated from 33 positive fecal samples in a sucrose gradient with a specific gravity of 0.85 M and then washed with distilled water. DNA extraction was carried out by one of two methods, the QIAamp Stool Mini Kit (QIAGEN, Germany) or the conventional phenol/chloroform/isoamyl alcohol (PCI) method. For pretreatment, the *Giardia* cyst walls were disrupted by glass beads, freeze-thaw cycles, or combinations of these methods, and DNA extraction was performed by one of two methods. Amplification of the *gdh* gene was performed as a single PCR.

**Results:** Without pretreatment of cysts, the *gdh* gene was not amplified from any DNA sample whether using the conventional PCI extraction method or the QIAamp DNA Stool Mini Kit. After pretreatment of the cysts with glass beads and freeze/thaw cycles, the target gene was amplified in all 33 extracted DNA samples. The 458 bp fragment of *gdh* gene was amplified in nine samples using PCI (31.0%), and in the 24 remaining extracted DNA samples with the QIAamp DNA Stool Mini Kit.

**Conclusion:** Consequently, the pretreatment of cysts with glass beads and freeze/thaw cycles followed by extraction of DNA with the QIAamp Stool Mini kit was the more effective protocol.

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**Type: Poster Presentation**

Can cystic Echinococcosis trigger autoimmunity?

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**Background:** Cystic echinococcosis(CE) is a zoonosis and disseminated disease in worldwide. Little is known about parasitic molecules that behave like immunomodulatory antigens and the mechanisms that they use to evade the host's immune response. Some authors suggested that immunomodulatory antigens of *Echinococcus granulosus* can directly inhibit the basic immune cell functions and stimulate the immune molecules for the development of CE. We aimed to evaluate the role of parasitic molecules of *E. granulosus* and also evaluate the link between immunomodulatory effects of these molecules and autoimmunity etiopathogenesis of the host by serology

**Methods:** Twenty-eight non-operated patients with hydatid cysts (NOP) and 88 operated patients with hydatid cysts (OP) were included as study group and 54 healthy individuals with no known chronic diseases were included as control group. The presence of ANA (Anti nuclear antibody), ASMA (Anti Smooth Muscle Antibody) and AMA/LKM (Anti Mitochondrial Antibody /Liver Kidney Microsomal Antibody) antibodies which were known as autoimmune parameters were investigated for all groups by using Euroimmune *indirect* fluorescent *antibody* (IFA) kits (Euroimmune Labor Diagnostica, Germany). The ages of patients were between 16- 83 years and patient and control groups were matched for age and gender. Patients with positivity in at least one out of three tests were considered positive for autoimmunity.

**Results:** The antibodies were detected in 16(57,1%), 32(36,3%) and 12(23%) of NOP, OP and control groups, respectively. It was detected a statistically higher significant difference between NOP and healthy controls ( $p < 0.01$ ). No difference was detected between OP and healthy controls ( $p > 0.05$ ). No statistically significant difference were detected between OP and control groups, but we detected a moderately higher positivity in results of OP. It was detected a significant difference between all patient groups (patients with NOP and OP) and control group ( $p < 0.05$ ).

**Conclusion:** As a result, detection of high autoantibodies in NOP regarding to our results suggested us, these chronic long-lasting disease process together with their immunoregulatory molecules may activate unknown autoimmune mechanisms of the host and lead to formation of autoimmunity. We also suggest that the decrease of autoantibodies in OP may be caused by the absence of antigenic stimulus originated from hydatid cysts.

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**Type: Poster Presentation**

Prevalence of intestinal parasitoses and factors related in the population infant of the community Santa Bárbara, Municipality Valley Guanape, State Anzoategui, Bolivarian Republic of Venezuela

**R. Martínez**

Instituto de Medicina Tropical "Pedro Kourí", Ciudad Habana, Cuba

**Background:** Intestinal parasitism affects mainly the infant population, on a worldwide scale; it is assessed that contributes the greatest number of infected among more than 1000000 of people who every year is declared. The objective was to determine the prevalence of intestinal parasitism in the infant population of the community Santa Barbara and to identify some risk factors.

**Methods:** A prevalence study was conducted. Universe: infant population from 0 to 14 years, including a total of 172. The collection of the primary datum was carried out under the total consent of the parents, who were interviewed, and some general data and of epidemiological interest was also gathered. Two stool samples were in addition collected by spontaneous defecation to each child. This study was conducted during the months from February to August 2008. For the analysis of association among the variables there were used the tests of comparison of proportions, and of X<sup>2</sup>, being considered a level of statistical significance when p value was equal or less than 0.05. In order to establish the strength of association among some variables, the Reason of Prevalence was used, there was taken into account the 95% confidence interval (CI).

**Results:** A total of 89 children were parasitized, for a prevalence of 51.7%, being the ages of 5-9 most affected (52.8%), as well as the male sex (56.1%). The risk factors associated with the intestinal parasite infection were water consumption without boiling, PR = 2.91 [1,8-4.4], not having in-house WC, (PR = 3.8 [1.61–6.31], the utilization of the collective well as source of water supply (RP=1.80 [1.1- 3.1] and the use of feeding-bottle, (PR = 3.85 [1.8 -8.1]. The symptom that most frequently we found was the abdominal pain (43.8%), the parasite that predominated was *Giardia lamblia* followed by *Entamoeba histolytica*.

**Conclusion:** The prevalence of intestinal parasitism in the infant population of the community studied was high, being corroborated a narrow relationship between the presence of parasites and some risk factors that predisposed to the intestinal parasitism.

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**Type: Poster Presentation**

Efficacy and safety of ivermectin versus high dose albendazole for chronic strongyloidiasis in immunocompromised patients

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<sup>1</sup>Department of Medicine, 10700, Thailand, <sup>2</sup>Department of Preventive Medicine, 10700, Thailand, <sup>3</sup>Department of Parasitology, 10700, Thailand

**Background:** Ivermectin and albendazole are effective against strongyloidiasis. However the efficacy and the most effective dosing regimen in immunocompromised patients are to be determined.

**Methods:** A prospective randomized open study compared a 7-day course of oral albendazole 800 mg daily with a single dose (200mg/kg), or two single doses given 2 weeks apart of ivermectin in Thai patients with chronic strongyloidiasis. The primary end points were relief of symptoms (if present) and clearance of *Strongyloides* larvae from faeces after treatment and at one year follow up.

**Results:** 90 patients were included in the analysis (30, 31, and 29 patients in albendazole, single dose, and two-single doses ivermectin group respectively). Only 3 patients in this study did not have any underlying diseases. Diabetes mellitus, SLE, nephrotic syndrome, hematologic malignancy, solid tumor, and HIV infection were common underlying diseases these patients. The median (range) duration of follow up were 15 (2-62) weeks in albendazole group, 30 (2- 59) weeks in single dose ivermectin group, and 24 (2-61) weeks in two single doses ivermectin group. Cure rates were 76.7%, 96.8% and 100% in albendazole, single dose oral ivermectin, and two single dose oral ivermectin respectively (P=0.003) in per-protocol analysis. No serious adverse event associated with treatment was found in all groups.

**Conclusion:** This study confirms the superiority of ivermectin compared to high dose of albendazole for the treatment of chronic strongyloidiasis in immunocompromised patients. A single oral dose was as effective as two single oral doses of ivermectin.

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**Session: Parasites**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Biochemical and immunological characterization of the EhCBP30 protein from *Entamoeba histolytica*

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CINVESTAV-IPN, México, Mexico

**Background:** *Entamoeba histolytica*, is the causative agent of the amoebiasis. The infection is asymptomatic, but only a few people develop complications such as amebic liver abscess. During this event, *E. histolytica* trophozoites survived in liver in anaerobic conditions, and they can survive high concentration of reactive oxygen species (ROS), this capacity for surviving to ROS is mediated by detoxifying enzymes like peroxiredoxins.

**Methods:** The EhCBP30 gene, with homology to peroxiredoxins, was amplified by PCR. The amplified DNA was cloned in the plasmid pRSET, and the His-tagged EhCBP30 recombinant protein was purified. The antioxidant capacity of the protein His::EhCBP30 was studied in MCO assays, and the peroxidase activity (PO) of this protein was also assayed. Mice were immunized (ip) with the protein His::EhCBP, and serum samples were obtained one week after the last immunization. Anti-EhCBP30 antibodies (isotype) was assayed by ELISA, and induced cytokines were evaluated by flow cytometry.

**Results:** In MCO assays, there is a iron-salt-dependent decomposition of hydrogen peroxide, generating the highly reactive hydroxyl radical that promote oxidative DNA damage (nicks). However, a protein with PO activity will protect DNA against the damaging effect of hydroxyl radicals. When the His::EhCBP30 protein was included in the MCO assays, protection of DNA nicking was clearly detected, suggesting a PO activity of the His::EhCBP30 protein. In the PO activity assays the protein His::EhCBP30 was able to eliminate hydrogen peroxide in a dose dependant reaction. Finally, to investigate whether the EhCBP is immunogenic, mice were immunized (ip) with the protein, and 15 days after the last immunization serum samples were obtained. Results showed that the EhCBP30 protein induces an immunoglobulin immune response with the Igs isotypes IgG2b>IgG1>IgG2a>IgG3, suggesting a non polarized Th1/Th2 immune response. However, when serum cytokines were examined, only IL-2, IL-6, IL-10 and IL-17 were detected, thus indicating that a Th1 immune response was polarized.

**Conclusion:** The findings of this study indicated that the EhCBP30 protein has antioxidant activity (peroxiredoxin), and is able to induce a Th1 immune response. Therefore, the EhCBP30 may be a good candidate for further immunoprophylaxis studies.

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**Type: Poster Presentation**

Soil-transmitted helminthiasis in rural South West China: Prevalence, intensity and risk factor analysis

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**Background:** Soil-transmitted helminth infections (STHs) are common across China and carry a significant disease burden, especially in children. Few studies have investigated the local epidemiology of soil-transmitted helminth infections (STHs) in rural China or the risk factors for transmission. The present study compared STH prevalences in Simao and Mengla counties, Yunnan province, China, in collaboration with the Yunnan Institute of Parasitic Diseases.

**Methods:** Faecal samples were collected from 317 school-aged children and from 94 inhabitants of a single village to determine age-prevalence. The Kato-Katz thick smear method was used to determine prevalence and intensities of the different STHs found. Data on socio-demographic and behavioural risk factors for the schoolchildren were collected using questionnaires.

**Results:** In Simao County the overall STH prevalence was 40.2% (2.7%, 5.4% and 35.7% for ascariasis, trichuriasis and hookworm infection, respectively). STHs were significantly higher in Mengla County, with an overall prevalence of 68.3% (19.0%, 34.6% and 47.3% for ascariasis, trichuriasis and hookworm infection, respectively). According to World Health Organization (WHO) STH intensity guidelines, the majority of infections were "light," but, 6.6% of hookworm infections were "heavy". Multiple logistic regression found that females were less likely to be infected with *Trichuris trichiura* (OR: 0.29, 95% CI: 0.15-0.56) and with hookworms (OR: 0.55, 95% CI: 0.33-0.93) than males. Children 12 years of age or older had increased risk of hookworm infection (OR: 2.9, 95% CI: 1.2-7.1). Children with mothers with educational attainment of secondary school or higher had a protective effect against *T. trichiura* (OR: 0.18, 95% CI: 0.06-0.54) and hookworm (OR: 0.21, 95% CI: 0.09-0.51) infections. The Jinuo ethnicity was most prevalent in Mengla County and had the highest risk of infection with *T. trichiura* (OR: 10.71, 95% CI: 2.78-41.28) and hookworm (OR: 4.64, 95% CI: 1.50-14.30). In the village survey for age prevalence, hookworm was again the most prevalent species (62.8%) with a high prevalence persisting in those 50 years of age and older.

**Conclusion:** The observed higher prevalences of STHs in Mengla County could be due to differences in cultural and hygiene practices among native ethnic groups. Based on recommended intervention strategies by the WHO, Simao County should opt for school-based deworming annually, while Mengla County should implement a similar strategy biannually. In addition, targeted strategies in Mengla County should include the elderly population.

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**Type: Poster Presentation**

Hymenolepiasis in institutionalized Romanian children

**T. R. Olariu<sup>1</sup>**, G. Darabus<sup>2</sup>, I. Marincu<sup>1</sup>, C. Balutescu<sup>3</sup>, V. Dumitrascu<sup>3</sup>, C. Petrescu<sup>3</sup>, A. Koreck<sup>3</sup>  
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**Background:** Parasitic diseases represent an important public health problem. Digestive parasitosis are characterized by high prevalence and a variety of clinical manifestations, being especially frequent in institutionalized children. The aim of the present study was to investigate the prevalence of intestinal parasitic infections in a Romanian Children Care Unit.

**Methods:** Stool examinations were performed using the iodine staining for the identification of protozoan cysts and the Willis-Hung method for the identification of helminth eggs. Eosinophil values were determined by differential white blood cell count with May Grunwald Giemsa staining.

**Results:** We investigated 64 institutionalized children aged 2 to 12 years.

Parasitic infections were identified in 44 cases (68.7%). *Hymenolepis nana* (31.2%), *Giardia lamblia* (28.1%), *Blastocystis hominis* (7.8%), *Entamoeba coli* (4.7%), *Ascaris lumbricoides* (4.7%), and *Trichuris trichiura* (3.1%) were diagnosed. We have determined associations of two (45.4%) and more than two parasites (18.2%) among the infected children; of the 20 with hymenolepiasis, 11 (55%) had multiple parasitic infections.

Clinical and laboratory examinations were conducted to investigate the presence of symptoms and eosinophilia in patients with parasitic infections.

Diarrhoea (38.6%), weight loss (47.7%), abdominal pain (25%), nervous disorders (34.1%), cutaneous manifestations (22.7%), fever (9.1%) and respiratory infections (59.1%) were reported in the infected children.

Eosinophilia was noted in 27 (61%) of the 44 patients with parasitosis. High eosinophil values were mostly observed among those diagnosed with helminth infections.

**Conclusion:** *Hymenolepis nana* infection was reported with an increased frequency among Romanian institutionalized children. The majority of the patients with hymenolepiasis were diagnosed with multiple parasitic infections.

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**Type: Poster Presentation**

Computational characterization of molecular mechanism of surface receptor binding with alpha-enolase of *Trichomonas vaginalis*

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**Background:** *Trichomonas vaginalis* is a parasitic protozoan causing the most common human sexually transmitted disease (STD), trichomoniasis. The exact mechanism of its pathogenesis is still obscure. Alpha-enolase plays a pivotal role in the host-pathogen interaction, and as a surface receptor of several protists mediating plasminogen binding. In view of identifying plasminogen binding sites of *T. vaginalis* alpha-enolase, homology modeling and docking studies were conducted to obtain modeled structures of the *T. vaginalis* alpha-enolase-plasminogen complex.

**Methods:** The protein sequence of *T. vaginalis* alpha-enolase used in this study was kindly provided by Dr. John F. Alderete (University of Texas, USA). Modeling templates were searched by using BLAST, followed by multiple sequence alignment. The atomic coordinates of *Escherichia coli* enolase was retrieved from Protein Data Bank. Molecular structures of *T. vaginalis* alpha-enolase were modeled by using restraint-based modeling, followed by energy minimization using MODELLER program. The quality and stereochemistry of the models were evaluated by program PROCHECK. After addition of Mg<sup>2+</sup>, the selected model further refined by energy minimization employing NAMD program. The VMD program was used to superimpose structure of *T. vaginalis* alpha-enolase model with crystal structures of enolases from *E. coli* and *S. pneumoniae*. *T. vaginalis* alpha-enolase model was docked to human plasminogen for protein-protein interaction using Hex 5.1. Mark Gerstein's calc-surface program was used to calculate the solvent accessibility at the interface of *T. vaginalis* alpha-enolase and human plasminogen before and after docking.

**Results:** Molecular docking revealed hydrogen bonding of eLys70-pgTyr50, eAsn165-pgThr66, eAla168-pgGlu21, eAsp17-pgLys70, and eAsn213-pgPro68/pgAsn69. Substantial decreases in accessible surface area (ASA) were observed and in concurrence with hydrogen bond pattern.

**Conclusion:** These findings provide new insights for interaction at the protein-protein interface. Our theoretical prediction is consistent with preexisting biochemical data. The predicted interaction complex can be of great assistance in understanding structural insights, probably being necessary to an interaction between pathogen and host-component. The ability of *T. vaginalis* alpha-enolase to bind plasminogen may be indicative of being a key player in invasion of this pathogen to host. Conclusively, this work theoretically establishes the *T. vaginalis* alpha-enolase as a novel surface-linked virulence factor.

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**Type: Poster Presentation**

Prevalence of *Dientamoeba fragilis* among asymptomatic individuals from North Central Venezuela

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**Background:** *Dientamoeba fragilis* is a protozoan parasite, with the presence of only trophozoites in its life cycle, with worldwide distribution, and that may be considered responsible for enteric disease in humans. Although described in 1918 by Dobell, many clinical and pathological aspects of *D. fragilis*, including its epidemiology, still need more study in order to improve the understanding of this emerging parasite.

**Methods:** In the context of a Venezuelan Study on Human Growth and Development (SENACREDH), a cross-sectional study of 3388 asymptomatic individuals (1656 male, 1732 female; mean age 13.05±0.22year-old) from 40 municipalities of North Central Venezuelan states (Aragua, Carabobo, Miranda, Vargas and Capital District) was carried out. *Dientamoeba fragilis* was diagnosed in stool samples after being preserved in MIF media.

**Results:** We found a prevalence of 1.8% (95%CI1.39-2.32%). From the total of individuals with *D. fragilis* infection (61), 52.5% were females and 47.5% males. Mean age was 14.37years-old(±12.59). Prevalence according to sex was not different(p=0.935), neither to age(p=0.144). From those infected, 10.9% presented anemia(6.54% microcytic hypochromic), 43.5% presented relative eosinophilia. Mean haemoglobin was 13.07g/dL(10.8-14.9 g/dL), mean %eosinophils 5.17%(0-23%), and mean eosinophils count 396.93 cells/mm<sup>3</sup>(0-1760 cells/mm<sup>3</sup>). Co-infections with other parasites were identified: 80.3% *Blastocystis hominis*, 27.9% *Endolimax nana*, 26.2% *Iodamoeba butschlii*, 13.1% *Entamoeba coli*, 9.8% *Giardia lamblia*, 8.2% *Ascaris lumbricoides*, 1.6% *Trichuris trichiura*, 1.6% *Hymenolepis nana* and 1.6% *Entamoeba histolytica/dispar*.

**Conclusion:** Eosinophilia has been occasionally described in cases of *D. fragilis* infection. However, herein a significant proportion of patients presented it suggesting a pathological role of this protozoan. As has been previously reported a significant proportion of helminth and other protozoan coinfections was found in this study, suggesting a shared transmission mode with these enteric parasites. There are great gaps in our present state of knowledge concerning the virulence, pathogenicity, and mode of transmission of *D. fragilis*. However, the neglect of *D. fragilis* as an emergent parasite should not longer be justified as there are a growing number of reports incriminating it in different enteric diseases.

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**Type: Poster Presentation**

Risk factors in patients with trichinellosis from Western Romania

**I. Marincu**, L. Negrutiu, A. M. Neghina, I. Iacobiciu, R. Neghina

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**Background:** Trichinellosis continues to be an important public health issue in Romania due to the consumption of unverified pork from unauthorized sources. Authors of the present paper established the risk factors in a study group of patients diagnosed with trichinellosis.

**Methods:** We have retrospectively analyzed the medical charts of 121 patients, inhabitants of four trichinellosis endemic areas in Timis County, Romania who were admitted to Hospital of Infectious Diseases Timisoara during 2002-2009. The diagnosis was based upon epidemiologic features (the onset of infection following consumption of infected pork with *Trichinella spiralis*), clinical features (fever, headache, chills, nausea, diarrhea, facial edema, myalgia, urticaria etc.) and laboratory tests (erythrocyte sedimentation rate, fibrinogen, antibodies against *Trichinella spiralis*, leukocyte counts, eosinophil counts etc). The results of the monitoring of the clinical, laboratory and therapeutic parameters were registered in the individual files of the patients. Epi Info soft was used for the statistical processing of data.

**Results:** There have been recorded two trichinellosis foci both in rural (Ilecea – 26 cases and Padureni – 5 cases) and urban regions (Kuncz – 57 cases and Ronat – 33 cases). According to the ethnic origin of the patients, 95.86% of them belonged to the gipsy minority, characterized by a low level of education (without respect to the basic individual or collective hygienic alimentary rules), and specific unhealthy and unsafe food habits. Although they were in the evidences of general practitioners, only 9 of them (7.75%) used to benefit of the regular medical visits. Nevertheless they didn't buy the prescribed drugs and didn't follow the indicated therapy. All of the patients had consumed unverified pork infected with *Trichinella spiralis* that was illegally traded. In Ilecea focus the consumed meat was taken by the gypsies from a septic tank where it was deposited for destruction. Thirty-eight patients (30.17%) had consumed undercooked pork and additional pork products (smoked sausages). During the hospitalization, 86.20% of the gipsy patients presented an inadequate and recalcitrant behavior.

**Conclusion:** The study of the risk factors for trichinellosis allows the implementation of programs for prophylaxis and decrease of the incidence of disease in regions where this severe zoonosis is widespread.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

The prevalence of giardiasis in patients with acute enterocolitis from Timis County, Romania

**I. Marincu**, L. Negrutiu, I. Iacobiciu, A. M. Neghina, R. Neghina

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**Background:** According to its increased incidence and severe complications, acute enterocolitis continues to represent a global major public health concern. The present study aimed to determine the prevalence of giardiasis in a group of patients diagnosed with acute enterocolitis in Timis County, Romania.

**Methods:** We have retrospectively analyzed the medical charts of 84 adult patients diagnosed with acute enterocolitis who were admitted at the Hospital of Infectious Disease in Timisoara. The diagnosis was established upon clinical features (fever, abdominal colic, repeated watery diarrheic stools, dehydration, asthenia, unspecific cutaneous eruption, muscular cramps etc.), laboratory tests (leukocyte counts, leukocytic formula, erythrocyte sedimentation rate, fibrinogen, C-reactive protein, stool culture, stool examination, natremia, kalemia, calcaemia etc.) and other explorations (abdominal echography, electrocardiography). Stool examinations, repeated after 1 to 2 days, were performed in all patients to detect *Giardia lamblia* cysts.

**Results:** Of the 84 patients included in the study group, in 8 cases (9.52%) were detected *Giardia lamblia* cysts. The epidemiologic investigation revealed that 5 patients were married, 2 were living in a student hostel, and one was living alone. All patients were professionally active and part of collective with over 10 individuals. Five patients presented a maculopapular urticarial eruption disseminated on thorax, abdomen and limbs, and accompanied by a moderately pruritus. All patients followed a diet and a treatment with albendazole (2x200 mg daily, 5 days), antispasmodics, antipyretics, calcium. Six of them required a hydroelectrolytic replacement with different infusions (Ringer, glucose, physiological serum). Three out of the 8 patients were ambulatory treated with antibiotics prior to hospitalization. Nevertheless the symptoms didn't remit and consequently they necessitated hospitalization. The stool exams of the family members (in 5 patients with families) showed the relevance of setting of the ambulatory antiparasitic therapy for the whole family. The outcome was favorable in all cases. Adverse reactions were minor and transitional: nausea in 3 cases, bitter taste 2 cases, and pyrosis 2 cases.

**Conclusion:** The early detection of giardiasis in patients with acute enterocolitis avoids unnecessary administration of antibiotics and allows the administration of the antiparasitic therapy with optimal clinical results and decrease the spread of this affection.

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**Type: Poster Presentation**

Immunological changes in children with giardiasis

**T. R. Olariu<sup>1</sup>**, V. Dumitrascu<sup>2</sup>, I. Marincu<sup>3</sup>, C. Petrescu<sup>2</sup>, A. Koreck<sup>2</sup>

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**Background:** *Giardia lamblia* is an intestinal parasite which has been diagnosed with an increase frequency among Romanian institutionalized children.

The aim of the present study was to assess peripheral blood lymphocytes populations and serum cytokine levels for IL-4 and IFN- $\gamma$  in children infected with *Giardia lamblia*.

**Methods:** Lymphocyte subpopulations were determined using flowcytometry and cytokines were quantified by sandwich ELISA.

**Results:** Total T cells, CD4+ helper T cells, activated T cells (HLA-DR+ or CD25+) and NK cells did not differ significantly between children with *Giardia lamblia* infection and aged-matched healthy controls. In contrast, CD8+ T cell were elevated in *Giardia lamblia* (34.42  $\pm$  6.08%) infected children compared to controls (27.66  $\pm$  2.15%). B cells were significantly reduced in the peripheral blood of children with giardiasis (16.89  $\pm$  2.44%) compared to healthy controls (26.41  $\pm$  4.12%). IL-4 was detected in higher concentration in the serum of children infected with *Giardia lamblia* (4.19  $\pm$  1.67 pg/ml) and was not detected in the serum of healthy children. IFN- $\gamma$  levels were elevated in children with giardiasis (5.7  $\pm$  5.04 pg/ml) compared to controls (0.79  $\pm$  0.47 pg/ml).

**Conclusion:** Our data suggest that *Giardia lamblia* infections in children may be accompanied by systemic immunological changes.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

IgG antibodies relative avidity as a phase-specific diagnosis for toxocariasis in populations of Venezuela

O. Delgado, J. Ortegoza, V. Coraspe, M. A. Rivas, S. Silvia, C. Duran, Y. Pascual, **A. Rodriguez-Morales**

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**Background:** IgG-Avidity ELISA has been recently developed in toxocariasis as a useful technique for ruling out recently acquired infections. Avidity of antibodies increases with time after antigen challenge and the measurement of the avidity has been used to diagnose various infections especially when differentiation of recent and distant infections is crucial.

**Methods:** In this study we focused on a method for distinguishing acute and chronic *Toxocara* infection in children and adults, the basis of which was the assessment of specific IgG antibodies avidity. We studied 400 serum samples collected from populations in Venezuela (aged 1–65y, 52% male).

**Results:** From this population screening with a validated ELISA (with locally prepared *T.canis* excretion-secretion antigens) we serologically diagnosed 71.25% of them (62% 1:128dils). From these serologically positive patients we evaluated the *Toxocara*-specific IgG avidity, which was measured employing the dissociative method using urea as a denaturing agent and the calculation of a relative avidity index. The relative avidity index was calculated as the ratio of IgG values in sera treated with urea and the value of IgG in nontreated sera ( $\times 100$ ). Values on this index  $\leq 50$  were considered as low avidity (indicating recently acquired infection) and those  $> 50$  were considered as indicative of high avidity (chronic infection). In the sera from these patients low index of IgG antibodies avidity was shown in 71.25%; 50% of these patients presented avidity values  $\leq 33$ . These values were independent of the ELISA titers of these patients ( $t=0.207$ ;  $p=0.837$ ), age ( $F=0.234$ ,  $p=0.631$ ), sex ( $t=0.624$ ;  $p=0.536$ ) and epidemiological risk factor such as dog ownership ( $t=0.955$ ;  $p=0.348$ ). In the group of patients with titers of 1:32dils mean avidity was 32.7 (low index of IgG), in those with 1:128dils mean avidity was 31.9 (low index of IgG) ( $p>0.05$ ). Compared with IgG avidity test, conventional ELISA had just 32.9% of specificity (95%CI 28.2–38.0).

**Conclusion:** These results, as other previous, suggest that measurement of specific IgG avidity (which is independent of the ELISA titers) may assist in the discrimination between recent and distant toxocariasis, and particularly to ruling out (by high avidity) a recently acquired infection.

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**Type: Poster Presentation**

Characterization of proteins of excretion/secretion (ES) of nematode parasite

*Mammomonogamus laryngeus*

**M. I. Giraldo**, J. C. Castaño

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**Background:** *Mammomonogamus laryngeus* is a hematophage nematode parasite that affects the respiratory tract of domestic mammals. The female and male are united in permanent copulation producing a distinctive "Y" form. So far, over 100 cases of human infection had been reported in the scientific literature. In Colombia in 2006 we reported the first case of human infection in the Quindio region, and the second report in bovine livestock with a high prevalence of 14.5% (Fig 1).



**In the micrograph are observed *Mammomonogamus laryngeus* specimens in copula permanent.**

**Methods:** The protein profile of excretion/secretion (ES) of *M. laryngeus* was determined by SDS-PAGE electrophoresis and the characterization of the enzyme activity was evaluated with a zymogram using casein and gelatin dissolved in 20 mM sodium phosphate buffer at pH 7.4 and copolymerization with polyacrylamide 10% and to 0.8% bisacrylamide, 1.5 M Tris-HCl, pH 8.8. The protein concentration was determined by bicinchoninic acid assay (BCA). To evaluate the type of protease on the ES proteins, a zymogram assay was performed with and without specific protease inhibitors (leupeptin trifluoro acetate, EDTA, pepstatin A, 4-2 amino ethyl benzene sulfonyl fluoride Hydrochloric (AEBSF) and N-tosyl-L-phenylalanine chloromethyl ketone (TPCK)) in 10% polyacrylamide gel and gelatin as substrate. To evaluate the effect of pH on the protein activity, the zymogram conditions were performed on pH from 4 to 11.

**Results:** The products of ES from adult *M. laryngeus* have protease activity showing four bands with molecular masses of 94.4 kDa the most dominant and a diffuse series of bands of 122 kDa, 108 kDa and 72 kDa.

The *M. laryngeus* ES shows a protease activity. For the specific family of proteases, a metalloprotease activity was found corresponding to the inhibition of protease activity by EDTA but no under other inhibitor. The pH did not change the protease activity over the range tested.

**Conclusion:** Our findings, suggest that maybe this ES proteins are involved in skin penetration process and migration through connective tissues in the host, therefore, it is mandatory to obtain more data about this parasite for the understanding of its infective process, and also look for ES homologies within the same family of nematodes for the searching of vaccine candidates and treatment strategies.

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**Session: Parasites**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Fibronectin increases the adherence of *Taenia solium* oncosphere in CHO –K1 cells *in vitro*

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**Background:** Neurocysticercosis is the infection caused by the larval stage of *Taenia solium* and is also the most common parasite of the nervous system in humans and is a public health problem in poor areas of Latin America, Asia and Africa and it is spreading worldwide due to increased migration of NC cases and tapeworm carriers. *T. solium* eggs contain an oncosphere that is released and penetrates in the host small intestine. This mechanism is not known.

Therefore, the elucidation of the molecules involved in the parasite-host cell recognition process is of extreme importance and that constitute a focal point of research. Previous works supported the idea that parasites can interact with extracellular matrix and basement membrane proteins, such as fibronectin (FN), laminin, collagen and vitronectin leading to enhanced adherence. The purpose of this study was evaluated the role of FN in the adherence mechanism of *T. solium* oncosphere using *in vitro* assay in CHO-K1 cell.

**Methods:** The *T. solium* oncospheres and CHO-K1 cell monolayer were incubated with different concentrations of FN (70, 80, 90 and 100 ug/ml) and the adherence was visualized by immunofluorescence using hyperimmune rabbit sera against *T. solium* activated oncospheres. In the other hand, only the CHO –K1 cells were preincubated with 80 ug/ml of FN for 20 hr and then the *T. solium* oncospheres with and without FN were added on monolayer cell. FN fibrils and oncospheres were visualized by immunofluorescence using polyclonal anti-FN antibody produced in rabbit by UV light microscopy.

**Results:** The adherence of *T. solium* oncospheres was increased from 70 ug/ml FN versus control. The fibrillar FN matrix on CHO–K1 cells were visualized over monolayer cell. Also, the adherence *T. solium* oncosphere with FN preincubate reacted with anti-FN antibody but with minor intensity versus control

**Conclusion:** These results suggest that Fn increases the adherence of oncosphere of *T. solium* serving as a bridge between the *T. solium* oncosphere and cell. Additional characterization of *T. solium* oncosphere fibronectin binding protein that interact with host components, will further our understanding of the adherence and could be markers as candidate a vaccines.

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**Type: Poster Presentation**

Ectopic knee tungiasis and historical aspects in Peru

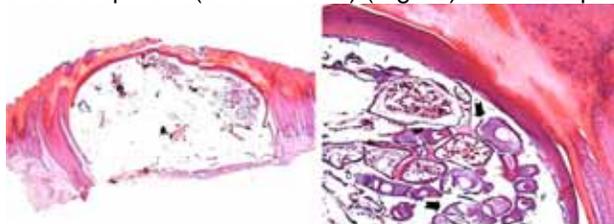
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**Background:** Tungiasis is a zoonotic ectoparasitism caused by the penetration of the female sand flea *Tunga spp.* into the skin. It can be caused by two species: *Tunga penetrans* or *T. trimamillata*, and affects commonly distal lower extremities of individuals living in poverty-stricken villages or suburban areas. Although it has been a well known entity to Peruvian physicians and other care providers alike for centuries, little is known about its current epidemiology.

**Methods:** We present a rare case of knee tungiasis which was diagnosed by excisional biopsy. Briefly, the patient was a 7 years old mestizo female with no significant PMH, who lived in Villa El Salvador, Lima, a district with high poverty index. She complained of a painful nodule in her right knee that was noticed 3 weeks prior. Her dwelling was made of straw mats and lacked electricity and water supply.

**Results:** On physical examination, a 0.5 cm tender nodule with a central black dot was noticed in the extensor aspect of her right knee, surrounded by a whitish halo and mild redness around it. On microscopic examination, the epidermis showed hyperplasia, hyperkeratosis, parakeratosis, and a hemorrhagic area in the dermis. Multiple structures compatible with eggs in different stages of development (arrow heads) (Figure) were compatible with *Tunga spp.*



**Conclusion:** To our knowledge, our case is the first human tungiasis located in the knee. Evidence of tungiasis in Peru dates back before the Inca civilization and the Conquest of the Americas. Pre-Columbian, autochthonous inhabitants of the coast depicted tungiasis on mud pottery called *huacos*, as can be appreciated in anthropomorphic ornaments. Moreover, the first references in the local literature were made by the Peruvian Indian Guaman Poma de Ayala (~1535/~1619).

Epidemiological data are scanty: a search on PubMed, Scielo, LILACS and MedLine produced no articles on epidemiology of tungiasis in Peru, except from a letter concerning the new species, and an isolated case report. With the description of a new species of *Tunga* and several Brazilian epidemiologic studies, tungiasis has regained the interest of the scientific community. Epidemiological studies with identification campaigns to understand the status of this ancient parasitism in Peru is our next step.

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**Type: Poster Presentation**

Blastocystis hominis infection among patients with and without gastrointestinal disorders

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**Background:** *Blastocystis hominis* is a common microscopic parasite found in human fecal samples both from symptomatic patients and from healthy people. The aim of this study was conducted to investigate the epidemiology and clinical features of *Blastocystis hominis* among Iranian patients with and without GI symptoms.

**Methods:** Six hundred and seventy patients without GI and 670 patients with GI symptoms recruited during 2006-07. Standard microscopic examinations following *in vitro* culture were used to examine the stool samples for presence of trophozoites and cysts of *B. hominis*.

**Results:** Infection with *B. hominis* occurred most commonly in those with GI symptoms (5.67 %) compared with those patients without GI symptoms (3.43 %). The most common symptom in case group was abdominal pain (86.84 %). *B. hominis* was mostly found with *Giardia lamblia* in case group and with *Entamoeba coli* in control group. In addition, there was no significant relation between the presence of GI symptoms and the incidence of *B. hominis*.

**Conclusion:** In the current study, with respect to no statically significant difference between case and control group in term of *B.hominis* prevalence ( $P>0.05$ ), we can express this hypothesis that support the idea which *B.hominis* is not a GI symptom ethological agent in contrast to other studies Iran. Thus, to confirm the complication is needed to additional study especially on molecular pathogenesis of this organism.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

A Portuguese couple with eosinophilia: From the diagnosis to the treatment

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**Background:** Zoonotic diseases are hard to control and eliminating as they require coordination among veterinary, livestock and human health departments and frequently are neglected diseases.

**Methods:** We describe the work up of a young couple presenting with vague abdominal complains and marked eosinophilia.

**Results:** A 35 years-old man, and his wife, 25 years-old, both previously healthy, without international travel, living in a small town in the north of Portugal, presented to their medical doctor in October 08 complaining from right upper quadrant pain, asthenia, nausea, cutaneous pruritus and urticaria; they had lost 4 pounds of weigh in 3 months. One month before becoming ill they ingested uncooked watercress from local production; vomiting and diarrhoea occurred for a few days after the ingestion. Physical examination was unremarkable. Liver and renal function tests were normal except for GGT that was two times elevated; eosinophilia was revealed. In both 3 stool samples were negative for parasites; abdominal ultrasonography and pulmonary XR were normal. Serology titer for *Fasciola hepatica* (hemagglutination test) was high positive. Praziquantel was prescribed (while waiting for triclabendazol (TCB) importation) with a short course of steroids. Two months after no clinical or analytical improvement occurred. Praziquantel was repeated without sucesss; in both stool samples remains negative and abdominal ultrasonography normal. Magnetic resonance imaging (MRI) revealed multiple ill-defined subcapsular clustered areas of low attenuation, more sharply delineated after contrast administration (later images), more pronounced in female patient. Two months after triclabendazol prescription (no elicited side effects) they gain weight and feel well; eosinophilia was absent and serology titer dropped in the male patient but not in the female. They are still on observation.

Date	WBC cmm / Eosinophilis (%)		Fasciola Serology (Cut-off : 1/320)		Treatment
	Male	Female	Male	Female	
February	16640 / 43	17220 / 58	1/640	1/5000	PZQ (March)
May	15000 / 32	12240 / 44	1/2560	1/5120	PZQ (May)
July	13280 / 32	10820 / 42	1/1280	1/1280	TCB (July)
October	10580 / 9	6860 / 10	1/520	1/2560	-

**Conclusion:** The diagnosis of acute/prolonged acute fascioliasis is high probable in both patients. The value of MRI in the diagnosis/evolution is still questionable. The access to triclabendazol is not so easy, although the WHO donation program can be applied for endemic countries. Treatment before the chronic stage is needed. The control of livestock disease is urgent.

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**Type: Poster Presentation**

Role of serology, neuroimaging and stool examination in diagnosis of neurocysticercosis

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**Background:** Neurocysticercosis(NCC) is a worldwide problem. It is the most common parasitic neurologic disease and the single most common cause of acquired epileptic seizures in the developing world. Many cases remain undiagnosed due to lack and expertise and lab facilities. and it remains a public health burden because of inability to identify and treat the intestinal carrier of the parasite. Currently it is diagnosed by detection of specific antibodies or by imaging techniques. The study is done to evaluate diagnostic significance of serology (ELISA) and neuroimaging technique in NCC and to determine the intestinal carrier of the parasite.

**Methods:** The tests (Neuroimaging, ELISA and stool routine and microscopy) was applied to neurocysticercosis patients, as well as to healthy controls and individuals with other parasitic infections. A total of 100 serum samples were obtained from patients meeting clinical, imaging and epidemiological criteria for neurocysticercosis. Samples were processed by enzyme-linked immune-sorbent assay. Controls included 50 serum samples from matched hospitalized controls with a diagnosis unrelated to Neurocysticercosis . Similarly, 50 serum samples from patients with parasitic infections different from cysticercosis.

**Results:** A total of 200 samples were analyzed. In samples from neurocysticercosis patients and healthy control individuals, the ELISA test showed an overall sensitivity of 80.7% (CI 95% ) and a specificity of 85.4% (CI 95%). Out of 50 samples from patients with parasitic infections different from taeniasis, 8% were positive in ELISA. Abnormal neuroimaging is seen in 100% of the cases whereas confirmation of diagnosis by neuroimaging alone could be made only in 40% of the cases based on diagnostic criteria. Stool microscopic examination showing eggs of *Taenia solium* is seen in 18% of the cases.

**Conclusion:** The study indicated that Combined serology and neuroimaging should be done to confirm the diagnosis. Serology can be used as screening test for the diagnosis of cysticercosis. And all the patient should be treated for the intestinal carrier of the parasite as well.

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**Session: Parasites**

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**Type: Poster Presentation**

*Ixodes persulcatus* tick salivary gland extract (SGE) inhibits IL-4 and IFN- $\gamma$  secretion and CD69 expression by mitogen-stimulated murine splenocytes

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**Background:** The tick *Ixodes persulcatus* are the main vector of Lyme disease and viral encephalitis in Europe and Asia. Immunosuppressive agents in tick saliva aid successful blood meal acquisition and appear critical to the transmission and establishment of pathogens, both viral and bacterial. Our present work was undertaken to investigate modulation of IL-4, IFN- $\gamma$  production and CD69 expression of murine splenocytes by *I. persulcatus* SGE, derived from unfed and partially fed (3 days) *I. persulcatus*.

**Methods:** Splenocytes were isolated from naive BALB/c mice. Cells ( $2 - 5 \times 10^6$  /mL) were cultured in RPMI supplemented with 10% foetal bovine serum, 2 mM glutamine, 1 mM sodium pyruvate, 100 U/mL penicillin and streptomycin. Splenocytes were stimulated in triplicate by addition of ConA, LPS (Sigma) and LAC GP (Leukocyte activation Golgi Plug, BD) at a final concentration of 5  $\mu$ g/mL, 1  $\mu$ g/mL and 1  $\mu$ g/mL, respectively. Concentration of SGE in cell culture were 5, 25 or 50 mkg/ml. Splenocytes were harvested after 24-h stimulation and stained for surface markers CD3, CD4, CD19, CD69 and intracellular cytokines IL-4 and IFN- $\gamma$ . Cells were analysed by flow cytometry.

**Results:** We found that SGE, derived from both unfed and partially fed *I. persulcatus*, reduced the early activation marker CD69 expression on ConA- or LPS-activated T- and B-cells in dose dependent manner. SGE reduced IL-4 and IFN- $\gamma$  production by LAC GP stimulated CD4+splenocytes. SGE, derived from unfed *I. persulcatus*, markedly suppressed IL-4 and IFN- $\gamma$  production by LPS-stimulated splenocytes by 35% and 55%, respectively, compared to the control condition without saliva. No significant modification of IL-4 and IFN- $\gamma$  production was observed when SGE, derived from partially fed ticks, was added.

**Conclusion:** Only SGE, derived from unfed *I. persulcatus*, inhibits IL-4 and IFN- $\gamma$  production by CD4+ T cells in naive murine splenocytes. The inhibition of IL-4 and IFN- $\gamma$  production by SGE appears to be due to a reduced level of activation in T and B cells: SGE treatment reduced the activation-induced CD69 expression on splenocytes. Transmission of pathogens into a site with lymphocyte unresponsiveness would have great advantages for the establishment and dissemination of the pathogen in the host.

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**Final Abstract Number: 58.030**

**Session: Parasites**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Seroprevalence of toxocariasis in hypereosinophilic individuals in Ahwaz city

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**Background:** Human toxocariasis is a helminthozoonosis due to the migration of *Toxocara* larvae in to human organs. Toxocariasis is one of the cause eosinophilia in peripheral blood, and provokes eosinophilic infiltration in internal organs. The aim of this study was to evaluate the prevalence of toxocariasis in hypereosinophilic individuals in Ahwaz city by Enzyme linked immunosorbent assay (ELISA) and Western blot techniques, using excretory-secretory *Toxocaracanis* antigen.

**Methods:** Serum samples from 100 persons presenting with peripheral blood eosinophilia (>500 eosinophils/ $\mu$ l or more, equal or >10% of total white blood cells), and from 100 blank persons (without eosinophilia) were examined using ELISA technique (IBL Hambur). Sera with *Toxocara* antibodies underwent further conformational evaluations through Western immuno-blotting. Results then, were analyzed using statistical descriptive and Chi-Square test.

**Results:** Seroprevalence antibodies against *Toxocara* in hypereosinophilic individuals, with ELISA test were obtained in 19% (19 persons) of which, 12 persons (63.15%) were female and 7 persons (36.85%) were male. Positive sera were subsequently confirmed by Western blot. All of the observed bands ranged from 24 to 100 KDa. Antibodies against *Toxocara* among blank persons was 1% which did not confirmed by Western blot. There were significant differences between the frequency of infection with age and gender ( $P>0.05$ ). The highest prevalence of infection was seen in adult men and women. Also, in this study significant differences was observed between the hypereosinophilic and healthy individuals, in term of *Toxocara* infection frequency ( $P>0.05$ ).

**Conclusion:** The present study confirms the significant prevalence of toxocariasis, as a hygiene problem, among hypereosinophilic individuals in this area, and it is necessary to examine the hypereosinophilic individuals for toxocariasis.

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**Session: Parasites**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Immunogenicity of the recombinant ASP1r protein of *A. caninum* in a murine model

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**Background:** Ancylostomiasis is a disease produced by the intestinal hematophagous nematode *Ancylostoma caninum*. This parasite has as major excretion/secretion protein ASP1, which has a molecular weight of 42KDa and is encoded by a gene of 1016 pb.

**Methods:** RNA of adults of *Ancylostoma caninum* was amplified by reverse transcription polymerase chain reaction. The ASP1 protein gene was inserted into the pcDNA3 vector. Plasmid was digested with Bamh1 and EcoR1 and cloning was performed directionally. Later on, a transformation and selection of *E. coli DH5a* cell competent with the product for the ligation was made. Then, a screening by PCR was made to confirm the presence of ASP1 gene. PcDNA3-ASP1 was inoculated by intraglandular parotide and intramuscular route in Balb/c mice. Determination of antibodies in these animals was measured in serum and saliva by ELISA and immunochemistry.

**Results:** PcDNA3-ASP1 was incorporated and expressed in *E. coli DH5a* cell. This recombinant plásmid induced production of antibodies anti-ASP1 specific in Balb/c mice.

**Conclusion:** It was possible to demonstrate that using of pcDNA3-ASP1 did not display reactogenicity and it did not produce unfavorable reactions, plus it induced a humoral response against the excreción/secretion protein of *Ancylostoma caninum* in mice.

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**Final Abstract Number: 58.032**

**Session: Parasites**

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**Type: Poster Presentation**

Prevalence of intestinal parasites in urban, rural and commercial farming communities in Zimbabwe

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**Background:** An estimated 3.5 billion people worldwide are infected with intestinal parasites mainly in the developing countries where poor water and sanitation facilities play a pivotal role in transmission of diseases. Previous studies on human protozoan parasites carried out in Zimbabwe by Mason and Patterson (1987) and Simango and Dindiwe, also in 1987 concentrated mainly on *Giardia duodenalis*. This study was done 21 years later, has provided the current status of intestinal parasitic infections in Zimbabwe, as societies change as a result of migration and constant change in socio-economic status.

**Methods:** Prevalence of parasitic infections in 1260 school pupils living in an urban, rural tribal trust land and commercial farming area of Zimbabwe was done. Stool specimens were collected on 3 alternative days and processed for parasites according to the standard operating procedures (SOPs) and a questionnaire was administered. The assays carried out were; wet preparations then the specimen was pooled in to sodium acetate formaldehyde preservative and the formal ether concentration method was done on the pooled specimen. The staining methods were the cold Ziehl Neelson stain and Gomori/trichrome staining.

**Results:** *Giardia duodenalis* and *Cyclospora cayetanensis* were prevalent in the rural area, the risk factor of being infected with *G. duodenalis* being, not washing fruits before eating and drinking water from deep protected wells. Parasites most prevalent in the farming area were *Entamoeba histolytica/dispar*, *Cryptosporidium parvum* and the helminthes. The risk factor of being infected with *E. histolytica/dispar* was not taking measures to make water safe for drinking. Rare protozoan parasites such as *Entamoeba polecki* and *Enteromonas hominis* were also identified in the rural and farming areas respectively. *Blastocystis hominis* was prevalent in the urban area.

**Conclusion:** Measures such as health education and provision of safe water can now be undertaken by policy makers to prevent infections by intestinal parasites.

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**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

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**Type: Poster Presentation**

Beijing genotype and other predominant *Mycobacterium tuberculosis* spoligotypes observed in Mashhad city, Iran

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**Background:** The molecular epidemiology of tuberculosis in Khorasan province of Iran was studied by Spoligotyping 113 *Mycobacterium tuberculosis* isolates. The Spoligotyping results were in comparison to the word Spoligotyping Database of Institute Pasteur de Guadeloupe (SpolDB4). Spoligotyping data from Iran have rarely been described up to the present moment, and there is limited information on the major circulating clades of *M. tuberculosis* in Iran was present.

**Methods:** Spoligotyping was performed on 113 *M. tuberculosis* isolates from Mashhad patients from November 2004 to September 2005.

**Results:** Fifty-seven Spoligopatterns were seen in this study and 17 different clusters and 32 true orphan genotype was seen. The biggest cluster has 13 isolates and had not been previously reported. The Beijing genotype was seen in 8 (7.1%) isolates.

**Conclusion:** The genotyping and Spoligotyping provides a unifying framework for both epidemiology and evolutionary analysis of *M. tuberculosis* populations.

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**Final Abstract Number: 59.002**

**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Integrating TB screening tool improved TB screening in HIV Patients in TASO

**S. Okoboi**

The Aids Support Organization, Tororo, tororoi, Uganda

**Background:** Globally 9.2 million new TB cases and 1.6 million deaths occur annually, 16% of new TB patients are HIV positive (WHO, 2007), Uganda estimated 70% of TB cases are HIV positive (NTLP, 2007). In 2008 out of 82114 (94%) of TASO registered clients screened for TB, 1960 (2.4%) were diagnosed with TB and treated (TASO MIS). Globally case target detection target is 70% of infectious TB and successfully treat 85%. Uganda detection rate is 60.1%, cure rate 62.2 % (HSSP II), integrating TB screening tool reminded clinicians to screen for active TB in TASO.

**Methods:** This involves completing clinical record forms by assessing if the patient enrolled for TASO care is symptomatic for TB using a screening tool. Patients who screened positive for one or more signs/symptoms were further investigated to confirm active TB by sputum analysis, chest X-ray, lymph node aspirates and pleural tap for analysis. Cases with confirmed TB received respective treatment as they continue to attend the usual medical review on appointment.

**Results:** Overall percentage of patients screened for TB improved from 78% to 98% between fourth quarter, 2008 and first quarter, 2009. Of those screened the percentage of patients with at least one or more positive signs/symptoms increased from 23.3% to 42.5% within the same period TASO Tororo MIS, out of those with a positive screen who took a test, the percentage of patients diagnosed with TB increased from (26/126)15.9% to (38/141) 27% within the same period.

**Conclusion:** Integrating screening tool in clinical record will; prompt clinicians to screen for active TB at each clinic visit, allow continuity and quality of TB care, prevent unmasking of TB through immune reconstitution syndrome in patients with lower CD4 cell count initiating ART and monitors TB investigation results, treatment, progress and outcomes.

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**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

A case report: Cutaneous tuberculosis presenting as chronic thoracic chest wall fistula

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**Background:** Cutaneous tuberculosis is now uncommon despite the rising incidence of extrapulmonary tuberculosis in areas with high prevalence of HIV infection. Nevertheless it is still important to know the multiple clinical manifestations of cutaneous tuberculosis .We report a rare form of cutaneous tuberculosis presenting as cutaneous fistula over the anterior thoracic chest wall.

**Methods: Patient presentation:**

A 50- year- old man from Afghanistan who presented with complaint cutaneous lesions over the anterior chest wall. There were two fistula with purulent discharge which started 3 month ago as a small papule without pain and pruritus the size of papules increased and there was no response to outpatient antimicrobial treatment There was history of diabetes mellitus type 1 , ischemic heart disease and hyperlipidemia .he migrated from Afghanistan to Iran 16 year ago and he had no travel to there during past years . There was no history of TB in patient and his family. The PPD test was negative. In laboratory examination there was significant increased of ESR (125mm/hr)and anemia (HB:11.1 g/dl). Cutaneous TB was suspected regarding to lack of response to antibacterial treatment and persistence of disease .Skin biopsy showed multiple granulomas consisting langhans giant cells compatible with tuberculosis and Ziehl-Neelsen staining of secretions revealed numerous acid fast bacilli .Anti tuberculosis treatment with Isoniasid,Rifampin, Ethambutol, Pirazinamid was administered. Culture of secretion was positive for mycobacterium tuberculosis after 4 week As a result of these medication the skin lesions started to heal and after 6 month healing was complete and there was no fistula.

**Results:** this is a csae report and it has no results

**Conclusion:** cutaneous tuberculosis can occur in an unusual form in an immunocompetent patient such as fistula formation .Regarding to high prevalence of TB among Afghanian people, TB must be suspected in any chronic cutaneous lesion and the disease deserves special attention because its prevalence can be expected to increase due to high rate of migration from Afghanistan to Iran.

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**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Use of Immunotest (tuberculin skin test) for early detection of pulmonary tuberculosis among health workers in a private hospital, 2008, Lima, Peru

**S. Manga**

Chemonics, Lima, Lima, Peru

**Background:** To determine the occupational risk of tuberculosis infection among health care workers. Examine the efficacy of intradermal tuberculin test in a population with high vaccination coverage rates with antibodies to Bacille Calmette Guerin.

**Methods:** After obtaining informed consent, to fill a questionnaire, TST test was performed Mantoux technique using 0.1 ml of pure protein intradermal tuberculin then we measured intradermal reaction between 304 workers include doctors, resident nurses, technical and nursing assistants, pharmacist, exclusion criteria pregnant or postpartum women Positives cases were include into a diagnosis confirmation protocol Radiological and Chest CT, serologic test of adenosine deaminase, erythrocyte sedimentation rate, Sputum Culture by staining Nielseen Zielh Nielseen method was used.

**Results:** 272 dose of 0.1 ml. pure protein derivative of tuberculin RT 23 were applied. Reading was 72 hours after. Were found 52 (19.11%) of positives cases when the cutoff was 10 mm and 50 (18.38%) when the cutoff was 15 mm. After a positive test greater than or equal to 15 mm, the worker was admitted into a deep diagnostic protocol by the pulmonologist, which consists on: radiological examination CT; erythrocyte sedimentation rate, serologic test of adenosine deaminase; test for AFB staining Nielseen Zielh or sputum culture and sensitivity or bronchoscopy in 03 workers

10.66% were positive to the test TB, and three cases of active lung tuberculosis (36x 100.000) was founded those started DOTS

23 workers were test positive they are receiving Isoniazid

**Conclusion:** On despite of high vaccination coverage rates with antibodies to Bacille Calmette Guerin (BCG) in a health care workers in Lima Peru; the intradermal reaction of a PPD showed high efficacy in early detection of occupational related lung TB cases, which is an important founding in a country living an multiresistant TB outbreak.

The intradermal test using pure protein derivative of tuberculin RT 23 were efficient on detecting early active lung TB cases

Accordingly with the results the occupational group at higher risk are nursing techniques (ODSS ratio 2.72), followed by Resident Nurse (ODSS ratio 2.02); Medical doctors (ODSS ratio 1.99) Medical Technologists (ODSS ratio 1.25). We strongly recommended using this simple, cheap easy to use, test at least every 9 months in all health care workers.

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**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Predictive value and cut off point of PPD in probable patients with pulmonary tuberculosis

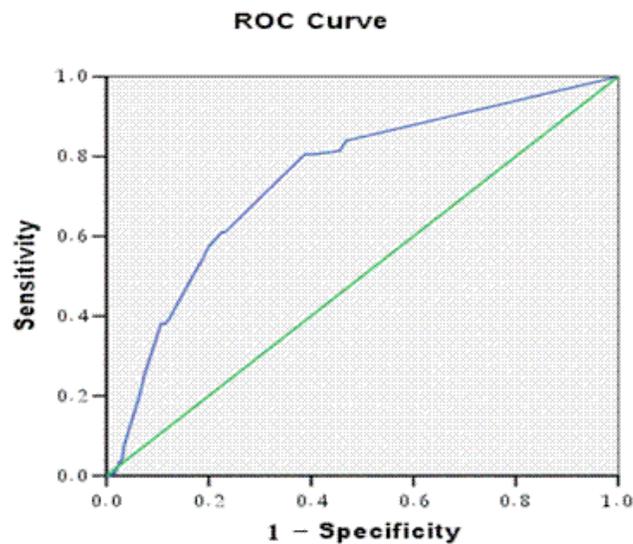
**M. sofian**<sup>1</sup>, L. MOINI<sup>1</sup>, S. Farahani<sup>2</sup>, A. rostampour<sup>3</sup>, H. Khalili<sup>2</sup>, A. Aghakhani<sup>4</sup>, M. Banifazi<sup>4</sup>, A. Ramezani<sup>5</sup>

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**Background:** Tuberculin skin testing (TST) is used for identification of tuberculosis infection. Interpretation of test is related to many factors such as prevalence of disease in community, use of vaccine, age of patients and exposure to *non-tuberculous mycobacteria*. Cut off point of TST is reported between 2 to 16 mm in different communities. In this study we aimed to determine the predictive value and cut off point of TST in probable patients with pulmonary tuberculosis (TB).

**Methods:** A total of 714 probable patients with pulmonary tuberculosis with mean age  $57 \pm 20.11$  years were included in this study. 52.9% of them were male. All subjects were tested using 5 tuberculin units of purified protein derivative (PPD). Gold standard for diagnosis of TB was considered positive smear or culture. Specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) of TST were determined. Cut off point of PPD was determined using Roc curve.

**Results:** 113 patients from 714 suspicious subjects (15.82%) had pulmonary tuberculosis. 80% of them had positive sputum smear. Sensitivity, specificity, PPV and NPV for  $PPD \geq 5$ mm were 80%, 61%, 28% and 94% respectively. Sensitivity, specificity, PPV and NPV for  $PPD \geq 10$ mm were 53%, 82%, 35% and 90% respectively. The calculated cut off point for PPD was  $\geq 4$  mm.



Diagonal segments are produced by ties.

**Conclusion:** Our results showed that a TST reaction smaller than 5 mm was seen in patients with bacteriological confirmed pulmonary tuberculosis. Predictably, as the cut-off is moved to larger reactions, sensitivity decreases and specificity increases. More estimates are needed to identify the accurate cut off point of PPD in tuberculosis patients.

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**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Challenges in the case detection of sputum smear-positive tuberculosis in Rwanda: A low-ressource setting

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National University of Rwanda/Faculty of Medicine, Butare, Rwanda

**Background:** The use of smear microscopy in patients suspected of tuberculosis presenting to health services is of great value in case detection and in reducing the spread of the infection throughout the population by treatment of such cases. The TB diagnosis using Smear microscopy places a burden on the patient. A patient with suspected TB typically has to visit the clinic at least twice before a diagnosis can be made, and then has to return again for the results. In the present study, we aimed to evaluate the performance of the sputum smear microscopy and its public implication with reference to recommendations for the number of specimen that have to be collected from TB suspect.

**Methods:** This study was conducted at the department of microbiology, University of Butare Teaching Hospital after obtaining clearance from the ethical committee. In this study, using TB register and laboratory sputum register, we review laboratory records of seven hundred and forty six patients who were TB suspect during the period from January 2007 to December 2008. Data processing and statistical analysis were performed using SPSS software (Windows version 16.0). The results were expressed as percentage, with significance at 5%.

**Results:** During the study period, 741 new TB suspects submitted sputum specimens for AFB smear. Among the suspects, 46% (341) of patients submitted sputum specimens in accordance with the guidelines (i.e., three sputum samples), 14.3% (106 of 741) had two sputa submitted for AFB smear and 39.7% (294 of 741) submitted only one sputum specimen. Of the 39.7% of TB suspects who did not show up after submitting the first on-spot specimen, 6.5 % (19) had a smear-positive first.

Of 341 patients submitting three sputum samples, 55 (16.1%) suspects were found to have at least one positive smear. Of these, 47 (85.5%) were detected from the first specimen and 8 (14.5%) were positive on the second specimen but not the first. The third specimen did not have any additional diagnostic value for the detection of AFB as shown in Fig. 2.

**Conclusion:** In conclusion, our result show that examining two sputa smears was sufficient for the detection of AFB in our laboratory. Further research involving different laboratories from all of the regions of Rwanda is needed to reassess these findings.

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**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Predictive factors for the indeterminate result on the QuantiFERON-TB Gold In-Tube test

**S. H. Han**, B. S. Chin, H. Choi, S. Y. Shin, Y. T. Chae, S. J. Jin, J.-H. Baek, C. O. Kim, J. Y. Choi, Y. G. Song, J. M. Kim

Yonsei University College of Medicine, Seoul, Korea, Republic of

**Background:** QuantiFERON-TB Gold In-Tube (QFT-G IT) test is recommended for screening of latent tuberculosis. However, this can reveal the indeterminate results, especially in the immunocompromised patients. Therefore, we investigated the predictive factors that can influence the indeterminate results on QFT-G IT.

**Methods:** We performed a retrospective case-control study with 144 cases with the indeterminate results on QFT-G IT from October 2006 to March 2009 at Severance Hospital. The controls with the positive or negative results were selected by matching 1:1 with the case for date of QFT-G IT assay. An indeterminate result was considered when the level in both the tuberculosis antigen and mitogen tube was less than 0.35 IU/ml, or the level in the nil tube was higher than 8.0 IU/ml. Through retrospective review of medical records, age, gender, total lymphocyte count at the QFT-G IT assay, underlying diseases, and medical conditions which can influence the immune function were collected as covariates. The predictive factors for the indeterminate results on QFT-G IT were assessed by multivariate logistic regression models.

**Results:** Age and gender did not show the significant differences between case and control patients ( $p=0.105$  and  $0.906$ ). The percentage of patients with hematologic malignancy (19.4% vs. 11.1%,  $p=0.049$ ), chronic renal disease (22.2% vs. 9.7%,  $p=0.004$ ), and autoimmune disease (16.0% vs. 6.3%,  $p=0.009$ ) in the case group were the significantly higher than those in the control group. Also, the case group had the significantly higher percentage of steroid use (16.0% vs. 8.3%,  $p=0.047$ ) and solid organ transplantation (11.1% vs. 2.1%,  $p=0.002$ ) than the control group. The total lymphocyte count was the significantly lower in the case group than in the control group (median [interquartile range], 540 [220-1,030] vs. 1,370 [870-1,970],  $p<0.001$ ). In the final regression model, clinical factors associated with the indeterminate results were chronic renal disease (odds ratio [OR] 2.26, 95% confidential interval [CI] 1.03-4.94,  $p=0.041$ ), autoimmune disease (OR 3.99, 95% CI 1.65-9.66,  $p=0.002$ ), and solid organ transplantation (OR 5.06, 95% CI 1.30-19.73,  $p=0.020$ ).

**Conclusion:** The patients with lower lymphocyte counts or immunosuppressive condition showed the higher possibility of the indeterminate results on QFT-G IT assay. Therefore, the combined assay such as tuberculin skin test and T-SOPT TB with QFT-G IT may be helpful to screen the latent tuberculosis in the patients with these conditions.

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**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

A retrospective study to assess treatment outcomes among TB patients taking DOTS treatment in South India

**P. Narasimhan**<sup>1</sup>, R. Peter<sup>2</sup>, A. Manoharan<sup>2</sup>, J. Wood<sup>1</sup>, R. MacIntyre<sup>1</sup>, D. Mathai<sup>2</sup>, K. John<sup>2</sup>  
<sup>1</sup>University of New South Wales, Sydney, Australia, <sup>2</sup>Christian Medical College and Hospital, Vellore, India

**Background:** In spite of the wide implementation of Directly Observed Treatment Short course (DOTS) strategy in India, Tuberculosis (TB) still remains a major public health problem in the country. There is a considerable doubt about the effectiveness of DOTS program in TB control in the country; a retrospective analysis was conducted to assess the response to treatment among individuals who take TB medications through the DOTS program in South India

**Methods:** Retrospective chart review of 136 consecutive TB cases who sought care at the DOTS clinic in Christian Medical College and Hospital, Vellore, South India during the period October 2008 to October 2009

**Results:** Of 136 patients who sought care there were 85 males (63%), median age was 39 years, 15 tested positive for HIV, 68 negative for HIV and 52 of them had no HIV status revealed. Of the total, 91 (67.4%) had pulmonary TB and 43 (32%) had extra pulmonary TB. Almost 104 (77%) of them were new cases of TB, 7 (5%) were defaulters from prior TB treatment. 99 of them had their sputum tested for AFB of which, 76 tested positive for AFB and the rest had negative smear results. There were no reports available for the rest.

At the end of intensive phase of treatment there were nearly 18 (27%) of them who remained smear positive in spite of supervised treatment. Similarly at the end of two months continuous phase there were 2 (8%) of them who still remained smear positive. There were a total of 6 patients who were completing 6 month DOTS therapy, among which one person had converted from smear negative to smear positive at the end of treatment.

**Conclusion:** Nearly one fourth of the cases remained smear positive at the end of intensive phase, in spite of supervised treatment, in our study sample. Cases which do not respond to treatment should be tested for drug resistance and susceptibility at the earliest. Unless there is rapid diagnosis and treatment of treatment failure and relapse cases there won't be any progress in our efforts to control TB.

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**Final Abstract Number: 59.009**

**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Population-based investigation on molecular characterization of Fluoroquinolones resistance of Mycobacterium tuberculosis in rural area of eastern China

**B. Xu, Y. Hu, W. Jiang, W. Wang**

School of Public Health, Fudan University, Shanghai, China

**Background:** Extensive use of fluoroquinolones in treating numerous bacterial infections might select for resistant strains of Mycobacterium tuberculosis (*M.TB*), especially in China where uses of fluoroquinolones in treating TB have not been standardized yet. The present study was to investigate the prevalence of fluoroquinolones resistant TB and its molecular characterization in rural areas of eastern China.

**Methods:** Deqing and Guanyun, two rural Chinese counties in neighboring provinces (Zhejiang and Jiangsu), were selected as the field site, where 164 and 187 isolates were collected from the pulmonary TB patients registered in local TB dispensaries during 2004/2005. The susceptibility to 1st line anti-TB drug and Fluoroquinolones was determined by the proportion methods. IS6110 restricted fragment polymorphism (RFLP) was performed for genotyping and DNA sequencing on hotspot region of *gyrA* gene was performed as well.

**Results:** Fluoroquinolones resistance was detected in 31 of 351 isolates including 24(6.8%), 11(3.1%) and 10(2.8%) resistant to ciprofloxacin, ofloxacin and levofloxacin respectively. Fluoroquinolones resistance was equally distributed in MDR-TB(15.8% and 11.8%), other combination(8.5% and 9.1%) and 1st line drug susceptible group(6.3% and 7.7%) both in Deqing and Guanyun. And the similar proportion of fluoroquinolones resistance has also been observed in groups of subjects with different social-demographic, clinical and bacteriological features. Mutations in the quinolone resistance determining regions(QRDRs) of *gyrA* were found in 17 of 31 fluoroquinolones resistant isolates in codon 94(45.2%), 90(12.9%) and 74(3.2%). IS6110-RFLP identified 2 clusters within fluoroquinolones resistant isolates and 3 clusters composed by both fluoroquinolones resistant isolates and fluoroquinolones susceptible isolates. The geographic distribution of the fluoroquinolones resistant TB patients noted their mainly distributed in central town areas of rural counties both in two counties, with 6(42.9%) from Deqing and 8(47.1%) from Guanyun respectively.

**Conclusion:** Fluoroquinolones resistance has emerged in *M.TB* circulating in rural China and mainly related to the mutation in QRDR region of *gyrA*. The selective growth of fluoroquinolones resistant strain might be the main cause for the epidemic of fluoroquinolones resistant TB in rural China. And the TB patients from central town areas deserve the special concern for the development of fluoroquinolones resistance.

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**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Usefulness of PCR in the differential diagnosis of tuberculosis in paraffin embedded tissues  
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<sup>1</sup>Universidad de Concepcion, Concepcion, Chile, <sup>2</sup>Hospital Guillermo Grant Benavente, concepcion, Chile, <sup>3</sup>Hospital Guillermo Grant Benavente, Concepcion, Chile

**Background:** Confirmation of differential diagnosis of *Mycobacterium tuberculosis* (MTB) is important on the finding of granulomatous inflammation that may or may not be associated with infectious agents. Conventional microbiological methods are suboptimal for samples formalin-fixed and paraffin-embedded (FFPE) for histological analysis. The usefulness of PCR for the diagnosis of MTB have been demonstrated with fresh samples, but still requires validation for FFPE. The aim of this study was to compare the diagnostic efficacy of a nested IS6110 PCR for MTB in FFPE and its correlation with histopathology finding and auramine / Ziehl-Neelsen stains.

**Methods:** We analyzed 110 FFPE (44 lymph nodes, 12 skin, 10 pleura, 10 lung, 5 breast, 4 intestine and 21 other tissues (brain, prostate, uterus, bone marrow, kidney, synovial fluid, larynx, mouth, appendix, among others).

FFPE Histologic Groups. According to histologic diagnostic of granulomatous lesion.

1. Granuloma with caseous necrosis (n=52); 2. Granuloma without caseous necrosis (n=25); 3. Granuloma tuberculoid-sarcoidal (n=9); 4. Suppurative granuloma (n=7); 5. Foreign body granuloma/chronic nonspecific inflammation (n = 17).

FFPE Processing. 2-5 sections of 5µm of identified lesion were deparaffinized, rehydrated and digested with proteinase K and DNA extracted with a Qiagen-Puregene commercial kit following manufacturer instructions. Quality of DNA was assessed by amplification of a 110 bp beta globin segment. Only FFPE beta globin positive samples were utilized for analysis.

Nested IS6110 PCR. Outer and inner primers were utilized to amplify a 123 bp segment of IS6110 repetitive insertion of MTB complex genome.

**Results:** Sensitivities of PCR versus staining for MTB for FFPE were: for granulomas with caseous necrosis, PCR 57.6%, auramine/ZN 19%. For granulomas without caseous necrosis: PCR 24.%, auramine/ZN 12.5%. No positives were found in the other histologic groups for PCR and stains. Clinical validity for PCR was estimated considering as most probable TB granulomas with caseous necrosis and those positive for stains. PCR had: 62% sensitivity, 93% specificity, 88% positive predictive value and 70% negative predictive value.

**Conclusion:** PCR is a useful complementary technique for diagnosing MTB in FFPE, with sensitivity and specificity considerably greater than auramine/ZN. Nested IS6110 PCR increased the accuracy of histological diagnoses associated with granulomatous tissue response.

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**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Genetic diversity and population structure of *M. tuberculosis* strains circulating in Central Russia

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Applied Microbiology and Biotechnology, Obolensk, Russian Federation

**Background:** All of 1200 collected strains with a unique spoligotype (57 of a total 72 spoligotypes) were included into the sample. Among the strains with shared spoligotypes, two to eight epidemiologically unlinked strains were chosen for analysis. The resulting panel of 107 strains should be representative of the diversity of *M. tuberculosis* strains circulating in the Central region of Russian Federation.

**Methods:** The genetic relatedness of the isolates in the sample was assessed using five genotyping methods: IS6110-RFLP, spoligotyping, MIRU-VNTR typing, SNP-based identification of principal genetic groups, PGGs, and SNP clustered groups.

**Results:** Members of 4 from 6 SCG were identified in the study sample. SCG6/PGG3 strains formed two groups. According to the IS6110 fingerprint patterns, SCG3/PGG2 was divided on at least 5 groups, including Haarlem strains and all strains with low IS6110 copy number.

MIRU copy number was identified in 12 microsatellite loci. A total of 58 distinct profiles were recognized with 38 unique profiles and 20 clusters comprising from 2 to 23 isolates.

Phospholipases C (PLC), have been shown to be involved in *M. tuberculosis* virulence. In this study, we analyzed the frequency of IS6110 insertions into the phospholipase C operon (*plcABC* genes) among clinical isolates of *M. tuberculosis* from Central Russia. Of the 107 isolates analyzed, 47 contained an IS6110 insertion. In 45 of the 47 isolates, the IS6110 element was inserted at the same position and in the same orientation within the *plcA* gene as determined by sequencing.

The stable association of the Ins2 IS6110 insertion in the *plcA* gene with the group supports the hypothesis of their clonal expansion from the common progenitor. The MDR TB level in previously treated patients in prison hospital approaches to 100% and exceeds 50% among civilians. Rate of M tuberculosis strains resistant to RIF INH and KAN was extremely high.

**Conclusion:** These data support the assumption that a parental strain had been circulating in the environment for a prolonged period of time and had been split into a number of variant strains after acquisition of resistance to many antituberculosis drugs.

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**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Evaluation of IS6110 PCR, BACTEC and conventional methods in rapid diagnosis of extra pulmonary tuberculosis cases attending two tertiary care hospitals in North India

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**Background:** In developing countries the diagnosis of extra pulmonary tuberculosis (EPTB) with conventional diagnostic tools is a major challenge. EPTB encounters many problems like paucibacillary nature, inadequate sample volume. All the limitations reflect in the poor contribution of conventional bacteriological technique in the establishment of diagnosis of EPTB.

The objective was to evaluate IS6110 PCR for detection of *Mycobacterium tuberculosis* complex for diagnosis of Extra Pulmonary Tuberculosis cases attending tertiary care hospitals.

**Setting:** Department of Pulmonary Medicine, C.S.M Medical University, Lucknow (Erstwhile King George Medical University) and Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India.

**Methods:** Specimens from sixty clinically suspected cases of EPTB were processed for Mycobacteria by conventional and BACTEC culture for *M. tuberculosis complex*. All the samples were also processed for PCR amplification with primers targeting 123 bp fragment of insertion element IS6110 of *M. tuberculosis complex*.

**Results:** Out of 60 cases, 47 (79%) were newly diagnosed; 13 (21%) cases were previous treated cases. Forty five (75 %) were male and fifteen were (25%) female. 11/60 (18.3 %) were positive for AFB by ZN staining. BACTEC culture was positive in 23/60 (38.8 %) and IS6110 PCR was positive in 37/60 (61.6%) cases.

**Conclusion:** IS6110 PCR can be highly useful in establishment of diagnosis of EPTB.

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**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Diagnosis of latent tuberculosis infection by using the QuantiFERON-TB Gold in-tube test in children whose household contact has contagious pulmonary tuberculosis disease

**T. Lee, N. Kim, J. Kim**

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**Background:** The QuantiFERON-TB Gold In-Tube test (Cellestis, Carnegie Victoria, Australia) was approved for the diagnosis of latent tuberculosis infection. Although it has been shown to be sensitive and specific in adults, limited data are available on its performance in children.

**Methods:** This was a case-control study of BCG-vaccinated children younger than 15 years of age in Seongnam, South Korea. We compared tuberculin skin test and QuantiFERON-TB In-Tube test results in children who had exposed to household with contagious pulmonary tuberculosis disease within the past 3 months. Controls with no known exposure history for *Mycobacterium tuberculosis* were matched in a 1:1 ratio to cases according to age and gender.

**Results:** Among the 27 children with a history of exposure to a household with contagious pulmonary tuberculosis disease, 21 (78%) had positive results for the tuberculin skin test, whereas 8 (30%) had positive results for the QuantiFERON-TB In-Tube test. Among the 27 children with no known exposure history for *M tuberculosis*, none had positive results for both tuberculin skin test and QuantiFERON-TB In-Tube test. There was moderate concordance between tuberculin skin test and QuantiFERON-TB In-Tube test results ( $\kappa = 0.43$ ).

**Conclusion:** The QuantiFERON-TB In-Tube test is a specific test for diagnosis of latent tuberculosis infection in children whose household contact has contagious pulmonary tuberculosis disease. Additional studies are needed to further assess the utility of the QuantiFERON-TB In-Tube test in the children with high risks of exposure to *M tuberculosis*.

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**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Clinical and epidemiological features of tuberculous meningitis

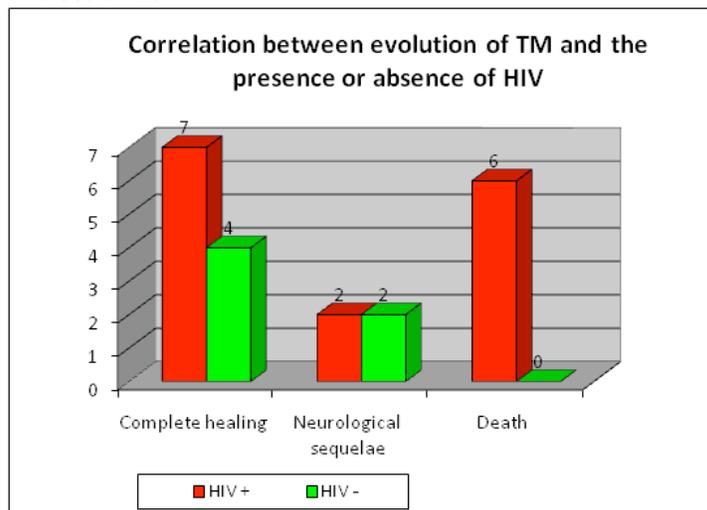
**M. E. FERREIRA**, N. E. Frassone

Hospital Rawson, Córdoba, Córdoba, Argentina

**Background:** Tuberculosis is common in people with HIV / AIDS, the main risk factor for tuberculous meningitis (TM) among individuals aged 25 to 45 years. It has a high fatality rate and serious neurologic sequelae.

**Methods:** Retrospective analytic study on patients over 15 years with TM assisted at the Hospital Rawson (Córdoba, Argentina) from 01/01/2001 to 01/01/2008, reviewing medical records. *Inclusion Criteria:*-High suspicion of TM: compatible clinical manifestations associated with CSF cytochemical parameters characteristic - *TM confirmed:* positive culture for M. tuberculosis CSF or brain tissue biopsy.

**Results:** We analyzed 21 medical records. Average age: 35 years. Sex: 90% male. Headache 95%, fever and stiff neck 67%. Stage 1 (S1) 48%, S2 38%, S3 14%. Hyponatremia occurred in 75% of those left with neurological sequelae and 50% of those who died. Chest Rx: 33% pathological (86% had positive culture of CSF). Neuroimaging: 43% hydrocephalus, cerebral edema 33%, stroke 29%, tuberculomas 24%, basilar arachnoiditis 5%. CSF: 95% hypoglycorrhachia, 90% high protein levels and hypercellular (89% had lymphocytic predominance). 100% smear negative. Positive culture: 55% (73% positive serology for HIV and 82% high protein levels). Associated cryptococcosis meningitis 29%. HIV + 71%. CD4 low, 100%. On 82% of patients who progressed to complete healing began tuberculostatic treatment in the 1st week of hospitalization. Complete healing 52%, neurological sequelae 19%, death 29%. Lethality 30% (100% aged 25 to 45). Lethality: 40% HIV +, 0% HIV-. Complete healing 80% of E1 and 0% of E3.



**Conclusion:** TM occurred most often in young men, where AIDS was more frequent and more lethal. Clinical: fever, headache and stiff neck. Hyponatremia was associated with worse evolution. Abnormal Rx, was associated with positive CSF cultures. Hydrocephalus, brain edema and cerebral infarction, more frequent injuries. Hypoglycorrhachia was characteristic. CSF culture positive: most had positive serology for HIV and high protein levels. Cryptococcosis meningitis, possible partnership in areas of high prevalence of TB and HIV. Evolution depended on the stage and the time when treatment was started. Lethality: 30%. The fatality rate in HIV + 40% vs. 0% of HIV-. HIV infection was associated with poor prognosis.

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**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Serum proteins in patients with pulmonary tuberculosis and controls in Nigeria

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<sup>3</sup>University of Maiduguri, Maiduguri, Peru, <sup>4</sup>University of Maiduguri, Maiduguri, Nigeria

**Background:** Tuberculosis (TB) remains an important communicable disease especially in the developing world. The complex relationship that exists between TB and poverty, homelessness, Human Immunodeficiency Virus and Acquired Immune Deficiency Syndrome (HIV/AIDS) and malnutrition has made the devastating effects of TB well known. We therefore undertook to evaluate this complex relationship.

**Methods:** A total of 420 subjects were studied, comprising 210 newly diagnosed sputum smear positive Pulmonary TB (PTB) patients (aged 18 years and above from Lagos metropolis) and 210 controls. We determined serum total proteins levels in the study population colorimetrically using the Biuret method, while serum albumin was measured by the bromocresol green method. Screening for HIV-1 and HIV-2 antibodies was done using ELISA (Wellcome Diagnostics, pasteur, Marnes-la-cognetee, France). Positive samples were subjected to Western Blotting (Dupont de Nemours, USA) for confirmation.

**Results:** Hypoalbuminaemia was found in 184 (87.60%) of the cases vs. 26 (12.38%) of the controls (P=0.001) (see Table 1). The protein sub-fraction, gammaglobulin was however, much higher in the cases than in the controls [mean 41.45 ± 11.21% vs. 29.42 ± 6.76% (p=0.005)] (see table 2). HIV antibody test was positive in 11/153 (7.19%) of those who accepted to be screened for HIV co-infection.

**Table 1: Distribution of serum protein pattern in the study population**

Protein fraction g/L	Cases n=210 (%)	Controls n=210 (%)	P value
Total Protein	73.25 ± 10.51	82.09 ± 63.57	0.04
Albumin	29.37 ± 9.22	40.18 ± 6.23	0.001
Globulin	45.63 ± 8.71	36.09 ± 6.43	0.001
Hypoalbuminaemia	184 (87.62)	26 (12.38)	0.001
Hypergammaglobulinaemia	189 (90.00)	90 (42.86)	0.001

**Legend:** Hypoalbuminaemia: Albumin < 35 g/L

Hypergammaglobulinaemia: Globulin > 35g/L

**Table 2: The Relative Sub-fractional Composition of Serum Proteins by densitometry in study subjects**

Sub-fraction	Cases n=21 (%)	Controls n=21 (%)	P Value
Albumin	28.41±10.88	42.55 ± 5.13	0.013
Globulins:	8.03 ± 5.04	11.19 ± 2.84	0.098
Alpha 1			
Alpha 2	11.18± 3.15	11.46 ± 2.03	0.810
Beta	10.26±6.61	10.54±5.41	0.810
Gamma	41.45±11.21	29.42±6.67	0.005

**Conclusion:** PTB cases had lower total serum proteins and lower serum albumin but higher plasma gammaglobulins than controls. This study therefore, tried to highlight on the inter-relationship between poverty and disease.

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**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

High ethionamide resistance in *Mycobacterium tuberculosis* strains isolated in Kenya

**V. Asiko**

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**Background:** Increasing development of tuberculosis (TB) resistance to the currently available drugs including second-line anti-TB drugs that are being used for treatment of Multi-Drug Resistant TB (MDR-TB) patients has frustrated efforts to control TB worldwide. Ethionamide (Eth) is one of the drugs used in the regimen for treatment of these patients.

**Methods:** A total of 216 MTB strains with pre-determined first-line drug susceptibility testing (DST) results were used including 78 first-line resistant to individual and combined drugs, and 138 susceptible to streptomycin, rifampicin, isoniazid and ethambutol. The strains were subjected to DST to ethionamide among other second-line.

**Results:** Thirty two [32/216 (14.8%)] strains showed resistance to second-line drugs. Resistance to Eth was the highest [18/32 (56.3%)] including co-resistance with isoniazid [8/18 (44.4%)]. Nine [9/18 (50%)] strains were fully resistant and 9 [9/18 (50%)] were intermediate resistant to Eth.

**Conclusion:** Unexplainable high levels of Eth resistance is a cause for concern. This will impact negatively on the outcome of management of MDR-TB especially in Kenya where the use of this drug is almost mandatory. Close monitoring of Eth before initiating individual patient management may be necessary.

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**Final Abstract Number: 59.017**

**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Pulmonary TB among elderly patients: Higher rates, difficulties for diagnosis and poorer prognosis

**L. P. Cruz Hervert**, R. A. Baez-Saldaña, L. D. Ferreyra-Reyes, E. E. Ferreira-Guerrero, B. Cano-Arellano, L. Juarez-Sandino, M. D. L. García-García  
Instituto Nacional de Salud Publica de México, Cuernavaca, Morelos, Mexico

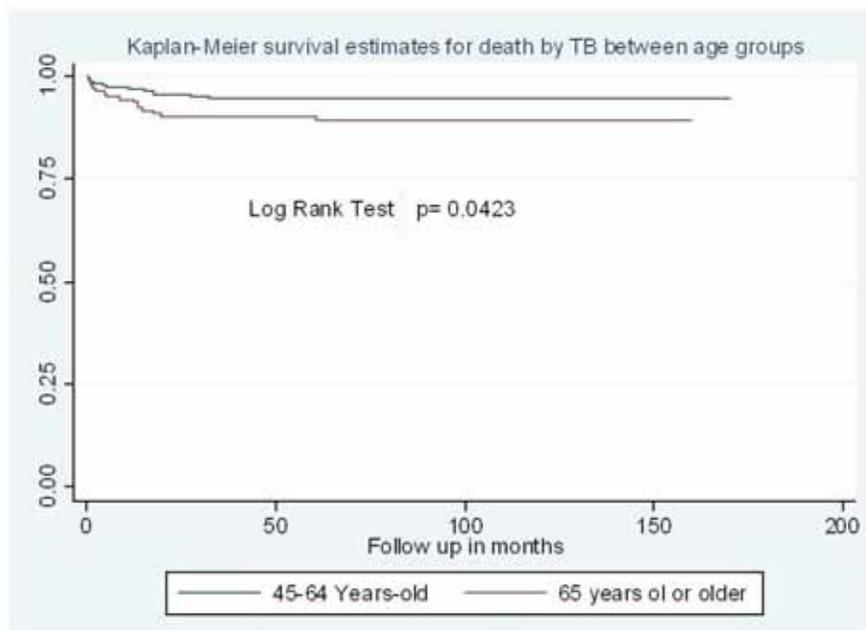
**Background:** Tuberculosis (TB) rates among the elderly (>65 yrs) in Mexico (31/100,000) are two-fold those among general population representing a major challenge to health systems. Complicating factors include immunosenescence, chronic diseases, and other infectious diseases.

**Methods:** Objective: To describe clinical and epidemiological consequences of pulmonary TB among the elderly.

We screened persons with more than 2 weeks cough in southern Mexico from March, 1995 to December, 2008. We collected clinical and mycobacteriological information (isolation, identification, drug-susceptibility testing and IS6110-based genotyping and spoligotyping) from individuals with bacteriologically confirmed pulmonary tuberculosis. Patients were treated in accordance with official norms and followed to ascertain treatment outcome, retreatment, and vital status. We present data for two age groups (45 to 64 years and 65 and more). We compared TB incidence and mortality rates. We used Cox proportional hazards models to assess the association of elderly age with death due to TB.

**Results:** 526 TB patients were older than 45 years of age; of these, 173 (33%) were 65 yrs of age or older. Individuals > 65 yrs had higher rates of recently transmitted TB rates, fewer symptoms and fewer bacilli in sputa as compared with 45 to 64 year old adults. Elderly age was associated to death due to TB (HR= 3.41; IC95% 1.4-8.3.; p=0.007) adjusting by gender, drug resistance, re-treatment and diabetes.

**Conclusion:** Although high, TB rates among the elderly are most probably underestimated. Pulmonary TB among >65 years entails difficulties for timely diagnosis and poses a higher risk of mortality due to TB. Specific strategies to achieve timely diagnosis and treatment are needed for vulnerable group.



**Graph 1. Kaplan-Meier survival estimates for death by TB between age groups.**

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**Final Abstract Number: 59.018**

**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Pleural effusion of patients with tuberculosis is characterized by accumulation of  $\gamma\delta$  T lymphocytes that expresses distinct surface markers

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<sup>1</sup>Instituto Clemente Ferreira, Sao Paulo, Brazil, <sup>2</sup>Universidade Federal de Sao Paulo, Sao Paulo, Brazil

**Background:** Tuberculosis (TB) causes about 3 million of death per year. It is very important to elucidate the precise mechanism of defense against *Mycobacterium tuberculosis*. The role of gamma-delta ( $\gamma\delta$ ) T lymphocytes in adaptative immunity in tuberculosis remains uncertain. We addressed the distribution of these T subsets at diagnosis of tuberculosis patients and suggest they must be involved in the pathogenesis of the disease.

**Methods:** The frequency of  $\gamma\delta$  T lymphocytes was evaluated in both pleural effusion and peripheral blood of 33 patients with pleural tuberculosis before treatment. Frozen PBMCs were surface stained for CD3, V $\delta$ 1, and V $\delta$ 2. Analyze of memory and naive subsets were performed by expression of CD11a and CD27 on  $\gamma\delta$  T cell. Also, the percentage of IFN- $\gamma$ -producing V $\delta$ 1 and V $\delta$ 2 cells after IPP stimulation was evaluated. Latently infected contacts and healthy subjects were used as controls.

**Results:** The latent infection group presented the highest proportion of V $\delta$ 1 T cells (12.9%,  $p < 0.05$ ) compared to healthy control (4.29%,  $p < 0.05$ ) and tuberculosis patients (6.2%,  $p < 0.05$ ). It was observed higher percentage of memory cells in latent infection than the other groups ( $p < 0.05$ ). Pleural effusion was characterized by higher frequency of memory phenotype cells than blood ( $p = 0.0342$ ). These subsets produced more IFN $\gamma$  (7.05% in memory versus 3.62% in naive cells,  $p < 0.05$ ) confirming that they are memory cells. We found that in tuberculosis the frequency of V $\delta$ 1 T cells are higher when compared with V $\delta$ 2 (5.07% versus 1.1%,  $p < 0.05$ ). These numbers reflected an inverted V $\delta$ 1:V $\delta$ 2 ratio associated with tuberculosis. The latent infection group presented higher frequency of V $\delta$ 2 T cells than healthy control (2.76% versus 1.29%,  $p = 0.03$ ), and than TB ( $p < 0.001$ ).

**Conclusion:** Marked accumulation of V $\delta$ 1 and V $\delta$ 2 in pleural effusion is seen in active tuberculosis, and  $\gamma\delta$  T lymphocytes are expanded in pleural effusion, and this site is mainly composed by memory subsets. Our data further indicate that  $\gamma\delta$  T cells participate in the immune response against *Mycobacterium tuberculosis*.

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**Final Abstract Number: 59.019**

**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Diagnostics of resistance mycobacterium tuberculosis (MR MT) to isoniazid (H) and rifampicin (R) by simultaneous identification of mutations in rpoB, katG, inhA and ahpC genes with use biological microchips (TB-biochip).

**V. Zhuravlev**

The Research Institute of Phthisiopulmonology, St.Petersburg, Russian Federation

**Background:** TB-biochip can simultaneously identify mutations in four MBT genes associated with MR to (R) and (H): rpoB, katG, inhA, ahpC.

**Methods:** We have studied opportunities of use of the TB-biochip for express diagnostic of genetic characteristic of MR MT in clinical samples (sputum) from 186 patients with disseminated tuberculosis.

**Results:** Mutations in rpoB gene were revealed in 125(67.2%) cases. Thus the replacement of codon 531 in 63(50.4%), including (Ser531-Leu) in 26(41.3%) was most often. This mutation is mostly distributed among R resistance strains in the world and commonly connected to Beijing genotype. This replacement causes resistance to high concentration R in vitro and does not affect MBT viability. Other variants of mutations in this codon were very seldom: Ser531-Cys(10), Ser531-Gln(19), Ser531-Trp(8) patients. Replacements in other codons: 511(12), 512(13), 513(9), 516(8), 526(15) and 533(5) cases. Drug resistance to H, caused by mutations in katG gene, was revealed in 95(51.0%) cases. Replacement in codon prevailed: isolated variant - in 41(43.2%) patients, combination with 328 codon replacement - 8(8.4%) or combination with 335 codon - 2(2.1%). We have revealed the replacement of nucleotides of inhA gene in 43(23.1%) patients. The ahpC gene has lowest number of mutations - 11(5.9%). Among the studied MBT we have revealed only 3(1.6%) with mutations in all studied gene. In 3 gene the mutations were - 16(8.6%) strains, in 2 gene - 59(31.7%), in 1 gene - 28(15.1%). The strains without any mutations revealed were only 37(19.9%). Our results had a good correlation with microbiological investigation of the same samples.

**Conclusion:** The prevalence of the strains with the combination of mutations of rpoB531 and katG315 once again confirms the presence of pool of drug resistant and multidrug resistant strains of Beijing genotype at territory of North-West region of Russia in conditions of wide application of R and H.

This the method of biological microchips is high sensitive and specific for express detection of MR MT. It allows detection of resistance MT to R and H with in 24 hours that is very important for choosing the adequate anti-tuberculosis chemotherapy.

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**Final Abstract Number: 59.020**

**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Fast identification of Mycobacteria from positive Mb/Bact bottles using a multiplex PCR

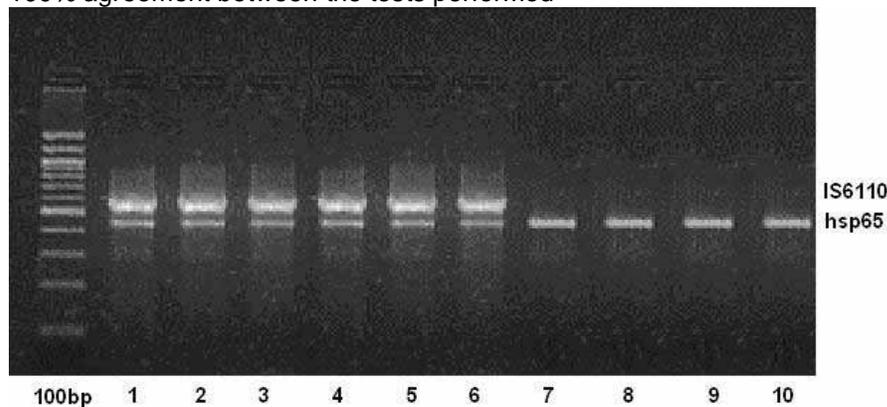
E. P. A. Bensi<sup>1</sup>, C. E. Levy<sup>2</sup>, **M. Ramos**<sup>3</sup>, P. C. Panunto<sup>1</sup>

<sup>1</sup>Universidade Estadual de Campinas, Campinas, SP, Brazil, <sup>2</sup>Universidade Estadual de Campinas, Campinas, Brazil, <sup>3</sup>Universidade Estadual de Campinas - UNICAMP, Campinas, SP, Brazil

**Background:** The rapid identification of mycobacteria from culture-positive Mb/Bact bottles is an important clinical issue. Furthermore, the availability of a non-expensive, technically simple, and accurate method also would benefit mycobacterial laboratories in developing countries. In this study, we aimed to develop an assay allowing the identification of the *Mycobacterium tuberculosis* complex (MTBC) and other frequently isolated nontuberculous mycobacteria (NTM) directly from positive Mb/Bact (Organon-Teknika) bottles. A multiplex PCR focusing the *hsp65* gene, and the *IS6110* was done and evaluated for its efficiency compared to PRA identification, and PNB inhibition.

**Methods:** Mycobacterial strains were grown from clinical samples and preserved in solid media (LJ). A panel of 91 *M. tuberculosis* strains, and 26 Non-Tuberculous-Mycobacteria were submitted to the following identification techniques: a) *hsp65* gene amplification followed by restriction (PRA); b) p-Nitrobenzoic acid (PNB) growth inhibition in liquid media (Mb/Bact); 3) Multiplex-PCR, using two sets of primers: TB284/TB850 (*IS6110* MTC specific primers), and TB11/TB12 (65kD antigen, Mycobacterium genus specific primers). The mycobacteria was inactivated being heated at 80°C for 20 min before DNA extraction. DNA was extracted by the freezing and thawing method. 50µl of the the supernatant was used for amplification. Identification using *hsp65* restriction was done according to international protocols (Telenti et al, 1993), and considered the gold-standard. PNB was added to liquid Mb/Bact bottles to a final concentration of 500µg/ml. p-aminobenzoic acid, and the Mycobacterial concentration was adjusted to the MacFarland 1 standard.

**Results:** The *hsp65* restriction analysis identified all Mycobacteria tested as: *M. tuberculosis* (61 strains), *M. avium*, *M. intracellulare*, *M. fortuitum*, *M. abscessus*, *M. goodii*, *M. szulgai*. There was 100% agreement between the tests performed



Multiplex-PCR showing on lanes 1-6 MTC strains, and on lanes 7-10 NTM strains

**Conclusion:** A simple and inexpensive Multiplex-PCR as described here, can readily distinguish among *M. tuberculosis* Complex strains, and Non-Tuberculous Mycobacteria.

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**Final Abstract Number: 59.021**

**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Identification and treatment of latent tuberculosis infection in haemodialysis patients in New Zealand

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Capital and Coast DHB, Wellington, New Zealand

**Background:** International and local reports indicate that tuberculosis (TB) is a major issue in haemodialysis units because of high rates, difficult and late diagnosis, high morbidity and mortality. Moreover, there is potential for nosocomial spread. The Mantoux test is unreliable in renal disease, and appears to have led to diagnostic nihilism with respect to latent TB infection (LTBI): no treatment of LTBI had been employed previously in our unit. The aims of this study were to describe the epidemiology of active TB within renal patients in the greater Wellington Region and to develop a LTBI testing and treating algorithm using an interferon-gamma release assay (IGRA)

**Methods:** A ten year review of notification data and clinical records was used to identify and describe renal patients with active TB. All patients receiving hospital haemodialysis (H-HD) were screened using QUANTIFERON-Gold (Cellistis, Australia). Chest radiographs and clinical notes were reviewed.

**Results:** Seven cases of active TB in H-HD patients were identified, approximating an incidence of 1.2/100 new dialysis patients. The overall annual incidence in New Zealand is 10.3/100,000. Six patients died (86% mortality), but mostly this was not directly due to TB. All 65 H-HD patients were screened for LTBI, 66% of whom were born outside NZ. Overall 6 (9%) were positive and all but one were from overseas, high prevalence, countries. Three results were repeatedly indeterminate due to weak mitogen test results. The mean mitogen response was lower in H-HD than in healthy staff and travellers (7.56 IU/mL vs 20.15 IU/mL,  $p < 0.001$ ).

**Conclusion:** The IGRA test gives fewer indeterminate and positive results than we expected from overseas reports. Those identified as having LTBI have all been commenced on isoniazid therapy, apart from two patients who are not expected to survive long term. Treatment has been simplified by using directly observed therapy when patients receive dialysis. Those with negative and indeterminate IGRA results will be carefully followed, enabling further clinical validation of the test. Most importantly, the IGRA test has given greater confidence to the renal unit staff and has resulted in active management of LTBI in a high risk group.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

The role of a whole blood interferon- $\gamma$  releasing assay for the tracing of tuberculosis infection in bacilli Calmette Guerin vaccinated children

**J.-K. Chun**, E. Soon, H. M. Kim

Wonju Christian Hospital, Wonju, Korea, Republic of

**Background:** There are limited data about new interferon- $\gamma$  releasing assay (IGRA) in pediatric ages. Paradoxically the screening of latent tuberculosis infection (LTBI) and the selection for isoniazid (INH) preventive treatment have significance in this age group. The high specificity of IGRA has a merit in younger children under BCG effects. The aim of this study was to evaluate the role of IGRA for the tracing of tuberculosis infection in BCG vaccinated children.

**Methods:** For 24 months, between Jul. 1, 2007 and Jul. 31, 2009, data were collected from children who visited for tuberculosis evaluation in Wonju Christian Hospital. The first group was composed of children who had close contact to active tuberculosis patients, the second group was the patients undergoing evaluations of tuberculosis with various diseases. Tuberculin skin test (TST) and QuantiFERON®-TB Gold In-Tube (QFT-G IT, Cellestis, Victoria, Australia) test were performed.

**Results:** A total of 70 children were evaluated and their median age was 5 year of age (range: 7 days – 16 years). There were 24 patients who had history of contact to active tuberculosis but no symptoms. Among them, 5 patients were QFT-G IT positive and 10 patients were TST positive when the cutoff value is  $\geq 5$  mm ( $\kappa = 0.53$ ), 5 patients were TST positive when the cutoff value is  $\geq 10$  mm ( $\kappa = 0.74$ ). Fourty six patients were evaluated to be ruled out tuberculosis infection and 16 patients were diagnosed as active tuberculosis and improved by antituberculosis medications. In 2nd group, TST was positive in 9/16 (56.2%) and QFT-G IT was positive in 11/16 (68.7%).

**Conclusion:** The results of IGRA seemed to be concordant with TST in pediatric age also in diagnosing LTBI and active tuberculosis. In BCG vaccinated children, using both methods would be useful for highly selective anti-tuberculosis treatments.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

An unusual case of esophageal tuberculosis in immunocompetent female patient

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**Background:** Esophageal tuberculosis is an extremely rare entity, totaling 3% of total patients with gastrointestinal tuberculosis (TB). Clinically these patients may be mistakenly diagnosed as esophageal carcinoma because dysphagia and weight loss are the more frequent symptoms. Esophageal TB involvement generally affects the middle-third of the esophagus around the carina. This usually is caused by direct extension and spread from mediastinal structures, the previous mechanism of spreading the inoculation of swallowed sputum, as well as by hematogenous or lymphatic spread. The goal of this case presentation is order to emphasize the importance of tuberculosis infection in the differential diagnosis of gastrointestinal symptoms because this form of TB can be successfully treated by antituberculous chemotherapy.

**Methods:** A 31-year-women was admitted to Clemente Ferreira Institute, a reference center for tuberculosis in Brazil, with complains of dysphagia and postprandial epigastria pain. It was associated with non-productive cough, weight loss and weakness. She otherwise was in good health, clinical examination appeared normal and, she reported no other relevant symptoms. No TB contacted informed prior history of tuberculosis. She had no immunosuppressive conditions.

**Results:** The barium swallow film revealed an extrinsic compression and erosion in the esophagus. She was submitted to esophagogastroduodenoscopy showing large ulcers in the esophagus, and the histopatological examination the mucous biopsy showed ulcerative inflammation and presence of granulomatous inflammation, but a negative staining for acid-fast bacilli. She had a positive tuberculin skin test (13mm). Chest X-ray showing infiltration image at right hilar region. Tubercle bacilli were isolated on Lowenstein-Jensen medium from sputum. Treatment with rifampin, isoniazid and pyrazinamide was initiated, having the patient shown favorable clinical response during a follow-up period of 6 months.

**Conclusion:** Esophageal tuberculosis is rare, and the diagnostic may be mistakenly considered as malignancy. The treatment with tuberculous drugs is effective, however the early diagnosis is important to avoid clinical complication or even association to malignance tumors.

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**Final Abstract Number: 59.024**

**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Pott's Spine in a 3-year-old child

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**Background:** Less than 1% of pediatric tuberculosis is associated with spinal disease. The infection is via hematogenous or lymphatic spread from a primary focus in the lungs.

**Methods:** A 3 year-old recently adopted Ethiopian girl presented with kyphosis. Imaging studies showed significant erosive defect in the lower thoracic spine, multiple eroded vertebral bodies, 30-degree kyphotic deformity, and a prevertebral abscess. The patient had a normal chest x-ray and a positive PPD test (17x14 mm induration). The patient had no constitutional symptoms, no lung disease and minimal elevation of ESR.

**Results:** Pott's Disease (Tuberculous Spondylitis) was suspected and confirmed by obtaining a specimen from the prevertebral abscess through interventional radiology. The specimen was positive for acid-fast bacilli, and the culture grew *mycobacterium tuberculosis*.

**Conclusion:** This case report highlights the patient's initial presentation, diagnostic work-up, and the management. Pott's Disease, relatively rare in children, will be discussed.

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**Session: Tuberculosis: Diagnosis, Treatment & Drug Resistance**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Status of HIV/TB co-infection in Cuba and strategies followed in the health care of patients with active tuberculosis and AIDS in the Institute of Tropical Medicine "Pedro Kourí"

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Instituto Pedro Kouri, La Lisa, Cuba

**Background:** There is a tendency to the reduction of tuberculosis cases in Cuba. However, an increase of tb cases in the HIV/AIDS population has been observed.

**Methods:** The data supplied by the Cuban Public Health Ministry were compiled in relation to the number of cases with tb and HIV/AIDS diagnosed during the last 8 years, and diagnostic and treatment procedures in the Institute of Tropical Medicine "Pedro Kourí" were reviewed.

**Results:** The number of tb patients with HIV/AIDS increased from 13 (1.2%) of 1133 tb patients reported for year 2000 in Cuba, to 50 (6.6%) of 754 tb patients reported for 2007.

Forty-one cases were diagnosed in the IPK hospital in 2007, 21 were confirmed by bacteriology tests, and 24 already had previous antiretroviral treatment.

The procedure followed with inpatients was to keep them in respiratory isolation until 3 smears were performed with cultures and antibiograms. In case these smears were positives, the tb treatment was given priority over the antiretroviral treatment.

For up to 60% of tb patients, negative smears have been reported even for sick patients.

Therefore, given the evidence that a patient had an active tb even with a negative smear, the case is analyzed in a staff meeting considering aspects such as previous contacts with tb patients, persistent respiratory symptoms, weight loss, and radiology studies compatible with the disease, among others, where it can be decided to report and start the tb treatment.

**Conclusion:** The number of patients with TB and HIV/AIDS in Cuba has increased with the years. Even in patients with negative smears, the disease cannot be ruled out and specific strategies are needed for its diagnosis and prompt treatment.

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**Final Abstract Number: 73.001**

**Session: Animal Models, Pathogenesis & Host Defenses**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Antibacterial effects of *Humulus lupulus L.* extract on topical staphylococcal infection in BALB/c Mice cornea

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**Background:** Staphylococcus aureus is an opportunistic pathogen and the most common cause of bacterial keratitis that can result in irreversible corneal scarring, a pathologic effect that reduces visual acuity and can lead to blindness. *Humulus lupulus L.* flower complex has multiple therapeutic properties such as antibiotic effects against some gram positive bacteria and fungi. In the present research, topical anti-staphylococcus aureus effects of hydroalcoholic extract of *Humulus lupulus L.* flowers in corneal infection induced by staphylococcus aureus were investigated in mice.

**Methods:** At first, staphylococcus aureus were inoculated into right cornea of animals under anesthesia by making parallel scars. One, three, five, seven and nine days post-inoculation, the eyes were observed carefully under microscope. In observations, eyes were scored according to the area and the degree of opacity. In order to assess the antibacterial effects of *Humulus lupulus L.*, the extract was administered in form of eye drop at 1%, 5% and 10% concentrations. Treatments were started twice daily as soon as the first opacity was observed and continued for one week. The first sign of corneal infection with opacity was observed after three days of bacterial inoculation as compared with the normal eye.

**Results:** The intensity of opacity was progressed time dependently in a manner that maximum opacity of the whole cornea was obvious in more than half of animals, after nine days. Administration of the *Humulus lupulus L.* extract 10% topically, reduced corneal opacity and consequently the infection. Introducing animal models of ocular diseases such as bacterial infection in cornea has special importance in ocular research.

**Conclusion:** Effective components existing in *Humulus lupulus L.* flower extract are mainly resins and essence which among them, resins has special importance and seems to be responsible for its antibiotic effects.

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**Session: Animal Models, Pathogenesis & Host Defenses**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Up regulation of IRF-2 in West Nile Virus infection: Implications for establishment of viremia in the brain leading to encephalitis

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National University of Singapore, Singapore, Singapore

**Background:** A proportion of patients afflicted with WNV can develop neuromuscular degenerative diseases and potentially fatal encephalitis. However, host immuno-modulatory factors involved in the establishment of viremia and subsequent infiltration of cytotoxic immune cells is yet well understood. This study thus investigates the role of IRF-2, an attenuator of interferon (IFN) response, on the establishment of viremia and immune cell infiltration during WNV infection.

**Methods:** Real-time PCR, Western blot, and FACS analyses were used to study the regulation of various IRFs and downstream targets during WNV infection of an astrocytic cell line, A172. Regulation of IRF-2 at the cellular level was then studied using immuno-fluorescence microscopy. Subsequently, cell lines over-expressing, or with a knockdown of IRF-2 were infected to study the effect(s) that IRF-2 has on WNV production. Finally NK cell assay was performed to investigate the ability of NK cells to lyse these infected cells.

**Results:** The IRFs 1, 2, 3 and 7 are highly up regulated post-WNV infection. Expectedly, downstream gene targets like IFN- $\beta$ , IL-12 and IL-6 are up regulated transcriptionally and translationally. In addition, concomitant increased mRNA expression of MHC Class I loading genes like TAP1, TAP2 and  $\beta$ 2m with that of HLA-E, translated to an increased surface expression of HLA-E. Interestingly, FACS dot plot analysis revealed that expression of IRF-2 was insufficient to suppress HLA-E expression. Immuno-fluorescence microscopy further showed the surprising preferential enhanced expression of IRF-2 in the non-infected cells. Finally, infection of IRF-2 over-expressing cells resulted in increased virus production, while a reduction in virus titer was observed in the IRF-2 knockdown cells.

**Conclusion:** Our results show that IRF-2 is preferentially up regulated in the neighboring non-infected cells, possibly in a homeostatic fashion to regulate pro-inflammatory genes like IFN and cytokines in these cells. On the other hand, the activated activators overwhelm the attenuation effect(s) of IRF-2 in the infected cells. In these infected cells, the inhibitor of NK cell lysis, HLA-E, is expressed. Its expression thus protects the infected cells from the cytotoxic effects of NK cells. Predisposition of neighboring cells to NK cell lysis and / or subsequent infection thus contributes to overall WNV pathogenesis.

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**Session: Animal Models, Pathogenesis & Host Defenses**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Crimean-Congo hemorrhagic fever virus infects human hepatocytes and induces IL-8 secretion  
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<sup>1</sup>Fondation Merieux, Lyons, France, <sup>2</sup>Fondation Merieux/Institut de recherche Biomedicale des Armees-antenne de Grenoble, Lyons, France

**Background:** Crimean-Congo hemorrhagic fever virus (CCHFV) is a very pathogenic tick-borne virus member of the Bunyaviridae family and the Nairovirus genus. The knowledge of CCHFV pathogenesis is improving: recently, new target cells were identified. We and others had demonstrated that CCHFV is able to infect and partially activate monocytes derived dendritic cells and macrophages. During one retrospective study, it was shown that CCHFV was detected in the liver of infected patients.

**Methods:** Attempting to find other target cells to better understand the pathogenesis of CCHFV, we analyzed the host response induced by CCHFV in hepatocytes infected *in vitro* during a kinetic study.

**Results:** We noticed that while in HuH7 CCHFV infection elicited a cytopathogenic effect, no visible effect was seen in CCHFV infected HepG2. This intriguing feature led us to analyse the viral parameters expecting a differential cellular response. HuH7 and HepG2 both were shown to be permissive to CCHFV and to replicate the virus at a high load as monitored by plaque titration assay, genomic and anti-genomic strand quantification. The high secretion of IL-8 but no other inflammatory cytokines such as TNF- $\alpha$ , IL-1b indicated that CCHFV induced a response in both hepatocytes. Interestingly, no type I IFN was detected during the kinetic study. In spite of these similarities, we observed a pro-apoptotic CCHF effect more significant in Huh7 than in HepG2 cell lines.

**Conclusion:** We found that hepatocytes could be considered as CCHFV target cells that could be involved in the pathogenesis disorders. The high IL-8 production by infected hepatocytes associated to the pro-apoptotic effect likely contribute to the disease progress.

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**Final Abstract Number: 73.004**

**Session: Animal Models, Pathogenesis & Host Defenses**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

The course of infection in respiratory infected chickens caused by avian influenza virus A/H5N1  
**A. Sergeev**, O. V. P'yankov, O. K. Demina, L. N. Shishkina , A. S. Safatov, A. N. Sergeev, I. G. Drozdov  
FSRI State research center of virology and biotechnology "Vector" , Novosibirsk, Russian Federation

**Background:** High pathogenic avian influenza virus (AIV) is an object of research by many scientists in the world. This disease agent is capable of infecting a wide range of varieties of wild and domestic birds. Among the known pathways of any infection the most effective believe airborne and fecal-oral routes. It is believed that chickens infected by the fecal-oral route, i.e. through the gastrointestinal tract. Do not exclude also the aerosol route of transmission of the disease in chickens. In the present study infectious properties of various AIV strains and the degree of sensitivity to this pathogen of respiratory and gastric-intestinal tract of the chickens, as well as the dynamics of dissemination in their body were studied.

**Methods:** We used eight highly pathogenic AIV A/H5N1, isolated in Russia and CIS countries in chickens that are infected by aerosol, intranasal, intra gastric and oral methods.

**Results:** All studied AIV strains showed same high virulence for chickens (LD50 is 2 - 15 EID50) for aerosol challenge. When aerosol challenge sensitivity of these animals to AIV 30 times higher than in the intranasal, 500 times higher than with oral, and 10000 times higher than intra gastric method of infection, indicating a higher susceptibility to AIV of respiratory organs of chickens compared to gastrointestinal tract. Replication of the virus in the membrane of the nasal cavity has already recorded 18 hours after infection (a.i.). The second wave reproduction of the pathogen observed in many organs and blood serum of chickens and begins a 30 hours a.i. with the highest rates of accumulation of virus in excess of 7 lg EID50/g observed in the lungs, blood serum and kidneys of animals.

**Conclusion:** According to the results of comparative analysis of LD50 of AIV strains at different options for infections have found that the highest susceptibility to the virus have respiratory organs of chickens compared with the gastrointestinal tract. The primary target organ to AIV in intranasal infected chickens is the mucous membrane of the nasal cavity. In addition to the results of research, we proposed a fecal-nasal transfer mechanism of AIV A/N5N1 in chickens for nature conditions.

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**Final Abstract Number: 73.005**

**Session: Animal Models, Pathogenesis & Host Defenses**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Role of leukotrienes in resistance and susceptibility to infection by *Histoplasma capsulatum*

**A. Secatto**<sup>1</sup>, E. M. Soares<sup>2</sup>, A. I. Medeiros<sup>3</sup>, L. H. Faccioli<sup>4</sup>

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<sup>4</sup>Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Ribeirão Preto, Brazil

**Background:** *Histoplasma capsulatum* (*H.capsulatum*) is a dimorphic pathogenic fungus that causes a wide spectrum of diseases. Macrophages are an important phagocytic cells in host defense against fungi. In order to enhance host defense, these resident cells secrete chemotactic substances such as leukotrienes (LTs) and cytokines that recruit effector cells to the focus of infection. LTs are potent lipid mediators of inflammation and host defense, derived from the 5-lipoxygenase (5-LO) pathway of arachidonic acid (AA) metabolism. We have been shown that the absence of leukotrienes in genetically modified mice (5-LO<sup>-/-</sup>) or by treating WT animals with pharmacological inhibitor MK886, have increased susceptibility to infection when they are infected with *H. capsulatum*. Recent studies show that susceptibility or resistance of different strains to certain infections, such as *Leishmania amazonensis*, is associated with differential production of LTs.

In the present study, we evaluated the production of LTB<sub>4</sub> by peritoneal macrophages (PM) from susceptible and resistant mice after challenge with *H. capsulatum* and the effect of LTs in phagocytosis by macrophages of both strains.

**Methods:** Macrophages from C57BL/6 (susceptible) and sv129 (resistant) mice were infected for 48h at a ratio of 1:5 (*H. capsulatum*:macrophage). Supernatants were collected and the production of LTB<sub>4</sub> was evaluated by ELISA. The phagocytosis was assessed by fluorescence using unopsonized or IgG-opsonized FITC-labeled *H.capsulatum* and MK886, a LTs inhibitor, was added to the cells previously to the infection.

**Results:** Interestingly, macrophages from resistant mice produced higher levels of LTB<sub>4</sub> upon *H. capsulatum* challenge than did those from susceptible mice. As expected, PMs from sv129 phagocytosed 1.9 fold-increased IgG-opsonized-*H.capsulatum* than PMs from C57BL/6. However, phagocytosis of IgG-opsonized-*H.capsulatum* by PMs from C57BL/6 and sv129 are both dependent on endogenous LTs, since when the LTs synthesis is pharmacologically inhibited, the phagocytosis was decreased 10 and 20 fold respectively.

**Conclusion:** LTs are important mediators involved in the mechanisms of host defense by participating in the patterns of resistance/susceptibility to infection of *H. capsulatum*.

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**Session: Animal Models, Pathogenesis & Host Defenses**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Different clinical isolates of *Mycobacterium tuberculosis* induced distinctive pulmonary inflammation in mice

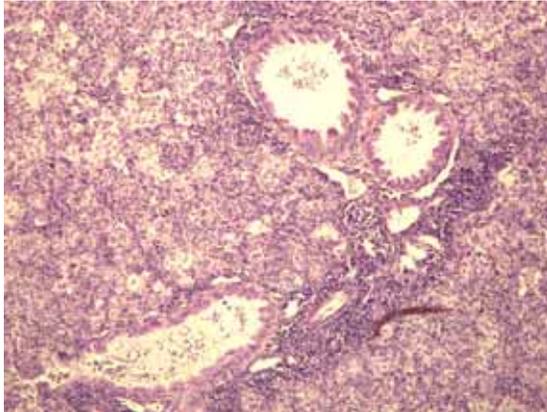
**E. Soares**<sup>1</sup>, C. M. Peres<sup>2</sup>, A. Secatto<sup>3</sup>, C. L. Silva<sup>4</sup>, L. H. Faccioli<sup>5</sup>

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**Background:** *Mycobacterium tuberculosis* (*Mtb*) is a virulent intracellular pathogen that infects and persist in host macrophages, resulting in granuloma formation and collagen deposition in the lung. The mechanisms that confer resistance to *Mtb* or results in establishment of disease are poor understood. Data from the literature suggest that differences in *Mtb* virulence contribute to setting up of the disease. In order clarify this aspect, our purpose is to investigate the immune response and lung pathology in mice infected with *Mtb* obtained from distinct clinical isolates. The isolates were recovered from patients with noncavitary (SV 009) or extrapulmonary (SV 068) active tuberculosis.

**Methods:** Female Balb/c mice were infected intratracheally with  $1 \times 10^5$  CFU/100  $\mu$ L of *Mtb* clinical isolates. Neutrophils and mononuclear cells recruitment to the lung were accessed by bronchoalveolar lavage at 30 days post infection and lung histology were evaluated on 30 and 60 days post infection.

**Results:** Mice infected with SV 068 showed 22% more neutrophils ( $9 \times 10^5$  mL) and 70% more mononuclear cells ( $6 \times 10^5$  mL) recruited to bronchoalveolar space 30 days post infection, when compared with mice infected with SV 009 that presented  $5 \times 10^5$  mL of neutrophils and  $4.5 \times 10^5$  mL of mononuclear cells. The histology analysis of lung tissue, demonstrated that animals infected with SV 068 present greater number of foamy macrophages containing aggregations of *Mtb*, especially at 60 days post infection. Also, in this period, we observed the presence of more intense infiltrate of neutrophils in perivascular and perialveolar spaces when compared with animals infected with SV 009.



**Histology Clinical Isolate SV068 Hematoxylin Eosin 60 days**

**Conclusion:** Our preliminary findings suggest that the host defense can vary accordingly to the type of clinical isolation, leading to a correlation between the virulence and the source of infection.

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**Session: Animal Models, Pathogenesis & Host Defenses**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Evaluation of the ferret as a model for influenza A/Brisbane/10/07 H3N2

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**Background:** During the 2008-2009 influenza season, an outbreak of the H3N2 A/Brisbane/10/07 strain occurred in several European countries. Great Britain experienced its worst influenza outbreak in eight years. Further, the World Health Organization (WHO) reports that influenza seasons in which H3N2 strains are predominate have been associated with a greater risk of severe illness and mortality rates. Thus, the need for effective vaccines and therapeutics against inter pandemic influenza strains remains a high priority. To provide an appropriate animal model for these novel products, the ferret was evaluated as a possible efficacy model for Influenza A/Brisbane/10/07 H3N2.

**Methods:** Forty-eight male ferrets were divided among 8 groups. Four groups of 8 ferrets were infected with approximately 1x10<sup>6</sup> TCID<sub>50</sub>/mL of A/Brisbane/10/07 in the nasal cavity. Sham control groups of 3 ferrets each were inoculated with allantoic fluid produced in embryonated chicken eggs and CMF-PBS. The ferrets were monitored for signs of infection by clinical observation, changes in weight and temperature, detection of viral shedding in nasal washes, and seroconversion as determined by microneutralization assay.

**Results:** All animals survived infection with only mild signs of illness. Weight loss was generally not observed after infection. Instances of febrility (defined as greater than two standard deviations from the baseline temperature) were present in some animals and persisted through the end of the study. Viral load in the nasal wash was present at 16 hours post-challenge. Nasal washes collected 24 to 72 hours post-challenge demonstrated an increased titer of 1 to 2 logs from the previous time-points, while samples collected 96 to 120 hours post-challenge exhibited a decrease in titer. Pre-challenge sera tested by the microneutralization assay (MN) lacked detectable neutralizing antibodies against A/Brisbane/10/07, however sera collected at the study end-point displayed a discernible increase in neutralizing antibody titer.

**Conclusion:** The most reliable indications of infection for an individual animal were viral recovery from nasal washes and observation of sneezing or coughing.

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**Session: Animal Models, Pathogenesis & Host Defenses**  
**Date: Friday, March 12, 2010**  
**Time: 12:30-13:30**  
**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Natural history study of a low dose HPAI (A/Vietnam/1203/04) infection in ferrets  
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<sup>1</sup>Battelle, Columbus, OH, USA, <sup>2</sup>Battelle, Columbus, OH, USA

**Background:** The objective of this study was to refine a low dose ferret therapeutic model of A/Vietnam/1203/04 influenza infection.

**Methods:** Forty-four male ferrets (*Mustela putorius furo*), 8-15 weeks in age, were divided into 4 groups of 8 ferrets (groups A, C, E and G; experimental) and 4 groups of 3 ferrets (groups B, D, F and H; controls). Animals in groups A, C, E and G were challenged with 5 x 10<sup>3</sup> TCID<sub>50</sub> of A/Vietnam/1203/04 via the intranasal route. Data collection was staggered by group pairs (groups A+B, C+D, E+F, G+H) so that clinical observations, blood (clinical hematology and clinical chemistry), nasal wash specimens (qPCR and TCID<sub>50</sub>), and temperature data were collected at approximately 8 hour intervals from 16-48 hours and at 24 hour intervals between 72 and 120 hours. After 120 hours, temperatures and clinical observations were recorded 2 times daily until the end of the study (Day 14) or death of the animal. Tissues were collected from any infected animal found dead or euthanized prior to day 14, for determination of viral load by a combination of qPCR and TCID<sub>50</sub>.

**Results:** All animals challenged with A/Vietnam/1203/04 succumbed. Observations for infected animals included: 1) significant decreases in body weight on all study days, 2) significant increases in body temperature as early as 1 day post-challenge, and 3) significant changes in clinical activity beginning at 40 hours. Analysis of clinical hematology data for peripheral blood specimens revealed significant differences between control and infected animals as early as 32 – 48 hours post-challenge for lymphocytes, white blood cells, and platelets. Significant persistent clinical chemistry parameter changes were limited to serum glucose, albumin, calcium and phosphorous. Results of TCID<sub>50</sub> assays and qPCR demonstrated virus shedding in nasal wash specimens as well as high viral titers in a number of tissues (brain, olfactory bulb, liver, lung, and nasal turbinates).

**Conclusion:** This study identified a combination of clinical parameters that can be used to characterize early onset of disease following a low dose A/Vietnam/1203/04 challenge.

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**Session: Animal Models, Pathogenesis & Host Defenses**  
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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

FcγR IIA polymorphism -131H/R and malaria severity in Ghanaian children  
**D. Amoako-Sakyi**  
University of Cape Coast, Cape Coast, C/R, Ghana

**Background:** *Plasmodium falciparum* malaria still remains a major public health problem in most parts of the world especially in Sub Saharan Africa. Pathogenesis of severe malaria is still not fully understood and several factors have been suggested to play a role. Fc receptors constitutes a crucial link between humoral and cellular immune responses and are thought to be important in the pathogenesis of severe malaria. *FcγRIIA* which belongs to the family of Fc receptors are predominantly expressed on neutrophils which have been shown to kill merozoites. Thus variants of *FcγRIIA* may influence the binding affinity of IgG and subsequently affect phagocytosis and parasite clearance. On the other hand, increased binding affinity and enhanced phagocytosis can also stimulate the release of some immune factors in quantities that are detrimental leading to severe malaria. The objective of this study was to investigate the role of the *FcγRIIA-131H/R* variant in the pathogenesis of severe malaria in Ghanaian children.

**Methods:** This study was a hospital based unmatched case-control study involving 290 malaria cases and controls. The study was conducted at the Korle-Bu Teaching Hospital in Accra, Ghana. PCR-RFLP was used to characterize the *FcγRIIA-131H/R* polymorphism in 210 Ghanaian children with uncomplicated malaria, severe malaria anaemia, and cerebral malaria.

**Results:** The study revealed that the *131HH* genotype is associated with susceptibility to cerebral malaria (OR = 4.0, 95% CI = 1.28 – 12.52;  $p = 0.014$ ). The results also revealed that carriers of the R allele had a significantly lower parasitaemia ( $p < 0.050$ ) than non-carriers.

**Conclusion:** The presence of *FcγRIIA-131HH* which is regarded as a low affinity variant may lead to decreased phagocytosis and poor control of parasitaemia in Ghanaian Children. This however does not exclude the possibility that IgG binding to the *FcγRIIA-131HH* variant can stimulate the release of factors that may contribute to the development cerebral malaria.

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**Final Abstract Number: 73.010**  
**Session: Animal Models, Pathogenesis & Host Defenses**  
**Date: Friday, March 12, 2010**  
**Time: 12:30-13:30**  
**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

An animal model to study antimicrobial effects on community-acquired methicillin-resistant *Staphylococcus aureus* infection

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**Background:** Outbreaks of Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections have been reported worldwide, and a main concern is the severity of infection with fatal necrotizing pneumonia and toxic shock. Studies showed that the expression of virulence proteins by *Staphylococcus aureus* depends on the strain types and could be modulated by different agents including antibiotics. The objective of the study was to establish a murine infection model to study the in-vivo effects of commonly used antimicrobials on the severity of CA-MRSA pneumonia.

**Methods:** 6-to-8-week-old BALB/c mice were divided into groups of six in individually ventilated cages and injected with intranasal injection of a representative strain of CA-MRSA. The bacterial inoculum was titrated with varying concentration ranging  $1 \times 10^7$  –  $5 \times 10^8$  cfu, with controls receiving same volume of broth medium. Mice were sacrificed on Day 2, 3 and 4 of infection and the lungs were dissected, placed in formalin, embedded in paraffin blocks and sectioned for histological examination. The severity of pneumonia was scored according to size of leukocyte infiltration and level of alveolar integrity and epidermis damage. Sub-inhibitory concentrations of antimicrobial agents (half of MIC per body weight) of vancomycin, ciprofloxacin, gentamicin were administered at different timepoints after bacterial inoculation.

**Results:** The severity of pneumonia varies with the bacterial inocula titrated. All infected mice developed pneumonia after 24 hours of infection after inoculation of at least  $1 \times 10^8$  cfu bacteria. The endpoint for outcome of pneumonia was interpreted at 24 hours. Incubation of 48 and 72 hours led to increasing mortality of mice and masked the histological effects of pneumonia. The most significant effects was in the ciprofloxacin group, with 33% (2/6) mice in ciprofloxacin-treated group died and the remainder (4/6) developed severe pneumonia of Grade 5 histologically (in terms of size and extent of necrotizing lesions) than the infected-control group. The non-infected-control group remained healthy.

**Conclusion:** A murine model for CA-MRSA pneumonia to study effects of different drugs was established. The choice of antimicrobials that minimizes release of toxins and reduces the severity of pneumonia is an invaluable approach in improving treatment outcome in CA-MRSA infections.

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**Type: Poster Presentation**

Increased killing of liver NK cells by Fas/FasL and NKG2D/NKG2DL contributes to hepatocyte necrosis in virus-induced liver failure

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<sup>1</sup>Tongji Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan, Hubei, China, <sup>2</sup>Tonji Hospital, Wuhan, China

**Background:** The role of liver NK cells in virus-induced severe viral hepatitis and subsequently hepatic failure is not well defined.

**Methods:** In this study, we investigated the role of liver NK cells in the development of hepatocyte necrosis in fulminant hepatic failure (FHF) and acute-on-chronic liver failure (ACLF) due to viral infection. A mouse model of FHF induced by murine hepatitis virus strain 3 (MHV-3) was used to study the role of liver NK cells. Samples from patients with hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF) were examined.

**Results:** Following MHV-3 infection, the number of NK cells in livers of Balb/cJ mice increased markedly, peaked at 48 h post-infection, and remained at a high level until sacrifice. In peripheral blood, spleen, and bone marrow, this number decreased significantly. Expression of CD69, cytotoxic activity, and intracellular IFN- $\gamma$  and TNF- $\alpha$  production by liver NK cells at 48 h post-infection were all significantly upregulated. Highly activated liver NK cells were cytotoxic to MHV-3-infected hepatocytes and this effect was markedly inhibited by anti-FasL plus anti-NKG2D monoclonal antibodies. Furthermore, the accumulation of hepatic NK cells and increase in the expression of natural cytotoxicity receptors (NKP30 and NKP46) on the peripheral NK cells from patients with HBV-ACLF were correlated with disease progression.

**Conclusion:** These results indicate the pivotal involvement of NK cells in the pathogenesis of FHF and HBV-ACLF, and the synergetic effect of Fas-FasL and NKG2D-NKG2DL pathway in liver NK cell mediation of hepatocyte toxicity.

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**Session: Animal Models, Pathogenesis & Host Defenses**

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**Type: Poster Presentation**

CMV infection causing Adult Onset Still's Disease: A clinical case

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**Background:** CMV normally causes a mononucleosis syndrome in immunocompetent persons. Immunologic aberrations recognized with this infection include hypogammaglobulinemia, rheumatoid factor (RF), antinuclear antibodies and anticomplementary activity. CMV has been associated with inflammatory bowel diseases, rheumatoid arthritis, psoriasis and Wegener's granulomatosis.

Adult Onset Still's Disease (AOSD) is an RF-negative inflammatory disorder of unknown aetiology, responsible for many cases of fever of unknown origin. It is marked by cytokine abnormalities and a predominant Th1-cell response.

**Methods:** A 34-year-old man presented himself to the ED on June 2007 with two weeks of fever, arthralgia and sore throat. After an empirically course with Amoxicillin Clavulanate he also experienced a self-limited morbilliform rash.

On admission he exhibited 39° temperature, cervical adenopathies and mild splenomegaly. Analysis showed leucocytosis, atypical lymphocytosis, C-RP 3.53 mg/dl and ESR 21 mm/h. There was an elevation of hepatic enzymes and positive CMV IgM and IgG. The search for Hbs, p24 antigen and antibodies against HIV, HCV, EBV, *Toxoplasma gondii* and Streptolysin-O was negative.

**Results:** In October 2007 he remained symptomatic. His liver was biopsed. Histological sections in H&E identified epithelioid granulomas, portal inflammation, nuclear and cytoplasmic inclusions in hepatocytes and Kupfer cells. Immunohistochemistry was compatible with CMV. He was started on Valganciclovir.

Fever remained intermittently. In February 2008 he maintained positive titers of CMV IgM and elevated liver enzymes. The search for other infections with cultures of peripheral blood and bone marrow was negative. Antibodies to other agents such as *Brucella* spp, *Borrelia* spp and *Coxiella burnetii* were negative. Echocardiogram, body CT scan and upper and lower GI endoscopy were normal.

Markers for autoimmune/inflammatory diseases such as ANA, ANCA and RF and tumor markers were also negative. Cytometry showed an inversed TCD4+/TCD8+ ratio and low CD19+ B lymphocytes. IgA, IgG and IgM levels were low. Tetanus vaccination resulted in antibody response, excluding Common Variable Immunodeficiency.

**Conclusion:** In June 2008 he maintained fever and arthralgia. Analysis registered leucocytosis, ESR 77 mm/h and ferritin 6400 ng/ml. CMV IgM was negative and liver enzymes were normal. AOSD was suspected and the patient started prednisone 1mg/kg/day. The patient had a marked improvement after institution of this therapy.

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**Session: Animal Models, Pathogenesis & Host Defenses**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Telbivudine preserves Th1 cytokine response and down regulates PD-L1 in MHV-3-induced viral hepatitis model

Z. Wu<sup>1</sup>, W. Yan<sup>1</sup>, W. Guo<sup>1</sup>, Y. Zou<sup>1</sup>, H. Wang<sup>1</sup>, X. Wang<sup>1</sup>, X. Yang<sup>1</sup>, Y. Lu<sup>1</sup>, X. Luo<sup>2</sup>, **Q. Ning<sup>1</sup>**

<sup>1</sup>Tongji Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan, Hubei, China, <sup>2</sup>Tonji Hospital, Wuhan, China

**Background:** Telbivudine is an orally bioavailable L-nucleoside with potent and specific anti-hepatitis B virus (HBV) activity. Recent studies have suggested a potential immunomodulatory effect of telbivudine. To address this, we sought to determine the effect of telbivudine on the immune system, particularly cytokine production and T-cell response, in the mouse hepatitis virus strain 3 (MHV-3)-induced hepatitis model.

**Methods:** The effect of telbivudine on virus replication and cytokines production in MHV-3 infected macrophages were investigated. In vivo the T cell response to Telbivudine treatment were also studied in MHV-3 induced viral hepatitis model.

**Results:** *In vitro* there was no significant difference in MHV-3 replication in macrophages with or without telbivudine treatment. The production of tumor necrosis factor- $\alpha$  and interleukin-12 was increased significantly in MHV-3-induced macrophages with telbivudine treatment. *In vivo* survival was enhanced in telbivudine-treated mice with marked normalization in clinical conditions and histologic lesions. Serum levels of interferon- $\gamma$  were elevated significantly after telbivudine treatment in MHV-3-infected C3H mice. In contrast, serum interleukin-4 levels were decreased significantly. Furthermore, telbivudine treatment had a beneficial effect on T cells, restoring their ability to undergo proliferation and secrete cytokines but not to enhance cytotoxicity on infected hepatocytes. Notably, we found that telbivudine treatment suppressed programmed death ligand 1 expression on T cells.

**Conclusion:** These data identify an immunomodulatory mechanism of telbivudine treatment in the MHV-3-induced viral hepatitis model and provide insights into a potential additional mode of action for the management of viral hepatitis infection.

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**Session: Animal Models, Pathogenesis & Host Defenses**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Transcriptome profile of the human endothelial cell response to high- and low-density infections of *Candida albicans*

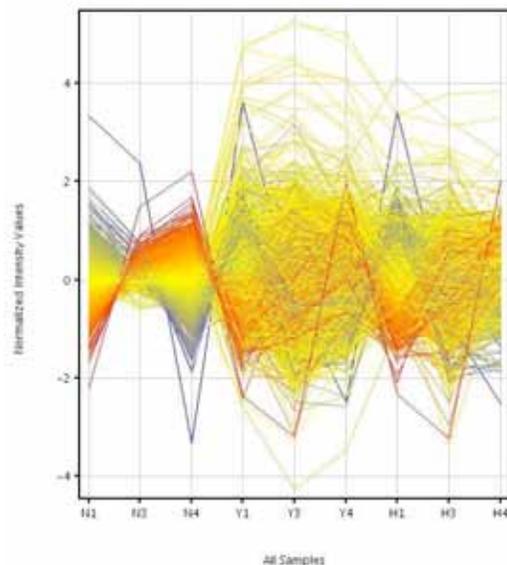
**P. P. Chong**<sup>1</sup>, C. S. Y. Lim<sup>1</sup>, Y.-H. Tan<sup>1</sup>, H. F. Seow<sup>2</sup>, R. Rosli<sup>1</sup>

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**Background:** *Candida albicans* morphology switching and quorum-sensing are important factors for pathogenicity and virulence in persons with a compromised or deficient immune system. This study investigates the *in vitro* response of human umbilical vein endothelial cells (HUVECs) to infections with low and high densities of *C. albicans*. We hypothesize that higher cell densities of *C. albicans* yeast-form cells (blastospores), are more detrimental to HUVECs than lower cell densities of hyphal forms.

**Methods:** Three biological replicates of confluent HUVECs in 6-well plates were challenged with 106 *C. albicans* blastospores (low-density infection) and 5 x 10<sup>7</sup> blastospores (high-density infection) for 8 hours. The low-density infection generated true hyphae, but in the high-density infection, *C. albicans* remained as blastospores. RNA from these samples were subjected to DNA microarray transcript profiling. For MTT and XTT cell proliferation assays, conditioned media from the co-cultures for microarray experiments were incubated with HUVECs in 96-well plates for 24 hours.

**Results:** The high-density blastospore-HUVEC co-cultures elicited significantly higher differential expression of genes involved in functional pathways of apoptosis, immune response, cell-cell signaling and cancer development, such as *ZC3HAV1*, *HES1*, *CSF2*, *CXCL2* and *PIM1*, compared to the low-density true hyphae-HUVEC co-cultures. Cell proliferation assays also show that HUVECs incubated with conditioned media from the high-density infection caused a higher percentage of cell death compared to incubation with conditioned media from the low-density infection. These results suggest that high densities of unattenuated, innate *C. albicans* blastospore cells can cause significant cellular toxicity, even though the cells are in the yeast form, not filamentous.



#### Microarray Data Analysis

**Conclusion:** Transcript profiling of this *in vitro* endothelial cell model may provide new insights into how *C. albicans* cell densities affect the host during the colonization and invasion through the bloodstream to the deep organs. We also suggest that quorum-sensing molecules and other unknown secretions from high-density *C. albicans* infections are strong inducers of cellular injury leading to cell death in systemic candidiasis.

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**Session: Animal Models, Pathogenesis & Host Defenses**

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**Type: Poster Presentation**

Levels of pro-inflammatory cytokines and soluble cell adhesion molecules serves as early markers for recognition of diseases severity in patients with dengue fever and dengue hemorrhagic fever

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**Background:** Several mechanisms have been proposed to explain the pathogenesis of dengue virus infection. One of the most important finding is the production of pro-inflammatory cytokines as responsible of activation of vascular wall, plasma leakage and subsequently disease severity. Our aim was to determine levels of pro-inflammatory cytokines during the course and severity of dengue infection.

**Methods:** Seventeen febrile patients with dengue infection (RT-PCR and IgM-anti-dengue) were included and classified by diseases severity (OMS). Dengue fever (DF) were observed in 7/17 cases (2 Adults, 22-56 years old,  $39 \pm 24.04$ ; and 5 children 3-11 years old,  $7.60 \pm 3.78$ ); and ten cases had Dengue Hemorrhagic Fever (DHF, 2 Adults; 22-47 years-old,  $34 \pm 17.67$  and 8 children, 5 months-12 year-olds,  $4.52 \pm 4.27$ ). Two afebrile patients were included as controls.

At least two serum samples were collected during either acute phase (<3 days post-infection) and critical phase (4 and more days) of the disease; and stored a -20°C until used. Serum levels of TNF- $\alpha$ , IL-8, sICAM-1 y sVCAM-1 were determined by commercially available ELISA kits following manufacture instruction.

**Results:** Levels of IL-8 ( $p > 0.05$ ) and sTNF- $\alpha$  ( $p = 0.026$ ) were significant higher in DHF cases during the critical phase ( $\geq 4d$ ) of the diseases. High soluble levels of ICAM-1 were observed in FD and DHF cases, with a significant differences ( $p = 0.025$ ) during critical phase of the disease. Moreover, significant soluble levels of VCAM-1 were observed in both cases of DF ( $p = 0.013$ ) and DHF ( $p = 0.011$ ) during the early stages of the clinical course (<3d).

**Conclusion:** High levels of TNF- $\alpha$  and IL-8, are correlated with activation of endothelial adhesion molecules sICAM-1 y sVCAM-1 during the critical stages of diseases, especially in DHF where plasma leakage plays a role in vascular damage. Finally, sVCAM-1 expression serves as a early marker for recognition of DHF and SSD.

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**Final Abstract Number: 73.016**

**Session: Animal Models, Pathogenesis & Host Defenses**

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**Type: Poster Presentation**

*Cavia porcellus* as a model for experimental infection by *Trypanosoma cruzi*

**Y. Castro Sesquen<sup>1</sup>**, R. H. Gilman<sup>2</sup>, M. Verastegui<sup>1</sup>, V. Yauri<sup>1</sup>, N. Angulo<sup>1</sup>, D. E. Velasquez Portocarrero<sup>1</sup>, C. Bern<sup>3</sup>

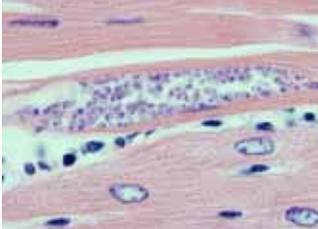
<sup>1</sup>Universidad Peruana Cayetano Heredia, Lima, Peru, <sup>2</sup>The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, <sup>3</sup>CDC, Atlanta, AT, USA

**Background:** Chagas disease remains a major public health concern with 8 to 10 million people already being infected, an annual incidence of 200 000 new cases in 15 countries, and 14.000 deaths associated with the infection per year.

The guinea pig (*Cavia porcellus*) is one of the major reservoirs of *T. cruzi* in Perú and Bolivia, however the number of studies using this mammal is reduced. The aim of this study was to evaluate the use of the guinea pig as an animal model for Chagas disease.

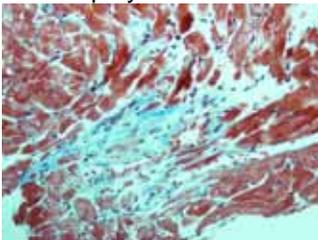
**Methods:** Seventy two guinea pigs were inoculated intradermally with 104 trypomastigotes form of *T. cruzi* strain Y (Experimental Group, EG) and 12 guinea pigs were used as Control Group (CG). Eight animals from EG and two from CG were sacrificed at different times (5, 15, 20, 25, 40, 55, 115, 165 and 365 days post infection).

**Results:** Clinical signs and mortality weren't observed. Peak of the parasitaemia was at 20 days post infection (pi), then decreased and since 55 days pi was negative. Specific IgM were detected from 15 to 115 days pi. Specific IgG were detected at 20 days pi and reached its maximum value at 115 days pi which were maintained until 365 days pi. k-DNA was detected in the blood samples from 100% of animals during the acute phase (5 to 55 days pi), during the chronic phase (115 to 365 days pi) the number of positives dropped to 37% (10/27). Lymphocytic infiltrate with some polymorphonuclear cells and amastigotes nests were observed by H&E in the heart, skeletal muscle, intestine, liver, kidney and brain. k-DNA was detected in the same tissue and in the skin, esophagus, lung and spleen. The most severe histopathological changes and the largest number of amastigotes nests were found in the heart. The major degree of inflammation and number of amastigotes were at day 25 pi. Fibrosis (62.5%) and vasculitis (62.5%) were observed in the heart at 365 days pi.



**Heart tissue from a guinea pig 25 days after *T. cruzi* infection; note the amastigote nests in a cardiac muscle fibers, surrounded by some lymphocytes (H&E, magnification 500X).**

**Conclusion:** These results indicate that the guinea pig is a good model for infection by *T. cruzi* as it displays similar characteristics to those found in humans.



**Heart tissue of a guinea pig 365 days after *T. cruzi* infection; note foci of fibrosis in the myocardium (Trichromic staining, magnification 200X)**

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**Session: Antibiotic Resistance: Gram-Positive**  
**Date: Friday, March 12, 2010**  
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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Prevalence of Vancomycin Intermediate *Staphylococcus aureus* (VISA) in a tertiary care hospital in Eastern Nepal

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BP Koirala Institute of Health Sciences, Dharan, Nepal

**Background:** *Staphylococcus aureus* is a leading pathogen causing many serious and life threatening infections. Resistant *S.aureus* has become a serious matter of concern over recent years. Knowledge of antimicrobial susceptibility profile of the local isolates is essential for selection of appropriate therapy for the effective management of Staphylococcal infections. Present study was undertaken to study the status of antimicrobial resistance among the clinical isolates of *Staphylococcus aureus* with special reference to the prevalence of Vancomycin Intermediate *Staphylococcus aureus*(VISA) .

**Methods:** *S.aureus* isolated from the clinical specimens submitted to the microbiology unit of clinical laboratory services, BP Koirala Institute of Health Sciences (BPKIHS) hospital were studied. Isolation and identification of *S.aureus* was done by standard microbiological technique. All the isolates were tested for antimicrobial susceptibility by disc diffusion method. Minimum concentration of vancomycin required to inhibit the *S.aureus* isolates was determined by using standard agar dilution technique.

**Results:** A total 300 *S.aureus* isolates were obtained from various clinical specimens .All the isolates were susceptible to vancomycin in disc diffusion method. However, in MIC testing 80(26.66%) were found to have intermediate susceptibility to vancomycin (VISA) with MIC (8-16mg/L).MRSA formed 26% of total isolates of which 18.42% were VISA. A significant number of VISA isolates were found to be multidrugresistant.Most of the VISA isolates were obtained from pus(71.25%),followed by blood(13%) suggesting association of VISA more commonly with abscesses and sepsis.

**Conclusion:** Resistant Staphylococci are prevalent in our set up. In addition, emergence of *S.aureus* with reduced susceptibility to vancomycin has added to the complexity of problem. Prudent use of antimicrobials and continuous surveillance are required for early recognition and containment of spread of this emerging pathogen. Further study on *S.aureus* infections, its antimicrobial resistance and its correlation with clinical conditions and epidemiological factors is recommended in future.

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**Final Abstract Number: 74.002**

**Session: Antibiotic Resistance: Gram-Positive**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

A worldwide surveillance program studying the *In Vitro* Activity of Tigecycline and 10 common therapeutic agents against methicillin-resistant *Staphylococcus aureus* and Vancomycin-resistant *Enterococcus* species from 2004-2009

**B. Johnson**<sup>1</sup>, M. Renteria<sup>1</sup>, J. Johnson<sup>1</sup>, R. Badal<sup>1</sup>, S. Bouchillon<sup>1</sup>, D. Hoban<sup>1</sup>, M. Dowzicky<sup>2</sup>

<sup>1</sup>International Health Management Associates, Inc., Schaumburg, IL, USA, <sup>2</sup>Pfizer Inc, Collegeville, PA, USA

**Background:** The T.E.S.T. program determined the *in vitro* activity against methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus* spp. of TIG and 10 antimicrobials commonly prescribed for serious gram-positive infections: amoxicillin-clavulanic acid (AUG), piperacillin-tazobactam (PT), levofloxacin (LVX), ceftriaxone (CAX), linezolid (LZD), minocycline (MIN), vancomycin (VAN), ampicillin (AMP), penicillin (P), and meropenem (MER). Study strains were collected from 697 laboratories in 55 countries globally throughout 2004-2009.

**Methods:** A total of 27,846 clinical isolates (10,806 enterococci, 17,040 *S. aureus*) were identified to the species level at each participating site and confirmed by a central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using broth microdilution panels. Antimicrobial resistance was interpreted according to CLSI breakpoints with TIG susceptible breakpoints defined as <0.5 mcg/ml for *S. aureus* and <0.25 mcg/ml for enterococci.

**Results:** 12.8% (1,381/10,806) of enterococci were resistant to vancomycin (VRE), and 42.1% (7,174/17,040) of *S. aureus* were resistant to oxacillin (MRSA). Among the vancomycin resistant *E. faecium* (VREF), % resistant rates to other study drugs were LVX 99.3, P 98.5, AMP 98.3, VAN 100, MIN 10.8 and LZD 0.2. Percent resistant rates for MRSA were P 100, AMP 100, AUG 75.4, LVX 74.9, PT 65.8 CAX 46.5, IMP 24.4, MIN 1.3, LZD 0.0, and VAN 0.0. TIG inhibited 100% of the enterococci and *S. aureus* resistant to other drugs. Modal TIG MICs for VRE/nonVRE were 0.12/0.12, and 0.12/0.12 for MRSA/MSSA.

**Conclusion:** TIG retained potent activity against drug-resistant *S. aureus* and enterococcal isolates, inhibiting 100% of all strains tested at their defined breakpoints of 0.5 and 0.25 mcg/ml, respectively.

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**Final Abstract Number: 74.003**  
**Session: Antibiotic Resistance: Gram-Positive**  
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**Type: Poster Presentation**

The survey of risk factors of multi drug resistant of *E. faecalis* isolated from clinical samples

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**Background:** During the last decade, enterococci have become important nosocomial pathogens, representing the second leading cause of urinary tract infections. This increasing prevalence has been paralleled by the occurrence of multi-drug resistant (MDR). The aim of this cross-sectional prevalence study was to determine the prevalence and risk factors of antibiotic resistance of *E. faecalis* isolated from clinical samples in hospitalized patients in Kashan, Iran.

**Methods:** This descriptive study was done on clinical specimens isolated from hospitalized patients. From September 2007 to 2008, a total of 106 *E. faecalis* isolates were collected from clinical specimens in Kashan hospitals. Antimicrobial susceptibility test was determined with disk diffusion and minimal inhibitory concentration of vancomycin assayed by E test.

**Results:** From 128 isolates were found to consist of *Enterococcus faecalis* 106( 82.8 %) and *Enterococcus faecium* 22 (17.2%). Resistance rates for *E. faecalis* were as follows: Erythromycin 52.8%; ciprofloxacin 40.6%; gentamicin, 38.7%; levofloxacin 34%; penicillin, 29.2%; nitrofurantoin 18.9% ; ampicillin, 11.3%; Imipenem 10.4% ; and vancomycin, 4.7% All isolate were sensitive to linezolid. Multidrug-resistant (MDR) phenotype (resistance to three or more of drugs) occurred in 37.7%. Risk factor for a VRE-positive culture were antimicrobial usage within 2 months before culture (P = 0.045).

**Conclusion:** Emergence of multi-resistant *E. faecalis* and high level resistance to vancomycin shown by *E. faecalis* strains is of concern because of the decrease in the therapeutic options for treatment of infections caused by enterococci.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

The relationship between macrolide resistance in *Streptococcus pneumoniae* and consumption of oral macrolides in Republic of Croatia and City of Zagreb

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**Background:** The aim of this study was to investigate the relationship between increased resistance of *Streptococcus pneumoniae* to macrolides and use of macrolides in Croatia from 2001 to 2008, with the special emphasis on differences in outpatient consumption of macrolides in City of Zagreb and total consumption in Republic of Croatia.

**Methods:** Zagreb Institute of Public Health in collaboration with Croatian Drug Agency collected and analyzed data on outpatient consumption of drugs by use of standardized methods of drug monitoring. Data on the number and size of packages were obtained from all pharmacies in Croatia, and based on these data the number of defined daily doses (DDD) and DDD per 1000 inhabitants per day (DDD/1000/day) were calculated. In the eight year study period upper respiratory isolates of *S. pneumoniae* were collected and antibiotics susceptibility was tested during October, November and December by use of standardized methods.

**Results:** The City of Zagreb led in drug consumption in Croatia according to counties, followed by Sibenik-Knin County and Dubrovnik-Neretva County. Outpatient utilization of macrolides was high in the City of Zagreb and varied from 4.67 in 2001 to 3.98 DDD/1000/day in 2008, while in the rest of Croatia varied from 2.12 to 2.92 DDD/1000/day. The observed macrolide resistance in *S. pneumoniae* significantly raised in 2005 in the City of Zagreb from 22% to 39%, and in 2008 recorded macrolide resistance in Zagreb was 38%. The significant increase in macrolide resistance in the rest of Croatia was observed in 2007 and 2008, from 25% in 2005 to 34% in 2007 and 40% in 2008, two years after the detection of increase of macrolide resistance in the City of Zagreb.

**Conclusion:** Further study is needed to clear whether the clonal spreading of resistant strains from Zagreb to other parts of Croatia is responsible for increasing macrolide resistance in Croatia, or genetically unrelated strains caused the high macrolide resistance due to increased macrolide consumption at national level.

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Blood culture isolates in a tertiary care hospital of Eastern Nepal: Trends in antimicrobial resistance

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**Background:** Bloodstream infections remain the major cause of morbidity and mortality. Knowledge of pattern of antimicrobial agents among local isolates and its changes over time plays a major role for selecting appropriate and effective therapy. Present study was undertaken to determine the type and frequency of bacterial isolates obtained from blood culture and their antimicrobial resistant pattern in BP Koirala Institute of Health Sciences (BPKIHS), a tertiary care hospital in eastern Nepal.

**Methods:** Blood culture specimens submitted to Department of Microbiology, BPKIHS over a duration of 5 years were evaluated. Isolation, identification and determination of antimicrobial susceptibility were performed by standard microbiological techniques.

**Results:** Out of total 39542 blood culture samples, 4889 (12.36%) yielded the growth. Range of culture positivity was 9.53-15.07%. Majority of isolates was obtained from neonates. Except in 1st year, Gram Positive cocci(GPC) were isolated more frequently than Gram Negative Bacilli (GNB). *Staphylococcus aureus* was the predominant organism among GPC throughout the study period. Enterococci replaced Coagulase negative staphylococci as 2nd most common GPC in last 3 years. *Acinetobacter* spp accounted for the most common GNB for last 3 years (32-38%). Isolation of *Salmonella* Typhi decreased from 11.37 to 0.86% and that of *Salmonella* Paratyphi A from 7.33 to 0.64% over the time period. Multidrug resistant decreased among *Salmonellae*, however reduced susceptibility to ciprofloxacin has emerged. Among *S.aureus*, around 26% were found to be Methicillin resistant. Vancomycin resistance was not observed in disc diffusion testing. Above 50% of Enterococci exhibited resistance to aminoglycosides. Most GNB were highly resistant to commonly used antimicrobial agents. For *Pseudomonas*, resistance to Carbenicillin and Piperacillin had increased and reached upto 100%.

**Conclusion:** Both gram positive and gram negative bacteria were responsible for bloodstream infections. Increased frequency of resistance observed in blood culture isolates towards commonly used antimicrobials in has emerged as serious matter of concern. Continuous surveillance antimicrobial resistance among local isolates is of utmost importance for monitoring and early detection of resistance.

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Multicenter evaluation of tigecycline activity in Latin America: Report from the SENTRY antimicrobial surveillance program (2009)

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**Background:** Tigecycline, the first glycylicycline, presents a therapy option for emerging multidrug-resistant (MDR) Gram-positive (GP) and -negative (GN) pathogens in complicated intra-abdominal, skin structure, and respiratory infections. Latin American countries have high and increasing prevalence of MDR isolates of Enterobacteriaceae (ESBLs), *Acinetobacter* spp. (carbapenem-resistant) and Gram-positive cocci (MRSA, VRE). The aim of this study was to assess the activity of tigecycline and comparator antimicrobials against recent (2009) isolates from Latin America.

**Methods:** Ten sites forwarded 2,672 strains to a central laboratory (JMI Laboratories, North Liberty, IA, USA). Infection types (n) were: bloodstream (1139), community respiratory (59), hospitalized pneumonia (424), skin and skin structure (514), GP miscellaneous (536). Country (n sites; n isolates) were: Argentina (2; 641), Brazil (4; 977), Chile (2; 557), and Mexico (2; 497). Susceptibility testing against a large panel of antimicrobials was performed by CLSI methods (M7-A8, 2009). Identifications were confirmed and interpretive/screening criteria were also by CLSI guidelines (M100-S19, 2009), except for tigecycline where United States - Food and Drug Administration breakpoints were applied.

**Results:** Tigecycline was active against 96-100% of indicated/tabulated species (see Table). Tigecycline MIC<sub>90</sub> values were not influenced by oxacillin or vancomycin susceptibility patterns for *S. aureus* and enterococci, respectively (0.25 mg/L for total *S. aureus* and enterococci, MSSA, MRSA, VRS, and VRE). Resistance patterns noted were: tetracycline (see Table), ESBL- and fluoroquinolone resistance in Enterobacteriaceae (28.8, 33.7%, respectively), VRE (9.9%), MRSA (47.7%) and *Acinetobacter* spp. carbapenem (imipenem)-resistant (76.1%).

Organism (no. tested)	Cum. % inhibited at tigecycline MIC (mg/L):							Tig	Tet
	≤0.06	0.12	0.25	0.5	1	2	4		
<i>S. aureus</i> (688)	10	46	99	>99	100			99.9	6.7
CoNS (221)	8	35	89	>99	100			99.5	7.7
Enterococci (292)	18	49	97	100				96.6	61.0
<i>E. coli</i> (291)	4	35	90	>99	>99	>99	100	99.7	47.8
<i>Enterobacter</i> (107)	0	4	41	81	98	100		100	16.8
<i>Klebsiella</i> (202)	0	3	43	85	95	99	100	98.5	29.2
<i>Acinetobacter</i> (205)	2	5	28	60	89	99	100	98.5	28.3

a. Tigecycline susceptibility by US-FDA and Jones et al. (2007) criteria.  
 b. Tetracycline resistance by CLSI criteria.

**Conclusion:** MDR rates across all GP and GN species have increased in Latin America. However, tigecycline remained very active against these MDR strains. Tigecycline exhibited promising spectrum/potency exceeding currently available agents against sampled isolates from Latin America.

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Genetic diversity of enterococci harboring high-level gentamicin resistance genes *aac(6')-Ie-aph(2'')-Ia* or *aph(2'')-Ie* in a Japanese hospital

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**Background:** Enterococci are important human pathogens implicated in various nosocomial infections. In Japan, prevalence of high-level gentamicin resistance (HLGR) is recognized as a potential concern for enterococcal infections, although vancomycin resistance is rarely found. In the present study, prevalence of two aminoglycoside-modifying enzyme (AME) genes *aac(6')-Ie-aph(2'')-Ia* and *aph(2'')-Ie* which confer HLGR to enterococci and clonal diversity of the enterococcal isolates with these resistance genes were analyzed.

**Methods:** A total of 1128 clinical isolates of enterococci obtained in a Japanese hospital during a period between 1997 and 2007 were analyzed for presence of *aac(6')-Ie-aph(2'')-Ia* and *aph(2'')-Ie* by PCR. IS256-flanking patterns, sequence diversity of these genes were analyzed by PCR and direct sequencing. *Enterococcus faecalis* and *Enterococcus faecium* strains with the HLGR genes were typed by multi-locus sequence typing (MLST).

**Results:** The *aac(6')-Ie-aph(2'')-Ia* was detected in 40.1%, 12.9%, and 3.6% of *E. faecalis*, *E. faecium* and other enterococcal species, respectively, and *aph(2'')-Ie* was detected in 3.3% of *E. faecium* isolates. Two IS256-flanking patterns, truncated forms of Tn5281 lacking IS256 at only 5'-end and both 5' and 3' ends of *aac(6')-Ie-aph(2'')-Ia* were the most prevalent. Among 14 *E. faecalis* and 10 *E. faecium* strains harboring *aac(6')-Ie-aph(2'')-Ia*, eight and six different sequence types (STs) were identified by MLST, respectively. STs of most of the *E. faecium* strains belonged to the clonal complex CC17 which is known as globally emerging lineage of vancomycin- or ampicillin-resistant *E. faecium* clones. In contrast, *E. faecium* strains with *aph(2'')-Ie* were classified into newly assigned STs (ST426 or its single locus variant ST427).

**Conclusion:** HLGR gene *aac(6')-Ie-aph(2'')-Ia* was distributed to *E. faecalis* with various genetic lineages and *E. faecium* with lineages in CC17 mostly. The *aph(2'')-Ie* was carried by *E. faecium* from a few limited lineages.

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The expansion of ST80-SCC*mec*-IV clone of community-acquired methicillin resistant *Staphylococcus aureus* in Kuwait hospitals

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Kuwait University, Health Science Center, Safat, Kuwait

**Background:** Community- acquired methicillin resistant *S. aureus* (CA-MRSA) that infects patients with no traditional risk factors for the acquisition of MRSA infections is increasing in many parts of the world. In this study, CA-MRSA obtained from patients in eight Kuwait hospitals were characterized for their antibiotic resistance and typed using pulsed-field gel electrophoresis (PFGE), SCC*mec* and multilocus sequence typing (MLST) to ascertain their relatedness.

**Methods:** In total 135 CA-MRSA isolates were obtained from eight hospitals in Kuwait between 1 January 2005 and 31 December 2006. Antibiotic susceptibility testing was performed by the disk diffusion method. MIC was determined using Etest strips. PFGE was performed utilizing *Sma*I digestion of genomic DNA followed by fragment separation in a CHEF- DRIII system. SCC*mec* typing and MLST were performed according to internationally standardized protocols.

**Results:** They were resistant to kanamycin (62%), fusidic acid (42%), tetracycline (39.3%), erythromycin and clindamycin (21.5%), gentamicin (5.9%), streptomycin (6.7%), trimethoprim (5.9%), mupirocin (6.6%) cadmium acetate (82.2%) and ethidium bromide (12.6%). All were susceptible to vancomycin, teicoplanin and linezolid. One hundred and three (76.3%) , 11 (8.14%), 9 (6.67%) and 12 (8.9%) isolates carried SCC*mec* type IV, SCC*mec* –Iva, SCC*mec*- IVc and SCC*mec* - V genetic elements respectively. PFGE yielded 10 PFGE types and subtypes with the majority of them belonging to PFGE type 1 and subtypes (47.4%), type 2 (22.2%), type 3 (3.7%), type 4 (14.0%). Other PFGE types were present in small numbers MLST revealed 10 sequence types comprising ST80 (46.6%), ST30 (10.7%), ST5 (19.3%), ST6 (10.7%), ST8 (3.6%), ST46 (3.6%), ST88 (3.6%), ST834 (3.6%) and ST950 (3.6%). Isolates belonging to the same PFGE pattern had the same sequence type

**Conclusion:** Although the isolates belonged to 10 different sequence types, the ST80-SCC*mec* IV clone belonging to PFGE type 1 and subtypes was the most prevalent clone. Its presence in all eight hospitals shows its continuing expansion in Kuwait hospitals

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A novel multiplex real-time PCR assay for CA-MRSA: Rapid typing of SCCmec type assignment with detection of the pathogenicity

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**Background:** Numerous community-associated MRSA (CA-MRSA) infections have been seen in healthy populations. To detect CA-MRSA is important for clinicians because many fatal cases were reported. Staphylococcal cassette chromosome mec (SCCmec) typing is useful for defining CA-MRSA clones. The rapid detection system for CA-MRSA is needed. We established a convenient multiplex real-time PCR for detection of SCCmec type assignment with detection of the pathogenicity analyzed MRSA clones in Nagasaki.

**Methods:** 776 MRSA isolated from different clinical specimens, sputum, pus and blood at Nagasaki University Hospital from Jan. 2000 to Dec. 2007. All isolates were subjected to MIC testing and PCR for *TSST-1* (toxic shock syndrome toxin 1), *sec* (enterotoxin type c), *etb* (exfoliative toxin type b), and *PVL* (Panton-Valentine Leucocidin). PCR was performed using a LightCycler 480 to amplify a total of 10 genes in the same run. The entire run time for this assay is approximately 4 hours. Based on these molecular typing methods, we characterized the genetic background of MRSA strains isolated in our hospital. The medical records were also reviewed for the determination of nosocomial infection or the community acquired infection.

**Results:** The 667 MRSA clones detected from pus were classified as SCCmec type II (77.7%), SCCmec type IV (19.2%), and SCCmec type I (3.0%). SCCmec type IV clones has been increasing for 8 years. 87.5% of SCCmec type II clone had *TSST-1* and *sec* genes. 15 isolates were *etb* positive, all of them isolated from pus. No isolate was *PVL* positive. Most patients infected SCCmec type IV clone were classified as nosocomial infection.

**Conclusion:** Our system was thus the convenient and reliable method for typing MRSA in Japan. SCCmec type II MRSA which possesses *TSST-1* and *sec* genes was the major nosocomial infection type in Japan. The present study indicated the high rates of *PVL* negative SCCmec type IV in Nagasaki, and reveals for the drift of MRSA clones mixed the type of nosocomial infection and the type of community acquired infection.

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Trend of vancomycin MIC values among MRSA clinical isolates and association with patient outcome

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**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a common cause of nosocomial and community-acquired infection. Vancomycin has become the drug of choice given the emergence of MRSA; however, studies have reported an increase in the vancomycin minimum inhibitory concentration (MIC) and vancomycin treatment failure despite MICs within the susceptible range ( $\leq 2$   $\mu\text{g}/\text{mL}$ ). Limited studies have examined whether this increase in vancomycin MIC is associated with patient outcome.

**Methods:** We reviewed the medical records of patients diagnosed with MRSA bloodstream or lower extremity wound infection from January 1, 1998 through December 31, 2008. Bivariate and multivariate analyses were conducted to examine the association between vancomycin MIC and other covariates.

**Results:** 97 patients were diagnosed with a MRSA infection; 65% (63/97) of patients had a bloodstream infection and 35% (34/97) of patients had a wound infection. From 1998 to 2003, MRSA with a low vancomycin MIC ( $\leq 1$   $\mu\text{g}/\text{mL}$ ) were in the majority; however, from 2004 to 2008, MRSA with a high MIC ( $\geq 2$   $\mu\text{g}/\text{mL}$ ) were in the majority. Therefore, over time, there was a significant upward trend in vancomycin MIC values ( $p=0.01$ ). Logistic regression analysis revealed that a high vancomycin MIC was significantly associated with a past medical history of malignancy ( $p=0.04$ ) and death within 30 days of infection ( $p=0.04$ ) compared to a low vancomycin MIC.

**Conclusion:** Our study has shown (1) vancomycin MIC values have displayed an upward 'creep' over time, and (2) high MIC values of vancomycin is significantly related to a past medical history of malignancy as well as higher mortality within 30 days of MRSA infection. Further prospective studies are needed to examine the clinical significance of an upward 'creep' in vancomycin MIC values.

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**Type: Poster Presentation**

Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in tertiary referral hospital in Nepal

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**Background:** Methicillin-resistant *S. aureus* (MRSA) was first detected in the 1960s, and since that time it has spread rapidly worldwide, becoming a leading cause of nosocomial infection. Because MRSA are also resistant to many antibiotics, the infections caused by them are particularly difficult to treat. Indiscriminate use of antibiotics both in hospital settings and in community may aid in higher prevalence of MRSA in a country like Nepal.

**Methods:** A total of 4690 clinical samples were microbiologically processed for the growth of *S. aureus* in tertiary referral hospital in Kathmandu, Nepal. Susceptibility testing was performed in Mueller Hinton agar medium at 35°C.

**Results:** 264 *S. aureus* were recovered from the clinical samples. Among the positive isolates, 73.1% (n=193) isolates were from outpatients, whereas 26.9% (n=71) were from admitted patients. We found 31.1% (82/264) *S. aureus* to be methicillin resistant. Among a total of 71 *S. aureus* isolated from admitted patients, 46.5% (n=33) were MRSA whereas among 193 *S. aureus* isolated from outpatient, only 25.4% (n=49) were MRSA. The association between the MRSA occurrence in admitted patient as compared to outpatient was found to be statistically significant ( $\chi^2 = 10.26$ ;  $P = < 0.05$ ).

The highest percentage of MRSA was isolated from sputum 100.0% (2/2), followed by Pus from wound 50.5% (55/109). The distribution of MRSA was highest in burn ward (75.0%) followed by ICU (66.7%), post operative ward (57.2%), orthopedic ward (57.1%), neuro-ward (50.0%) and female surgical ward (41.2%).

The antibiotic resistance of MRSA was highest with Amoxicillin (100.0%) followed by Cloxacillin (90.2%), Cotrimoxazole (68.3%) and Erythromycin (61.0%). This pattern varied with Methicillin Sensitive *S. aureus* (MSSA). MSSA showed the highest resistance to Cotrimoxazole (42.9%) followed by Gentamicin (34.6%), Erythromycin (30.8%) and Amoxicillin (23.1%). All the clinical isolates were sensitive to Vancomycin. MSSA constituted 46.2% (84/182) whereas MRSA constituted 100.0% (82/82) multidrug resistant (MDR) strains.

**Conclusion:** MRSA was isolated from variety of clinical specimens. Good communication should be established among laboratory, hospital wards and physicians for effective infection control against MRSA. The laboratory personnel should notify inpatient MRSA cases to the physician and nurses in charge of the ward to prevent the spread of MRSA. Prudent use of antibiotics, proper universal precautions, regular environmental screening and good hospital practices may reduce MRSA colonization and cross infection in patients.

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Emergence of linezolid-resistant coagulase-negative staphylococci in an Intensive Care Unit  
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**Background:** Linezolid resistance is rare among coagulase-negative staphylococci (CoNS). Such isolates have been firstly detected in our institution at November 2008. Despite perceived low virulence of CoNS, infection in critically ill patients is of potential concern. We performed an epidemiological and laboratory investigation to assess this finding.

**Methods:** For a 12-month period (November 2008-October 2009), we evaluated all patients harboring linezolid-resistant (LZ-R) CoNS clinical isolates. Clinical and laboratory records were reviewed. Species identification and MIC values determination were performed by Vitek 2 Compact (bioMerieux) and/or Etest (AB Biodisk). Environmental and staff screening was carried out as part of the investigation.

**Results:** During the study period, 24 patients (16 males, mean age 45.9±20.4 years) harboring LZ-R CoNS were identified. A total of 46 LZ-R CoNS clinical isolates were recovered. The majority of them (41/46) were isolated from blood cultures, while the remaining 5 isolates recovered from central venous catheter (CVC) tips. *Staphylococcus epidermidis* was the predominant species (38/46). Linezolid MICs were >256 µg/ml for 43/46 isolates. All isolates were methicillin-resistant and glycopeptides- and daptomycin-susceptible. The mean time from admission to LZ-R CoNS isolation was 28±15.9 days. Records of patients showed that 7/24 had >2 comorbidities, 9/24 had prior hospitalizations and 9/24 had foreign materials placed. At index positive culture for LZ-R CoNS, all patients had CVCs, had received prolonged broad-spectrum antibiotic therapy and 16/24 were mechanically ventilated. Prior administration of linezolid with mean duration 11.2±7.5 days was noted for 21/24 patients. Overall in-hospital mortality was 25%. There was no attributed death. Most infections were treated with teicoplanin. Only 1/58 environmental samples grew LZ-R *S. epidermidis*. Screening of ICU-personnel was negative for LZ-R CoNS carriage. Strict infection control measures were established in order to restrain further dissemination of LZ-R strains, without significant reduction of the number of new cases.

**Conclusion:** Isolation of LZ-R CoNS, though rare, may be observed in critically ill patients after prolonged administration of linezolid. Intense infection control measures, continuous surveillance and judicious use of linezolid are essential for preserving its effectiveness. Further study is necessary in order to determine the clonal profile of these isolates.

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**Final Abstract Number: 74.013**

**Session: Antibiotic Resistance: Gram-Positive**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Inducible clindamycin-resistance in clinical staphylococcal isolates with reduced vancomycin susceptibility in a University Teaching Hospital

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**Background:** The chemotherapy of staphylococcal infections has been complicated by evolution of multidrug resistant strains, especially the methicillin-resistant *Staphylococcus aureus* (MRSA) now treated with various groups of antimicrobial agents, including, the glycopeptides (vancomycin); and the macrolides, lincosamides and streptogramin B (MLSB) class of antibiotics. The risk of clinical failure during therapy is increasingly being reported while therapeutic failures due to MLSB-inducible resistance (MLSB-i) is becoming more frequent. The study determined prevalence of inducible *erm*-mediated clindamycin resistance (MLSB-i) in clinical staphylococcal isolates with reduced vancomycin susceptibility in a University Teaching Hospital was determined.

**Methods:** Suspected staphylococcal isolates from specimen submitted to the Medical Microbiology laboratory were screened for vancomycin resistance on vancomycin-agar screening plates; Brain Heart agar supplemented with 6µg/ml vancomycin (BHI-V6); Mannitol Salt agar supplemented with 4 µg/ml vancomycin (MSA-V4) and confirmed by E-test. All isolates with vancomycin MIC of 1.5 to 2 were tested for MLSB-i by the double-disc diffusion, D-test using erythromycin (ER, 15µg) and clindamycin (CL, 2 µg) discs placed 15mm-18mm apart on Muller-Hinton agar plates inoculated with the test organism.

**Results:** A total of 43/195 (22.1%) grew on BHI-V6 within 24hrs while 66/195 (33.8%) grew on MSA-V4 in 24-48hrs of incubation. However, only 36 isolates had vancomycin MIC of between 1.5 to 2.0. Only 41.6% (15/36) of the isolates gave a positive D-test indicative of MLSB-i.

**Conclusion:** This is of clinical importance because MLSB-i *S. aureus* undergo spontaneous mutation to constitutive clindamycin resistance at high rate and with potential to develop resistance during treatment. Routine testing of staphylococcal isolates for inducible clindamycin resistance is recommended in the 2006 CLSI guidelines and help to chose treatment failures especially for patients infected with inducible MLSB isolates as in our hospital.

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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Different antibiotic treatments in patients suffering from MRSA-mediastinitis after cardiac surgery  
**P. Dohmen**, J. Schaefer, W. Konertz  
Charite Hospital, Medical University Berlin, Berlin, Germany

**Background:** Methicillin-resistant Staphylococcus aureus (MRSA) poststernotomy mediastinitis is a rare but serious complication, which has a great impact on patient's morbidity and mortality. This retrospective study evaluated different antibiotic therapies, after similar aggressive surgical treatment was performed.

**Methods:** Between January 2005 and August 2008, 37 patients suffered postoperative from MRSA-mediastinitis. The mean age was  $70.6 \pm 6.5$  years, median logistic EuroSCORE was 27.0 % (range 5.3 to 62.9%). In all patient's samples Vancomycin MIC was  $<1.0 \mu\text{g/mL}$ . There were 3 groups initiated, Vancomycin 2g/d (group 1;n=15), Daptomycin 6mg/kg (group 2; n=11) and Linezolid 1200mg/d (group 3;n=11). The pre-operative risk scores for surgical site infection were measured in all patients. End-points were morbidity and mortality.

**Results:** The total mortality was 13.5% (5/37 pts). Results are shown in table1. There was a significant lower rate of acute renal failure by using Daptomycin compared to Vancomycin (\*p=0.016). Antibiotic therapy was changed in five patients of group 1 due to therapy failure. In group 2, antibiotic therapy was deescalated in one patient and one patient was switched in oral therapy. In group 3, therapy was changed due to thrombocytopenia in two patients and in one patient due to therapy failure. There was a trend of lower mortality in group 2 compared to group 1 and 3, however this was not statistical significant.

	<b>Group 1 (n=15)</b>	<b>Group 2 (n=11)</b>	<b>Group 3 n=11)</b>
acute renal failure	46.7%	0%*	27.3%
dialysis	20.0%	0%	27.3%
therapy change	33.3%	18.2%	27.3%
mortality	26.7%	0%	9.1%

**Conclusion:** Daptomycin decrease significantly acute renal failure compared to Vancomycin therapy in patients suffering from MRSA-mediastinitis. Furthermore there is a positive trend seen by using Daptomycin in MRSA-mediastinitis, however future studies with larger patient's numbers are needed to confirm this.

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**Final Abstract Number: 74.015**

**Session: Antibiotic Resistance: Gram-Positive**

**Date: Friday, March 12, 2010**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Trends in Methicillin resistant staphylococcus aureus (MRSA) minimal inhibitory concentration (MIC) to Vancomycin over a 2 year period in a community based hospital

**J. Murillo**<sup>1</sup>, **C. Garcia**<sup>2</sup>, **F. Ordieres**<sup>3</sup>

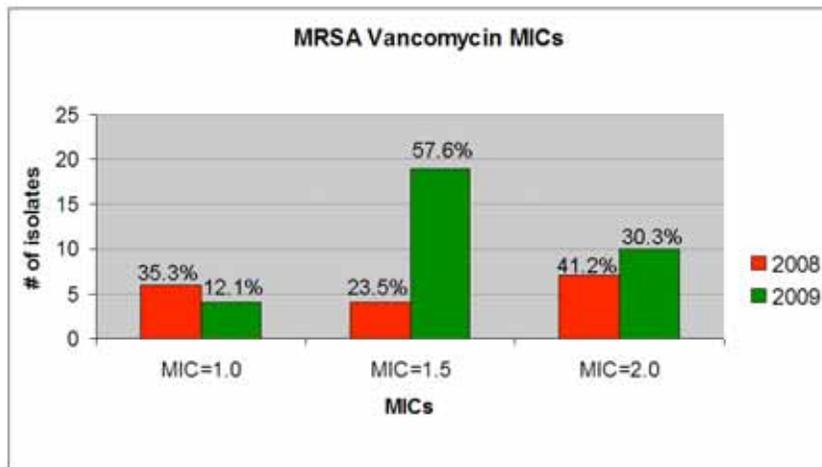
<sup>1</sup>Miami, USA, <sup>2</sup>South Miami hospital, Miami, FI, USA, <sup>3</sup>South Miami hospital, 33143, FI, USA

**Background:** Clinical and laboratory standards institute (CLSI) susceptible range for MRSA isolates to Vancomycin is established at MIC of 2mcg/ml or less, however MRSA isolates with higher MIC within the susceptible range are being reported more frequently. It has been reported that the clinical response to therapy with vancomycin for patients infected with MRSA isolates with MIC's  $\geq 1.5$  to 2mcg/ml is decreased.

The objectives of the present study were to determine the MIC to Vancomycin of MRSA blood isolates over a 2 year period in a Community based hospital in Miami, Florida.

**Methods:** All blood cultures that were found to have MRSA isolates, from patients treated at our institution during the years 2008 and 2009 were evaluated. Minimum inhibitory concentrations (MIC's) were initially obtained by the Vitek system, however by the last quarter of 2008 and all of 2009 the MIC's were confirmed by Etest strips and results were then reported.

**Results:** 64.7% of the strains tested in 2008 had an MIC between 1.5 and 2.0mcg/ml, whereas 87.9% of the strains had MIC's within the same range in 2009.



#### **MRSA vancomycin MIC 2008-09**

**Conclusion:** There seems to be a trend to higher MIC's of MRSA blood isolates to vancomycin, although still within susceptible range. The findings suggest that monitoring of MRSA susceptibility to vancomycin should continued in our institution.

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**Final Abstract Number: 74.016**  
**Session: Antibiotic Resistance: Gram-Positive**  
**Date: Friday, March 12, 2010**  
**Time: 12:30-13:30**  
**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Antimicrobial susceptibilities, and *SCCmec* and multi-locus VNTR analyses of polymorphism and genetic relationships of clinical isolates of MRSA

**P. Brown**, S.-L. Peart, J.-A. Dookie

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**Background:** MRSA is a major cause of both hospital- and community-acquired infections. In addition to their antibiotic resistance, MRSA can affect various anatomical sites, resulting in significant morbidity, long hospital stays and high treatment costs. The aim of this study was to determine the antimicrobial susceptibilities and the presence of inducible macrolide-lincosamide-streptogramin B (MLSB) resistance, and the genetic relationships based on *SCCmec* and multi-locus VNTR analyses (MLVA).

**Methods:** Sixty-one MRSA isolates obtained from hospital and community-based patients with staphylococcal infections were assessed for antimicrobial susceptibilities and the presence of inducible MLSB resistance using the disk diffusion technique. *SCCmec* types were determined by PCR-restriction endonuclease analysis of the *ccrB* locus while MLVA relationships were assessed by multiplex PCR amplification of the *spa*, *sspA*, *clfAB* and *sdrCDE* genes.

**Results:** Resistance to linezolid, teicoplanin and rifampicin (8%), quinopristin-dalfopristin (13%), erythromycin (87%) and lincomycin (80%) highlighted the antimicrobial susceptibility testing. Overall, 11 isolates were resistant to seven or more antibiotics, and no isolate was resistant to vancomycin. Of the 53 erythromycin-resistant MRSA isolates, 25 (47%) expressed constitutive MLSB resistance. Of the 40 isolates for which *SCCmec* types were available, 32 (80%) were type IV. MLVA typing of these 40 isolates produced 16 distinct profiles. While clusters were identified among *SCCmec* type IV isolates, all type I, II and III isolates yielded distinct MVLA patterns.

**Conclusion:** Significant levels of resistance were noted, even to antimicrobials not licensed for use in Jamaica, and many of the isolates exhibited cMLS resistance. While there is diversity among CA-MRSA, it is likely that many infections are caused by genetically similar MRSA organisms. It is imperative that surveillance initiatives be improved to monitor and limit the spread of this organism in the hospital and community environments.

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**Session: Antibiotic Resistance: Gram-Positive**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Resistance to antibiotics of acinetbacter strains isolated from hospital associated infections

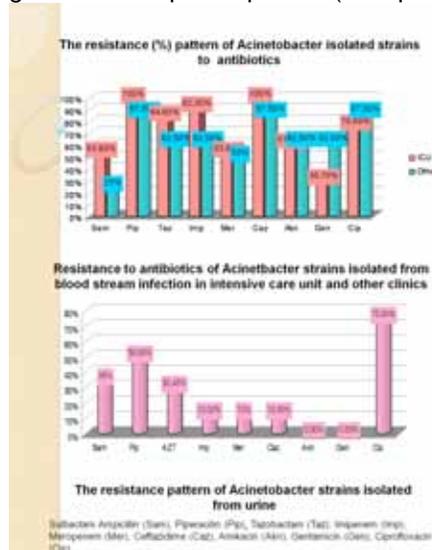
L. M. Junie<sup>1</sup>, E. Papadomanolaki<sup>2</sup>, G. Aleuraki<sup>2</sup>, P. Karagianni<sup>2</sup>, A. Tsafaraki<sup>2</sup>, A. Tsouri<sup>2</sup>, D. Labousaki<sup>2</sup>, E. Volanis<sup>3</sup>, **S. KASTANAKIS<sup>2</sup>**

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**Background:** Acinetobacter infection is an emerging problem, as multiple resistant strains to antibiotics colonize Intensive Care Units. The objective was to study the resistance patterns of Acinetobacter strains isolated in ICU and other wards.

**Methods:** Acinetobacter strains were isolated from Intensive Care Unit (ICU) and from others clinics (OC) in Chania, Greece, representing 3.06% of the total positive blood cultures. Three blood samples were taken from each patient in bottles of Bact-Alert system (Bio-Merieux). 68 strains were isolated from patients with urinary tract infections. Identification was carried out with API 20E and Vitek2 method. Antibiotic sensitivity testing of strains was performed by disk diffusion method (Kirby Bauer) as recommended by CLSI and Vitek 2 system.

**Results:** Acinetobacter strains isolated in ICU showed a high percent of resistance (84.6-100%) to Pip, Caz, Imp, Taz, Cip (76.9%), Akn (61.5%), lower percent of resistance (53.8) to Mer and Sam and only 30.7% were resistant to Gen. 87.5% of Acinetobacter strains isolated in OC were resistant to Pip, Caz and Cip, 62.5% to Akn, Gen, Taz, Imp, 50% to Mer and only 25% to Sam. Acinetobacter strains isolated in OC showed lower percent of resistance to Sam, Imp, Taz, Caz, Pip and higher to Cip and Gen. No significant difference exists in resistance among strains isolated in ICU and OC to Mer, Akn. 25%-50% of Acinetobacter strains isolated from urine were resistant to Am, Pip, Azt, Caz, Gen, Cip. They were resistant also to Nalidixic acid and Nitrofurantoin. They remain still susceptible to Imp, Mer, Ureidopenicillin+ $\beta$  lactamase inhibitor, Ticarcillin/Clavulanic acid (CA), to Cefpodoxime and some third (Ceftriaxone) and fourth generation cephalosporin's (Cefepime).



**Conclusion:** Aminoglycosides, Quinolones and most of  $\beta$  Lactamins are no more indicated in the therapy of Acinetobacter produced infections. The presence of multiple resistant strains in ICU is attributed to invasive procedures and the use of broad-spectrum antimicrobials. It is rather difficult to distinguish morbidity and mortality attributable to Acinetobacter from that attributable to the common and severe co-morbidity in these patients (ICU). Therefore good clinical evaluation is essential to avoid unnecessary treatment. Infection control measures are crucial for limiting spread and alternative therapies with ampicillin/sulbactam are an option that needs further study.

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**Final Abstract Number: 74.018**

**Session: Antibiotic Resistance: Gram-Positive**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Real time PCR resolution of community acquired MRSA reservoirs: A strategy for the reduction of time to detection of hospital acquired MRSA

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**Background:** Community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) are infiltrating hospitals and becoming the dominant colonising strains. While the optimal MRSA detection strategy remains debatable reliance on conventional microbiological methods causes delay in identifying MRSA carriers culminating in cross infection and dissemination of hospital acquired MRSA infection.

**Methods:** Nasal swab specimens were subjected to routine culture based and selective chromogenic screening, antibiotic susceptibility testing, as well as molecular detection using standard PCR and SYBR Green real-time PCR assays. MRSA was identified through the amplification of staphylococcal 16S rRNA, *mecA* and PVL genes.

**Results:** Time to detection was within 5 hours of admission using the real-time method versus 2 days for standard PCR and 4 days for microbiological methods. All hospital acquired MRSA strains carried the *mecA* gene and showed multiple resistance to a panel of antibiotics. The community source of the existing hospital strains was established through amplification of the PVL gene, identified both singularly and in multiplex PCR assays with *mecA*, demonstrating that HA-MRSA originated through the dissemination of CA-MRSA by cross infection of the carriers. PVL positive multiply resistant MRSA strains were identified from nasal specimens of healthy individuals who had not recently visited hospitals, while healthy MRSA carriers from care home facilities did not contain the PVL gene and these strains demonstrated sensitivity towards most antibiotics.

**Conclusion:** Thus, MRSA strains with specialised PVL-encoded virulence determinants persist in the hospital environment. This is in contrast to care home facilities where, in the absence of the selective pressure of antibiotics, low level resistance and PVL negative CA-MRSA strains are selected, whereas dominance of the more virulent PVL positive MRSA is curtailed. PCR assays, particularly SYBR Green real-time PCR, of *mecA* and PVL genes are preferential procedures in contrast to conventional methods for the rapid detection of CA-MRSA as a means of control of cross infection and the dissemination of HA-MRSA

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**Final Abstract Number: 74.019**

**Session: Antibiotic Resistance: Gram-Positive**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Study of Vancomycin (VA) and Trimethoprim/sulfamethoxazole (TMP-SMX) activity on community-associated Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) biofilms (Bf) in vitro

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<sup>1</sup>Facultad de Medicina, Universidad del Salvador, Buenos Aires, Argentina, <sup>2</sup>Laboratorio CIBIC, Rosario, Santa FE, Argentina, <sup>3</sup>Laboratorio CIBIC, Rosario, Santa Fe, Argentina

**Background:** Empirical CA-MRSA treatment could be affected by Bf development. There is an increasing appreciation that planktonic microbes account for only a very small proportion of microbial life, the bulk are found in a sessile form in Bf. Therefore, we study the influence of VA and TMP-SMX in early Bf development.

**Methods:** To better elucidate this, we work with 6 CA-MRSA. We employ the MIC (1.5 mg/l-0.125 mg/l) and sub-MIC (0.5 mg/l-0.06 mg/l) of VA and TMP-SMX respectively. As control we use one HA-MRSA with similar VA MIC and sub-MIC but with 20 mg/l (MIC) and 10 mg/l (sub-MIC) to TMP-SMX. Aliquots of overnight cultures in trypticase soy broth were incubated with glass coupons during 3 h for cell attachment. Coupons were transferred to fresh media with and without corresponding antibiotic (AM) concentrations, incubated for 24 h and evaluation previous staining with cristal violet

**Results:** Visual observations revealed that CA-MRSA isolates are less effective to form Bf than HA-MRSA. Both AMs (MIC and sub-MIC) didn't affect CA-MRSA but affected in different degrees HA-MRSA Bf development. Microscopically CA-MRSA with both AM produced more extracellular polymeric substances (EPS) than CA-MRSA without AM and similar to HA-MRSA with or without AM. Microcolonies structures were similar in all glass coupons for all isolates. The results showed that the presence of both AM seems not to affect early CA-MRSA Bf formation and there was an increase of EPS production. Recent reports showed a relationship between VA MIC and failure among patients with MRSA bacteremia treated with VA, atributing this failure to > 1.5 mg/l MIC.

**Conclusion:** Our experiment might explain controversies about effectiveness of AM treatment due to Bf formation rather than presence of planktonic CA-MRSA in the patients. It is necessary to use or add other AM with activity in early stage of Bf different of VA or TMP-SMX in patients with suspected or established CA-MRSA infections.

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**Final Abstract Number: 74.020**

**Session: Antibiotic Resistance: Gram-Positive**

**Date: Friday, March 12, 2010**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Disc diffusion methods versus PCR for *mecA* gene in detection of Methicillin Resistant *Staphylococcus aureus*

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<sup>1</sup>National Research Institute of Tuberculosis and Lung Disease, Tehran, Iran, Islamic Republic of,

<sup>2</sup>National Research Institute of Tuberculosis and Lung Diseases, Tehran, Iran, Islamic Republic of

**Background:** Accuracy and promptness in the detection of methicillin resistance are of key importance in ensuring the correct antibiotic treatment in infected patients and control of methicillin resistant *staphylococcus aureus* (MRSA) in the hospital environment. The aim of our study was to evaluate the efficacy of disc diffusion tests to characterize MRSA and compare it with oxacillin agar screening and detection of *mecA* gene by PCR.

**Methods:** Methicillin resistance of *staphylococcal* isolates derived from patients of a university hospital in Iran was assessed using the CLSI disk-diffusion method with a cefoxitin 30- $\mu$ g disk in comparison with an oxacillin 1- $\mu$ g disk. Oxacillin screen agar plates with 4% NaCl and 6 microg/ml of oxacillin were inoculated and interpreted as per standard guidelines. PCR-based detection of *mecA* gene was considered as the reference standard.

**Results:** Out of 200 *Staphylococcus* isolates 195 (97.5 %) were methicillin resistant by oxacillin disc diffusion test, 151 (75%) were resistant by cefoxitin disc diffusion test, 153 (76.5%) were detected as MRSA by oxacillin agar test and 126 (63%) were *mec A* gene positive by PCR. The cefoxitin showed 96% sensitivity and 61% specificity with a positive predictive value of 80% and a negative predictive value of 92%. However, the sensitivity and the specificity for oxacillin agar test were 89% and 36%, and for oxacillin were 98% and 14%, respectively.

**Conclusion:** Overall, the MRSA rate is so high in our hospital with any test in this research. Comparing different phenotypic methods for MRSA screening in routine microbiology laboratory, Cefoxitin disc and Oxacillin agar screening have better sensitivity and specificity in comparison with Oxacillin disc. However, according to the different results of these tests and PCR, it seems that phenotypic expression of methicillin resistance may alter depending on the growth conditions for *S. aureus*, such as temperature or osmolarity of the medium and some other factors besides *mecA* gene that may affect the accuracy of the methods used to detect methicillin resistance.

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**Final Abstract Number: 75.001**

**Session: Diagnostics**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Molecular diagnosis of *Helicobacter pylori* infection and risk factor of the presence of *cagA* and *vacA* genes

**A. Hassan**

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**Background:** Several *H. pylori* genes that are related to the risk of disease have been identified. The cytotoxin-associated gene (*cagA*) is a marker for a genomic pathogenicity (*cag*) island of about 40 kbp whose presence is associated with a more severe clinical outcome. A cytotoxin that injures epithelial cells is encoded by vacuolating cytotoxin A gene (*vacA*). *vacA* is present in all *H. pylori* strains and contains at least two variable parts.

The aim of our study was to evaluate the efficiency of using PCR technique as a powerful tool besides being an easy and low cost method for diagnosis of *H. pylori* infection through molecular detection of *cagA* and *vacAs1* genes in gastric tissue biopsies obtained from patients diagnosed as *H. pylori* positive

**Methods:** Fifty cases were enrolled in our study that underwent endoscopy.

The following investigations were done:

- Paraffin-embedded tissue sections were stained (H&E) to grade the severity of gastritis to detect *H. pylori* as a gold standard.
- CLO-Rapid urease test was carried out for all gastric biopsies.
- PCR analysis for detection of *cagA* and *vacAs1* genes in gastric tissue biopsies obtained from patients diagnosed as *H. pylori* positive.

**Results:** Biochemical results: For positive histology cases, 36/41 (87.8%) were positive, while for negative histology cases, 8/9 (88.9%) were negative by rapid urease test.

PCR results: For positive histology cases, 40/41 (97.6%) were positive by the PCR test for *vacAs1* gene. On the other hand, out of 9 that were negative by PCR test for *vacAs1* gene 9 (100%) were negative by histology.

Out of 41 positive cases by histology, only 40 (97.6%) were also positive by the PCR test for *cagA* gene. On the other hand, out of 9 that were negative by PCR test for *cagA* gene 9 (100%) were negative by histology.

Strong positive and statistically significant correlation between the expressions of these two genes (*vacAs1* and *cagA*) in patient of *H. pylori*. (P<.000)

**Conclusion:** We can conclude that:

- Rapid urease test is good screening test when multiple biopsies are used.
- Analysis of *H. pylori* virulence gene *vacAs1* and *cagA* by conventional PCR method, emphasizing high sensitivity of PCR method.

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**Final Abstract Number: 75.002**

**Session: Diagnostics**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Competitive ELISA for *Toxoplasma gondii* Zoonoses

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CENPALAB, La Habana, Cuba

**Background:** The diagnosis of *Toxoplasmosis gondii* in veterinary medicine is affected, one hand that limited of commercial offers and on the other hand the diversity of species, that which would require the employment of different species specific conjugated for the Indirect Immunofluorescencia and the indirect or sandwich ELISA, therefore to have a competitive ELISA system that allows the simultaneous diagnosis of big quantities of samples of any animal species, would be of incalculable value for epizootic and epidemiologist studies, simplifying the laboratory work

**Methods:** By means of this work we give to know the methodology used in the evaluation of competitive ELISA , by means of the use of indirect Immunofluorescence with employment of commercial fluorescent conjugated anti species (SIGMA anti Rabbit, Primates, Sheep, Dogs, Mouse and Humans) as reference systems developed in the Tropical Medicine Institute. (IPK) and the commercial agglutination latex system, used to evaluate 1500 sanguine serums of different species (Primates, Rabbits, Sheep, Dogs, Mouse and Humans)

**Results:** Our results of 99.3% of Sensibility, Specificity of 98.3%, Efficiency of 98.7%, VPN of 99.5%, VPP of 97.6% and Kappa index of the 0.97, indicate that our competitive ELISA system is comparable to the commercial systems used in the evaluation.

**Conclusion:** Our competitive ELISA system can be used in any animals species and humans.

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**Final Abstract Number: 75.003**

**Session: Diagnostics**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Sensitivity and specificity of ELISA test for diagnosis of brucellosis

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**Background:** Brucellosis is a common disease with different clinical features and diagnostic methods .Regarding to increasing use of ELISA as a new method this study was conducted to determine sensitivity and specificity of ELISA method for diagnosis of brucellosis in Kashan –Iran in 2007.

**Methods:** This study was done on 457 of persons with clinically suspected brucellosisThe agglutination tests wright, coombs wright and 2ME as a standard tests and IgG and IgM ELISA were done. Specificity and sensitivity of ELISA was compared with standard tests.

**Results:** Sensitivity of IgG and IgM ELISA was 93.7% &12.5%. Specificity of IgG and IgM ELISA was 70.6% & 100%.positive predictive value IgG and IgM ELISA was 19.3% &100%.negative predictive value of IgG and IgM ELISA was 99.3% &94%.

**Conclusion:** Regarding to high sensitivity and false positivity of IgG ELISA and Low sensitivity of IgM ELISA, strict rely to ELISA for diagnosis of brucellosis may cause confusion of physicans.So it must be used along by agglutination tests and clinical findings for confirmation of diagnosis of brucellosis.

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**Type: Poster Presentation**

Molecular epidemiology of selected sexually transmitted bacterial infections

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**Background:** Sexually transmitted infections are a major global cause of acute illness, infertility, long-term disability and death, with severe medical and psychological consequences for millions of men, women and infants. There are more than 20 pathogens that are transmissible through sexual intercourse. Many of them are curable by appropriate antimicrobial treatment. WHO estimated that 340 million new cases of trichomoniasis (174 million), chlamydia (92 million), gonorrhoea (62 million) and syphilis (12 million) have occurred throughout the world in 1999 in men and women aged 15-49 years. Epidemiological information plays an important role in the development of public health services. This information is not available for the catchment area of our laboratory.

**Methods:** Clinical Microbiology & Public Health Laboratory developed an assay to investigate the molecular epidemiology of genital infections caused by 6 different bacteria i.e. *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Ureaplasma urealyticum* and *Ureaplasma parvum*. In this assay, two amplification reactions were performed on each clinical specimen. This in-house real-time PCR assay tested genital swabs from 1,812 consecutive patients attending GU Medicine Clinic at Addenbrooke's Hospital from October to December 2008.

**Results:** Nine hundred and forty six (52%) of 1,812 specimens were positive for the DNA of one or more of the above bacteria. The DNA of *T. vaginalis* (4 swabs), *C. trachomatis* (129 swabs), *N. gonorrhoeae* (11 swabs), *M. genitalium* (18 swabs), *U. urealyticum* (292 swabs) and *U. parvum* (645 swabs) was detected. Single, dual and triple infections were detected in 807, 125 and 14 swabs respectively.

**Conclusion:** Our in-house assay is a useful tool to investigate the molecular epidemiology of sexually transmitted infections.

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Burden of infection with *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis* and HR-HPV in homeless youth determined by APTIMA testing

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**Background:** Homeless youth living on the streets of large cities and engaging in unprotected sex are at risk of infections with *C. trachomatis* [CT], *N. gonorrhoeae* [GC], *T. vaginalis* [TV], and human papillomavirus [HPV]. The APTIMA transcription mediated amplification [TMA] assays are sensitive and specific for detecting genital infections using various sample types.

**Methods:** In Group A, 296 women signed informed consent for collection of 2 cervical swabs [CS] and a liquid-based Pap [L-Pap] [SurePath] sample. In Group 2, 289 women self-collected a vaginal swab [VS]. CS and VS were tested for CT and GC using APTIMA Combo 2 [AC2] confirmed with ACT and AGC assays. VS were also tested for TV using analyte specific reagents [ASR] in an APTIMA protocol and confirmed using a research TMA directed against alternate targets. The L-Pap sample and CS were tested for 14 high-risk [HR] HPV genotypes using the APTIMA HPV [AHPV] mRNA test [Gen-Probe] and confirmed using HC2 [Qiagen] or HPV Linear Array [LA] [Roche].

**Results:** Prevalences were as follows: CT 12% [70/585]; GC 2.6% [15/585]; CT and GC 1.5% [9/585]; TV 13.5% [39/289]; HR-HPV 35.5% [105/296] and HR-HPV and CT were present in 7.1% [21/296]. CT, GC and HR-HPV were present in 2 patients. The most common HPV genotypes in descending order were 16, 51, 52, 56, 18, 31, 39, 58, 33, 45, 35, 59, 68, 66. Many samples contained more than 1 genotype, and the greatest number in a patient was 6.

**Conclusion:** Using APTIMA assays to screen genital infections in this younger, largely asymptomatic population demonstrated a high burden of infection. The use of multiple assays enabled confirmation of results and early treatment. Infections with several organisms and HPV genotypes indicate a public health concern for the sexual health of these homeless individuals.

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Evaluation of PCR versus EIA for diagnosis of *Clostridium difficile* infection

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**Background:** Despite the rising morbidity and mortality of *Clostridium difficile* infection (CDI), a rapid and reliable test to confirm the diagnosis remains elusive. The cell culture cytotoxin assay (CCCA), considered the gold standard, is complex and time consuming. Commonly used EIA methods for detecting toxins A & B have sensitivities of only 50-90%, leading to confusion regarding the interpretation of negative tests. The Cepheid Xpert *Clostridium difficile* PCR test was recently FDA approved and may improve sensitivity and specificity with faster turnaround time. We sought to evaluate the clinical utility of this new PCR test compared to the standard Meridian Premier EIA toxin A/B test.

**Methods:** Consecutive stool samples submitted for detection of *C. difficile* toxin at our 808 bed medical center were analyzed with both Meridian Premier EIA and Cepheid's Xpert PCR assay. The first submitted stool sample for each adult hospitalized patient was included. True CDI was defined as a positive test by either the PCR or EIA methods, along with the presence of clinical symptoms of infection (ie, diarrhea, fever, abdominal pain, elevated WBC count). If the test were discordant, CCCA was performed at a reference laboratory.

**Results:** 161 cases met inclusion criteria; 30 cases (18.6%) were diagnosed with CDI. The sensitivity, specificity, positive & negative predictive values of the PCR test (97%, 100%, 100%, and 99%) were superior to the EIA test (43%, 97%, 76%, and 88%). Four cases were EIA+/PCR-; only one of these had clinical symptoms consistent with CDI. EIA-/PCR+ was noted in 16 cases; all clinically had CDI. Of 16 discordant samples that underwent CCCA, only 1 (6%) tested positive.

**Conclusion:** PCR significantly improved the diagnosis of patients with suspected CDI; 16 additional patients with clinically evident disease were detected by PCR that were missed by EIA. CCCA was insensitive, with no correlation to other testing methods or clinical disease. PCR testing appears superior to EIA and allows for rapid, reliable diagnosis and timely institution of therapy and initiation of infection control measures. The enhanced sensitivity of PCR may obviate the need for additional testing.

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Serodiagnosis of tuberculosis using nine *in silico* predicted B-cell epitopes peptides derived from *Mycobacterium tuberculosis* proteins

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**Background:** Rapid, simple, and new diagnostic tools are needed for the diagnosis of tuberculosis (TB). The aim of this study is to identify a combination of *Mycobacterium tuberculosis* (*Mtb*) peptides useful for the serodiagnosis of active pulmonary tuberculosis (TBp).

**Methods:** Fifty-seven B-cell epitopes peptides of *Mtb* were selected by *in silico* methods and evaluated by immunoenzymatic assay. Two sample panels were used for evaluation: (i) twenty pulmonary tuberculosis (TBp) patients and ten healthy subjects (HS) from a country with low incidence of TB (Italy) and (ii) forty-seven TBp patients and 26 HS from a country with high incidence of TB (Morocco) and the data were analyzed using logistic regression analysis and Random Forest method.

**Results:** The best discriminating peptide between TBp patients and HS from the sample of the country with low incidence of TB has been the 23 amino acid peptide of the Rv3878 protein. Thus, the sensitivity and specificity was 65 % and 100 %, respectively. In contrast, the same peptide showed 47% and 100% as sensitivity and specificity respectively in the country with high incidence of TB. In addition, the best peptides combination was a pool of nine peptides which showed a sensitivity of 70.2% and a specificity of 100% in the country with high incidence of TB.

**Conclusion:** The present study showed that the 9-peptides pool can be useful in identifying patients with active pulmonary tuberculosis.

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Cerebrospinal and blood nitric oxide in tubercular meningitis

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**Background:** The role of nitric oxide (NO) in central nervous system infections is controversial. Nitrites, the stable end product of NO metabolism as reactive nitrogen intermediates (RNI), an index of NO synthesis, were measured in CSF and blood of patients with tubercular meningitis (TBM).

**Methods:** 40 patients with TBM and 50 matched controls were recruited for the study. The diagnosis was based on clinical features, CSF features (pleocytosis of more than 10 cells/mm<sup>3</sup> with >50% lymphocytes, sugar < 50% of blood sugar, protein > 40mg% and Adenosine deaminase (ADA) > 8U/L) or a positive PCR or culture for Mycobacterium tuberculosis in CSF or a suggestive CT Scan of brain. Controls were patients undergoing elective surgery for non-neurological conditions under spinal anaesthesia.

**Results:** The clinical profile of the patients (mean age 30.6±12.3 years) included fever in 38 (95%), headache in 36 (88%), seizures in 11 (28%) and focal neurological deficits in 6 (15%) patients. Total leukocyte and relative lymphocyte counts in CSF of patient's were 1650±157 /mm<sup>3</sup> and 76±31% respectively. ADA was more than 8U/L in 31 (77.5%) and PCR for M.tuberculosis was positive in 10 (19 samples). CT brain showed basal exudates in 13 (32.5%), gyral enhancement in 8 (20%), infarcts in 12 (30%) and hydrocephalus in 19 (47.5%) patients. CSF-RNI levels in patients (16.9±19.5µmol/L) were higher than in controls (1.3±1.1µmol/L) (p<0.01). Serum RNI levels of patients (53.5±13.8µmol/L) were higher than controls (3.8±1.9µmol/L) (p<0.01). There was no correlation between CSF-RNI and blood-RNI or between CSF-RNI levels and the biochemical or clinical parameters. Two patients died on follow up related to the disease.

**Conclusion:** CSF and blood NO levels are increased in tubercular meningitis and are not related to clinical outcome suggesting that NO is a non-specific marker of CNS inflammation and can not be incriminated as an agent of tissue damage.

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Combinatorial use of IgG antibodies to secreted mycobacterial proteins to create a screening test for childhood tuberculosis

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**Background:** To create a rapid, simple and relatively inexpensive screening strategy for childhood tuberculosis (TB) that includes antibody detection assays to improve the accuracy of microscopic examination of sputum for acid-fast bacilli (AFB smear) in Warao indigenous childhood TB given that TB is difficult to diagnose, and invasive procedures cannot be used to select samples in these communities.

**Methods:** TB diagnosis was established by *Mycobacterium tuberculosis* culture, clinical diagnosis, thorax radiography and smears stained with Ziehl-Neelsen. HIV status was determined by commercial serologic test. Efficacy and diagnostic potential of different secretory antigens of *Mycobacterium tuberculosis* (rESAT-6, (17) Ag85A and (5) ESAT-6 synthetic peptides) in the detection of specific IgG antibody profiles of childhood TB cases were evaluated using ELISA technique and sensitivity was compared with the gold standards (smear and culture). A total population of 117 children under the age of 15 years old residing in the visited indigenous communities was examined. Secretions of the pharynx and attempts to obtain samples of sputum by expectoration in children older than 10 years old was carried out in all highly suspected pulmonary TB cases. Serum samples were obtained from 39 untreated children patients, and 78 healthy children. ROC curve analysis was used to calculate the sensitivity and specificity of each antigen for antibody detection.

**Results:** The results revealed no case of HIV-positive TB among Warao children. Bacteriological confirmation had 8.8% sensitivity, Ag85A peptides showed better sensitivity and specificity than ESAT-6 peptides; anti-29880 peptide test was found to be showing highest sensitivity of 100.0% (Negative Predictive Value, NPV=100) but a low specificity of 20.8%. Two tests were highly specific, anti-11003 had 97.4% specificity (Positive predictive Value, PPV=85.7) and 32.4% sensitivity and anti-10999 had 96.2% specificity (PPV=86.4) and 48.7% sensitivity. Compared to bacteriological tests, sensitivity of a combination that included a two-antigen ELISA (29880 and 11003 synthetic peptides) was significantly higher,  $p < 0.0001$ .

**Conclusion:** Our results demonstrate that the potential of combinatorial use of antibodies directed at different epitopes of Ag85A protein could provide a screening strategy for developing a multi-antigen ELISA, which allows an increase in the diagnostic accuracy of pulmonary TB in Warao childhood population.

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Specificities of the APTIMA Combo 2 and ProbeTec for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in oropharyngeal and rectal specimens from MSM

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**Background:** Nucleic acid amplification tests (NAATs) are not FDA-cleared for diagnostic testing of chlamydial (CT) or gonococcal (GC) infection in extragenital sites. In men who have sex with men (MSM), CT and GC infections of the oropharynx or rectum may be common. There have been some concerns about false-positive NAAT results from these sites. We evaluated the APTIMA Combo2 (AC2, Gen-Probe Inc.) and ProbeTec (SDA, Becton Dickinson, Co.) performance on these specimens. Here we present the specificity results.

**Methods:** Oropharyngeal and rectal swabs were obtained from MSM in an STD clinic and SDA and AC2 were performed on all specimens. NAAT positive specimens were retested by AC2, SDA and by APTIMA CT Assay (ACT), or APTIMA GC Assay (AGC) which target rRNA sequences different from AC2.

**Results:** We tested 1110 MSM. AC2 had more positive results than SDA for both organisms at both sites. The number of positive results, and the percentage confirmed are presented in the table. Only 11 oropharyngeal CT infections were detected, but the number of positive GC specimens and rectal CT specimens was larger allowing more confidence in the reliability of the confirmation data. Using a combination of all 3 tests confirmed >90% of positive samples, resulting in high specificities (>99.6%) for both AC2 and SDA.

	SDA				AC2			
	CT		GC		CT		GC	
(N)	Phx (6)	Rec (43)	Phx (69)	Rec (70)	Phx (11)	Rec (67)	Phx (81)	Rec (85)
Confirmed by (%):								
AC2 or SDA	100*	93	83	99	55	60	70	81
ACT or AGC	100	91	98**	99†	82	93	91	94
Repeat test	100	98	91	97	82	93	91	94
All 3	100	100	94	99	91	94	98	98

% confirmed, \*\*only 57 tested by AGC, † 1 not tested by AGC.

**Conclusion:** In our MSM population, positive results obtained with AC2 and SDA are reliable, with PPVs >90%. The CT and GC NAAT positives were confirmed to a high degree. As expected, repeat testing, or using the ACT or AGC test, confirm more of the AC2 positive results than did the less sensitive SDA.

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Reverse transcriptase multiplex PCR for detection of viral agents in central nervous system infections

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**Background:** Viral infections of the central nervous system (CNS) may result in clinical syndromes like aseptic meningitis, encephalitis, and myelitis. These are often difficult to diagnose using conventional laboratory methods, such as viral culture and serology, because they are time consuming and unsatisfactory. Therefore rapid techniques should be employed to detect the etiologic agent. The study was aimed to standardize reverse transcriptase (RT) multiplex PCR aimed to detect viral etiology in CNS infections.

**Methods:** An RT multiplex PCR designed to detect, viral etiologies, enterovirus, herpes simplex and varicella zoster viruses in CNS infections has been standardized. Three sets of primers were been employed for their detection. Amplification of target sequences was qualitatively analyzed by looking for the presence or absence of amplicons on agarose gel. The RT multiplex PCR was standardized. Sensitivity of the PCR has been ascertained.

**Results:** Analysis of cerebrospinal fluid samples from pediatric patients is underway.

**Conclusion:** The RT multiplex PCR standardized can be employed to detect CNS infections caused by herpes, varicella and entero viruses.

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An improve dot enzyme immunoassay for serodiagnosis of melioidosis

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**Background:** Melioidosis in an infectious disease affecting multi-organ system of man and animals caused by a bacterium, *Burkholderia pseudomallei*. The disease is potentially fatal and early detection is crucial for institution of life saving antimicrobial therapy. Culture isolation remains as a mainstay for definitive laboratory diagnosis of melioidosis. But it is less sensitive, laborious and time-consuming task. In this report, an improved dot EIA test (patent pending) with user friendly assay protocol for serodiagnosis of melioidosis is described.

**Methods:** Two optimised concentrations of antigens were dotted onto a nitro-cellulose membrane (Microfiltration System, Ca, USA, 0.45uM) divided into 9mm x 4mm. The membranes were blocked for 30 minutes in tris-buffered saline (TBS) wiht 5% non-fat skim milk. The membranes were rinsed and cut according to the divided size into test strips and placed in 48 well flat tissue culture plates (Costar 3548, UK.) The test strips were then incubated with patient serum at 1:100 in TBS for 1 hour at room temperature. After incubation, the test strips were washed with TBS for three times and each washing was done for 5 minutes. Subsequently, the diluted alkaline phosphatase conjugated goat anti-human immunoglobulin IgM, IgG and IgA isotypes were added into the respective wells and incubated for 1 hour at room temperature. After incubation the strips were washed three times in TBS as described above. Chromogenic substrate was used in the colour development reaction. Aftwer after 15 minutes the strips washing with distilled water to stop coloué development. The positive and negative results were determined by visual comparison of the dot intensity for each test with the positive cut off control.

**Results:**

The sensitivity and the specificity of the assay was 95% and 92%, respectively for the detection of IgG, IgM and IgA antibody isotypes. However, detection of IgG alone showed sensitivity value of 89.2%. The sensitivity value for the detection of IgM alone and IgA alone were 47.7% and 60% respectively.

No. Lab No.	IgG	IgM	IgA	Final Results
Positive control	+++	+++	+++	Positive
Negative control	-	-	-	Negative
1. SPS 1	+++	+++	+++	Positive
2. SPS 2	+++	+++	+++	Positive
3. SPS 3	+++	+++	+++	Positive
4. SPS 4	+++	+++	+++	Positive
5. SPS 5	+++	+++	+++	Positive
6. SPS 6	+++	+++	+++	Positive
7. SPS 7	+++	+++	+++	Positive
8. SPS 8	+++	+++	+++	Positive
9. SPS 9	+++	+++	+++	Positive
10. SPS 10	+++	+++	+++	Positive
11. SPS 11	+++	+++	+++	Positive
12. SPS 12	+++	+++	+++	Positive
13. SPS 13	+++	+++	+++	Positive
14. SPS 14	+++	+++	+++	Positive
15. SPS 15	+++	+++	+++	Positive
16. SPS 16	+++	+++	+++	Positive
17. SPS 17	+++	+++	+++	Positive
18. SPS 18	+++	+++	+++	Positive
19. SPS 19	+++	+++	+++	Positive
20. SPS 20	+++	+++	+++	Positive
21. SPS 21	+++	+++	+++	Positive
22. SPS 22	+++	+++	+++	Positive
23. SPS 23	+++	+++	+++	Positive
24. SPS 24	+++	+++	+++	Positive
25. SPS 25	+++	+++	+++	Positive
26. SPS 26	+++	+++	+++	Positive
27. SPS 27	+++	+++	+++	Positive
28. SPS 28	+++	+++	+++	Positive
29. SPS 29	+++	+++	+++	Positive
30. SPS 30	+++	+++	+++	Positive
31. SPS 31	+++	+++	+++	Positive
32. SPS 32	+++	+++	+++	Positive
33. SPS 33	+++	+++	+++	Positive
34. SPS 34	+++	+++	+++	Positive
35. SPS 35	+++	+++	+++	Positive
36. SPS 36	+++	+++	+++	Positive
37. SPS 37	+++	+++	+++	Positive
38. SPS 38	+++	+++	+++	Positive
39. SPS 39	+++	+++	+++	Positive
40. SPS 40	+++	+++	+++	Positive
41. SPS 41	+++	+++	+++	Positive
42. SPS 42	+++	+++	+++	Positive
43. SPS 43	+++	+++	+++	Positive
44. SPS 44	+++	+++	+++	Positive
45. SPS 45	+++	+++	+++	Positive
46. SPS 46	+++	+++	+++	Positive
47. SPS 47	+++	+++	+++	Positive
48. SPS 48	+++	+++	+++	Positive
49. SPS 49	+++	+++	+++	Positive
50. SPS 50	+++	+++	+++	Positive
51. SPS 51	+++	+++	+++	Positive
52. SPS 52	+++	+++	+++	Positive
53. SPS 53	+++	+++	+++	Positive
54. SPS 54	+++	+++	+++	Positive
55. SPS 55	+++	+++	+++	Positive
56. SPS 56	+++	+++	+++	Positive
57. SPS 57	+++	+++	+++	Positive
58. SPS 58	+++	+++	+++	Positive
59. SPS 59	+++	+++	+++	Positive
60. SPS 60	+++	+++	+++	Positive
61. SPS 61	+++	+++	+++	Positive
62. SPS 62	+++	+++	+++	Positive
63. SPS 63	+++	+++	+++	Positive
64. SPS 64	+++	+++	+++	Positive
65. SPS 65	+++	+++	+++	Positive
66. SPS 66	+++	+++	+++	Positive
67. SPS 67	+++	+++	+++	Positive
68. SPS 68	+++	+++	+++	Positive
69. SPS 69	+++	+++	+++	Positive
70. SPS 70	+++	+++	+++	Positive
71. SPS 71	+++	+++	+++	Positive
72. SPS 72	+++	+++	+++	Positive
73. SPS 73	+++	+++	+++	Positive
74. SPS 74	+++	+++	+++	Positive
75. SPS 75	+++	+++	+++	Positive
76. SPS 76	+++	+++	+++	Positive
77. SPS 77	+++	+++	+++	Positive
78. SPS 78	+++	+++	+++	Positive
79. SPS 79	+++	+++	+++	Positive
80. SPS 80	+++	+++	+++	Positive
81. SPS 81	+++	+++	+++	Positive
82. SPS 82	+++	+++	+++	Positive
83. SPS 83	+++	+++	+++	Positive
84. SPS 84	+++	+++	+++	Positive
85. SPS 85	+++	+++	+++	Positive
86. SPS 86	+++	+++	+++	Positive
87. SPS 87	+++	+++	+++	Positive
88. SPS 88	+++	+++	+++	Positive
89. SPS 89	+++	+++	+++	Positive
90. SPS 90	+++	+++	+++	Positive
91. SPS 91	+++	+++	+++	Positive
92. SPS 92	+++	+++	+++	Positive
93. SPS 93	+++	+++	+++	Positive
94. SPS 94	+++	+++	+++	Positive
95. SPS 95	+++	+++	+++	Positive
96. SPS 96	+++	+++	+++	Positive
97. SPS 97	+++	+++	+++	Positive
98. SPS 98	+++	+++	+++	Positive
99. SPS 99	+++	+++	+++	Positive
100. SPS 100	+++	+++	+++	Positive

**Dot EIA test results**

**Conclusion:** The results showed that combined detection of all three antibody isotypes are required to increase the sensitivity of the assay. In addition, the result from the propective study showed that the test was very useful for early and accurate diagnosis melioidosis in clinical cases

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**Session: Diagnostics**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Diagnostic value of Elisa serological test using synthetic peptides of *Mycobacterium tuberculosis* antigens in childhood tuberculosis

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**Background:** One study was conducted to evaluate the efficacy of ELISA serological test for the detection of IgG antibodies against ESAT-6 synthetic peptides and the recombinant ESAT-6 antigen (rESAT-6).

**Methods:** The study was carried out on children with pulmonary tuberculosis and healthy controls (13 cases and 63 controls). Informed consents of their parents or guardians were taken. They were subjected to clinical examination, relevant laboratory investigations, tuberculin test (epidemiological link with confirmed case), chest radiograph and therapeutic positive test. Relevant body fluids (sputum and gastric fluid) were subjected to biochemical test adenosine deaminase (ADA) and bacteriological tests (Lowëstein-Jensen (LJ) and Ziehl Neelson (ZN)) and PCR. Sera samples were analyzed for antibodies against 5 ESAT-6 synthetic peptides (12033, 12034, 12035, 12036 and 12037) and rESAT-6. The Utility of these methods was evaluated using receiver operating characteristic curve (ROC) analysis.

**Results:** ELISA test using ESAT-6 peptide (anti-12036) showed a significantly higher sensitivity (100%) than ADA, LJ medium culture method and ZN staining and PCR tests (30% ADA, 0% ZN staining, 0% LJ culture and 12.5% PCR) in all patients ( $p < 0.001$ ). ADA biochemical test was highly specific (100%), followed by anti-12033 test (95.2%). Anti-rESAT-6 showed been lowly sensible and specific.

**Conclusion:** In view of the convenience, low cost and high sensitivity and specificity, the ELISA tests using a combination of ESAT-6 peptides could have a promising future in the diagnosis of childhood tuberculosis.

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**Type: Poster Presentation**

Can the nucleic acid amplification test (NAT) be an alternative to the serologic tests? A prospective study results of 18,200 blood donors from Turkish Red Crescent

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**Background:** Serologic tests having high sensitivity and specificity are used in order to prevent contamination with infectious agents from blood and blood products for transfusion safety.. Present serologic tests have problems like having low sensitivity and weak detection capacity of infectious agents in "window period". We aimed to test the use of NAT (Nucleic Acid Amplification Test) in routine blood screening in Blood Banking.

**Methods:** We used Procleix ultrio (Chiron, USA) test kit based TMA (Transcription Mediated Amplification) for NAT study in serum samples of 18200 donors who were applied to the Turkish Red Crescent between February 2007-September 2008. The NAT positive samples were studied twice. The discrimination of HIV, HCV and HBV of NAT positive samples were performed by Procleix Discrimination (Chiron, USA) test. Otherwise Micro ELISA were used parallelly for routine serological screening of Anti-HIV, Anti-HCV and HBsAg with Vironoste HIV Uni-form, AG/Ab innotest HCV Ab and Hepanostica ultra HBsAg test kits.

**Results:** The results of serum samples with serology(+) and NAT(+) (13/18200 and 0.05%) for Anti-HIV, Anti-HCV and HBsAg were detected higher than other NAT studies and we also detected that a transfusion risk can be occurred in every 1400 transfusions

**Conclusion:** In this work, we conclude that number of serologic-negative and NAT positive donors was higher than those of other studies and viral risk (HBV, HCV) was found in every 1 of 1400 transfusion. All these were due to high percentage of first time blood donors, nearly all of them were first time donors. If we consider about the high costs of NAT test compared with the other serologic tests; NAT was not found to be efficient in all studies on cost-based effectiveness. Therefore, NAT should be applicable only for the first time donors at regional blood centers established at national level. We conclude that gaining more regular blood donors are more beneficial for the blood centers.

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**Type: Poster Presentation**

Comparison of Bactec MGIT 960 and new culture system VersaTREK for isolation rate, time of detection and and contamination rate

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**Background:** Mycobacteria Growth Indicator Tube (MGIT) 960 method is a rapid test system which contains liquid media to determine mycobacteria culture and drug susceptibility. VersaTREK system contains Middlebrook 7H9 media with cellulose sponge and it detects microbial growth by measuring gas pressure changes in the bottle. We aimed to compare the two systems for incubation time and contamination rate.

**Methods:** In our study we inoculated various samples of 50 patients, which have been received from Istanbul University Cerrahpasa Medical Faculty Microbiology Department Tuberculosis Laboratory, into MGIT 960 and VersaTREK media. Both media compared for *Mycobacterium tuberculosis* for isolation rate, time of detection and and contamination rate.

**Results:** Isolation rates for MGIT and VersaTREK were detected as 4%. Additionally, both systems have been compared for contamination rate and it was found to be 10% for MGIT and 6% for VersaTREK media.

**Conclusion:** In conclusion, no substantial difference has been observed between MGIT and VersaTREK media for *M.tuberculosis* isolation and contamination rate. We suggest that VersaTREK media, which is going to be put into use in Turkey, is easy for application and follow up, gives rapid and sensitive results and that it can be used in routine alike MGIT media.

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**Type: Poster Presentation**

Development of genetic typing method to assign staphylocoagulase (SC) serotype I-X and two SC genotypes for *Staphylococcus aureus*

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**Background:** *Staphylococcus aureus*, including methicillin-resistant *S. aureus*, is the most common pathogen of hospital- and community-acquired infections worldwide. Staphylocoagulase (SC) serotyping is a serological classification system of *S. aureus* based on antigenicity of SC and has been used for a basic epidemiologic typing method for *S. aureus*. However, some strains have been untypeable because of low amount of SC expressed from bacterial cells or presence of putative novel or variant types. Recently, gene sequences of SC defining SC serotypes I-X were determined and divergent portions in SC genes were clarified. We found also novel SC genotypes, XI and XII. In the present study, based on the findings of SC genes, a novel genetic typing of SC (gene) types by PCR assay was developed.

**Methods:** Two PCR reactions 1 and 2 were designed. In both reactions, a common primer which is complementary to 5'-end conserved region of SC gene is included. Reaction 1 contains SC serotypes I through VI-specific primers, and reaction 2 contains SC serotypes VII, VIII, and X-specific primers. SC serotype-specific primers were mostly complementary to D1 or D2 region sequences which are divergent among different SC serotypes. Depending on the SC serotype, only a single PCR product with the size specific to each SC serotype is amplified in either the reaction 1 or 2. When no product is amplified, an additional PCR reaction (reaction 3) is carried out to determine SC serotype IX or genotype XI. When type IV was assigned by reaction 1, an additional reaction (reaction 4) is performed to discriminate between SC serotype IV and genotype XII.

**Results:** With the established SC types I-XII *S. aureus* strains, a single PCR product with the specific size to each type was amplified. When 63 *S. aureus* strains isolated during a period between 1993 and 2003 which were untypeable serologically were tested by this genetic typing method, about 90% of the strains were successfully typed. When 117 *S. aureus* strains isolated in 2008 were examined, SC types were identified for 96% of the strains.

**Conclusion:** The PCR assay established in this study could assign SC types for *S. aureus* accurately with high determination rate, and was considered to be useful for epidemiologic characterization of *S. aureus* clinical isolates.

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**Type: Poster Presentation**

Serological diagnosis of lyme disease in Valencia (Spain)

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Lyme borreliosis (BL) is the tick-borne disease with the highest incidence worldwide. Its geographical distribution shows a pattern of "endemic areas", where infected *Ixodes ricinus* in enough density to have an epidemiological impact. In Spain, it is a low incidence process although the problems inherent in their clinical and laboratory diagnosis are probably due to an underdiagnosis. Studies are needed to supplement information on the distribution of the etiologic agent in different areas of our country, which are not considered endemic but where their presence is suspected.

**Methods:** From 1 January 2008 to August 31, 2009, 1422 samples were analyzed in patients from the influence area of the Valencia University General Hospital with clinical suspicion of BL. As a screening test, we used an enzyme immunoassay that uses chemiluminescence technology (Dia Sorin Borrelia IgG) for the quantitative determination of IgG antibodies.

We used as a confirmatory test the Western blot (European Borrelia plus Virotech), considering positive findings, the presence of at least two bands of the following: p83/100, BmpA (p39), Ospc (p23), DbpA or vice-Mix -Mix. Indeterminate results were considered in the presence of a band; p83/100, BmpA (p39), OspC (p23), DbpA or vice-Mix-Mix.

In all samples detection of antibodies to *Treponema pallidum* was performed.

Positive results were found by the screening test in 137 samples (9.63%), corresponding to 105 patients. The Western blot showed positive results in 39 samples of 28 patients, indeterminate in 16 samples from 12 patients and negative in 82 samples.

The 39 positive samples were from patients (28) from the services of Neurology N = 16, Internal Medicine N = 13, N = 6 N = 4 Dermatology and Psychiatry.

Luetic serology were negative in all samples.

In 8 patients administered ceftriaxone 2mg/día for three weeks with good clinical and serological survey.

**Conclusion:** Our diagnostic methods, show a group of patients with specific characteristics and clinical outcome, and support the existence of probable cases of Lyme borreliosis.

Screening methods have high sensitivity but are not very specific, because only 28 (26.6%) of 105 patients tested positive by Western blot.

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**Type: Poster Presentation**

Multicenter evaluation of Ziehl-Neelsen bleach sedimentation method for diagnosis of smear negative tuberculosis in Kenya

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**Background:** World Health Organization (WHO) recommends direct smear microscopy using Ziehl-Neelsen (ZN) technique for tuberculosis (TB) case finding in resource poor settings. This method has a low sensitivity. Bleach methods have been shown to increase sensitivity in some settings. However, no evaluation has been carried out in settings where TB burden is high and improved diagnosis is desperately needed.

**Objective:** To evaluate the ZN bleach sedimentation technique for diagnosis of smear negative tuberculosis in peripheral canters.

**Methods:** 1122 direct ZN smear negative sputum specimens from new TB suspects attending three peripheral health centers in Nairobi were collected. At each center, sputum specimens were homogenized then divided into two equal portions. One portion was processed for culture. The other portion was treated with 3.5% bleach. The treated specimens were kept at room temperature and left overnight for at least 15 hours. Smears were prepared from the deposit and examined using ZN method.

**Results:** Of the 1112 smear negative specimens, 968 were analyzed. Of these, 84(8.7%) were culture positive. Of these, 23 (27.3%) were both culture and smear positive. The sensitivity was (27.4%) and specificity of (98.4%) with a PPV and NPV of 66% and 92% respectively. There was a significant increase in sensitivity ( $p>0.005$ ).

**Conclusion:** Bleach with sedimentation significantly increased the sensitivity of ZN smear microscopy and should be recommended for use in smear negative specimens to enhance case detection in TB control programmes especially in settings with high burden of dual TB/HIV infection.

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**Type: Poster Presentation**

Multilocus PCR in real time for detection of highly dangerous and dangerous viral infections

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FSRI State research center of virology and biotechnology "Vector" , Novosibirsk, Russian Federation

**Background:** Viral hemorrhagic fever plays a special role in infectious pathology of man. At the present time a constant threat of outbreaks and major epidemics of hemorrhagic fevers with high mortality in many tropical countries is remained. In connection with the development of transport links between states and the increase in passenger traffic as well as being highly contagious, dangerous disease causing virus, is becoming a real drift of infection to non-endemic areas and outbreaks with serious consequences. Emergency identification of pathogens hemorrhagic fever is highly relevant in relation to the current bio-terrorist threat. The purpose of this study was to develop a rapid and highly sensitive multilocus PCR analysis in real time to diagnose dangerous viral infections causing of hemorrhagic fever.

**Methods:** At the initial stage of study for each of the viruses were selected the most conservative regions of the genomes. A pair of specific oligonucleotide primers and probes for hybridization-fluorescence detection of PCR products was picked up. The complete sequence of two virus strains for successful selection of primers to the Japanese Encephalitis virus has been sequenced. Recombinant plasmids carrying fragments of the genomes of Marburg virus (MBV), Ebola virus (EBV), Junín virus (JV), Machupo virus (MV), virus of Lassa fever (LFV), virus of yellow fever (YFV), virus of Japanese Encephalitis (JEV), virus of Venezuelan equine encephalitis (VEEV) as a positive control samples were used and constructed.

**Results:** Parameters for the RT-PCR in real time, the laboratory mono versions of PCR test systems have been optimized. The data on detection of RNA flaviviruses (Marburg virus and strains of Sudan Ebola virus, Zaire Ebola virus) in multiplex format was obtained. The analytical sensitivity of the mono versions of PCR detection of genomes of viruses, which amounts to 102-103 copies of the plasmid in the sample, was determined. Experimental laboratory-based test systems for detection of high-risk and dangerous viral infections on the basis of xMAP-technology were obtained.

**Conclusion:** Thus, test systems for detection of genetic material of MBV, EBV, JV, MV, LFV, YFV, JEV, VEEV which will detect RNA viruses in clinical material from infected persons and patients were developed.

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**Type: Poster Presentation**

Expression of matrix metalloproteinases and tissue inhibitors in the serum and cerebrospinal fluid of patients with meningitis

**H.-C. Tsai**<sup>1</sup>, M.-H. Shi<sup>1</sup>, S. S.-J. Lee<sup>2</sup>, Y.-S. Chen<sup>1</sup>

<sup>1</sup>Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, R.O.C., <sup>2</sup>Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, R.O.C.

**Background:** The mortality rate in meningitis is relative high and the survivors may suffer from long-term severe neurological sequelae. Infectious meningitis is associated with an imbalance between matrix metalloproteinases (MMPs) and endogenous tissue inhibitors of MMP (TIMPs). The aim of the study was to determine the levels of matrix metalloproteinase-2, 9 (MMP-2, 9) and tissue inhibitors of matrix metalloproteinase1, 2, 4 (TIMP-1, 2, 4) profiles of patients enrolled in a clinical cohort of infectious meningitis.

**Methods:** Serum and CSF were collected prospectively on all patients with infectious meningitis between Jan 2008 to Dec 2008 to measure the concentrations of MMP/TIMP in those patients undergoing lumbar puncture for a presumptive diagnosis of meningitis. Levels of serum/CSF MMP-2, 9, and TIMP-1, 2, 4 were determined by ELISA. Gelatin zymography was used to detect the expression of MMP-2 and MMP-9 in CSF. The CSF control group consisted of patients without meningitis presenting with headache or disturbance of consciousness, who underwent a diagnostic lumbar puncture.

**Results:** A total of 201 patients were enrolled into the study. The concentrations of CSF MMP-9, and TIMP-1 were significantly higher in the meningitis group compared to the control group ( $p=0.032$ , and  $p<0.001$ , respectively). However the CSF TIMP-4 levels were significantly lower in the meningitis groups compared to the control groups ( $p<0.001$ ). Patients with bacterial meningitis had higher CSF MMP-9 and TIMP-1 levels than those who had aseptic meningitis and controls. There were no significant difference in serum MMPs/TIMPs levels were detected between meningitis group and controls. Patients who had different infectious meningitis etiologies tended to have higher CSF MMP-9 expression by gelatin zymography when compared to the controls

**Conclusion:** MMP/TIMP system dys-regulation was found in patients with meningitis and CSF MMP and TIMP system might act as a novel indicator in patients with infectious meningitis.

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**Type: Poster Presentation**

Combination of PCR and electrical microarray allows rapid and sensitive multiplex detection of mosquito-transmitted pathogens

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**Background:** More than 300 million people are infected every year with pathogens transmitted by mosquitoes, with 3.3 billion at risk. The majority of cases are infections with different *Flaviviridae*, *Chikungunya Virus* or *Plasmodia*. Standard tests to detect these pathogens are serological assays like IgM and IgG ELISAs for viral infections and the blood smear test in malaria diagnosis for the detection of *Plasmodia*. An early, fast and specific diagnosis often is vital for efficient treatment of these infections, but serological tests usually remain negative during the viremic and early symptomatic phase and in addition antibodies show broad cross-reactivity between *Flaviviruses*. While smear tests are a cheap and rapid method for diagnosis of malaria the limited sensitivity of this test does not allow detection of low level *Plasmodia* infections.

**Methods:** Real-time RT-PCR is a diagnostic tool with high specificity and sensitivity but has limitations in the parallel detection of multiple pathogens. Micro arrays tend to be less sensitive but have the potential to detect and discriminate a broad range of pathogens.

**Results:** We developed and optimized a highly sensitive multiplex PCR approach designing a combination of PCR primers for parallel amplification of *Dengue viruses*, *Yellow Fever Virus*, *West Nile Virus*, *Japanese Encephalitis Virus*, *Chikungunya Virus* and *Plasmodia*. Amplicons are differentiated on an electrical microarray. Specificity and sensitivity of the multiplex assay was determined and compared to specific real-time RT-PCR assays.

**Conclusion:** Our results demonstrate that combination of a broad PCR approach with differentiation on the electrical microarray is a simple, specific, and sensitive tool for early differential diagnosis for the majority of mosquito-transmitted tropical fevers.

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**Type: Poster Presentation**

Detection and differentiation of Dengue infections with highly sensitive real-time PCRs

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**Background:** Dengue is one of the most important arboviral diseases in tropical and sub-tropical countries caused by infection with one of the four dengue virus (DENV) serotypes. The disease is endemic in more than 100 countries around the globe and an estimated 50 to 100 million cases of acute febrile disease are caused by DENV each year. For treatment rapid, early diagnosis is a prerequisite for efficient treatment of infected individuals. Routine clinical diagnosis is based on serological assays as well as on RT-PCR.

**Methods:** We developed a real-time RT-PCR generic assay for highly sensitive detection of all four DENV serotypes. DENV-positive samples can be differentiated with a new real-time multiplex assay allowing differentiation of all 4 serotypes.

**Results:** The assay was validated with clinical samples. Serum from patients from the island of Saint Martin was collected for routine diagnosis of DENV infection with IgM and/or IgG ELISA or NS1 antigen capture assay and in addition RT-PCR at the hospital. DENV positive samples were re-analyzed with the new generic real-time RT-PCR assay and differentiated with the multiplex assay. Comparing these real-time RT-PCR results with the clinical data demonstrated a significantly increased sensitivity and specificity for the new assay. 6 out of 36 samples positive in ELISA but previously negative in RT-PCR were tested positive with the new real-time RT-PCR assays. In addition one sample, previously characterized as DENV1-positive was shown to be DENV2 infected. This was confirmed by sequencing data.

**Conclusion:** We could demonstrate that our new real-time PCR based approach resulted in improved detection and differentiation of Dengue viruses.

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**Type: Poster Presentation**

An automated workflow for high throughput MLVA using the BioNumerics® software, able to deal with varying experimental settings

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**Background:** Multi Locus VNTR Analysis (MLVA) is a method for high -resolution typing of microbial isolates based upon Variable copy Numbers of Tandem Repeats (VNTR). The use of capillary sequencers for determining fragment lengths allows for a cost- and time-effective determination of repeat numbers, since multiple VNTRs with non-overlapping size ranges can easily be pooled and loaded in a single capillary. However, the resulting complexity of the pooling schemes and the amount of data generated require an automated workflow for acquisition and processing of fragment files. Moreover, it is well-known that fragment sizes, compared between two genotyping laboratories using capillary electrophoresis (CE), can differ considerably because different laboratories may use different CE machines and running conditions.

**Methods:** Data processing: The software handles any pooling strategy (combination of dyes and size-compatible VNTRs) which can be parsed from the file name. Thus, each VNTR is defined by a pool, dye and expected size range, defined by the repeat length, offset and expected copy range. Using that information the software will automatically screen (in batch) all theoretical ranges for each VNTR and report on the peak(s) found (or not) in those ranges.

**Results:** The resulting VNTR information is stored in integer-type character sets where each VNTR represents one character. For comparisons within the same laboratory or between laboratories with compatible instruments and procedures, the software allows the user to handle calculations based on an expected band size range, depending on the known offset, repeat length, expected copy number variation and a user-defined tolerance. In order to deal with a possible experimental size shift linked to the CE system used, a custom 'mapping' tool was developed, allowing observed sizes for a specific instrument to be mapped to real sizes and exact copy numbers.

**Conclusion:** Data analysis: VNTR data can be analyzed as categorical characters (each different copy number is a different allele) or as quantitative characters. In the latter case, the larger the difference between copy numbers, the less related the organisms are considered. The Minimum Spanning Tree algorithm applied on VNTR data in BioNumerics has proven to be extremely useful for epidemiological study and population genetics.

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**Session: Diagnostics**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Navigating Dante's inferno: Creation of signatures for the rapid detection of heamorrhagic fever agents

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**Background:** Heamorrhagic fevers remain some of the most horrific diseases to impact human beings due to their deadly symptoms, limited treatment options and lack of successful vaccines. Heamorrhagic fever agents include Ebola, Marburg, Machupo, Lassa, Junin, Rift Valley Fever, and Crimean Congo Heamorrhagic Fever viruses, Yellow Fever virus and the bacterial agent *Rickettsia prowazekii*. Infection by a Heamorrhagic fever agent leads to an acute illness where multiple organs of the body are affected and can result in death. Today outbreaks of the disease are sporadic and occur only within the agent's host species territory. Humans are not the natural reservoir for any of these agents but once infected, we can spread the disease through personal contact. Since we have no vaccines for these agents early detection remains the most important factor in preventing a localized outbreak from spreading internationally. Since outbreaks are unpredictable, a cost effective way of screening human populations for the disease would enable quick identification of new outbreaks. Furthermore, once an outbreak of Heamorrhagic fever is identified, correct identification of the responsible agent is essential for treatment. Unfortunately the responsible agent is difficult to distinguish in a clinical setting by symptoms alone. A system for rapid identification of Heamorrhagic fevers is required for quick containment and response to these diseases.

**Methods:** At the request of the Department of Homeland Security (DHS), Lawrence Livermore National Laboratory (LLNL) has developed signatures for one-step TaqMan RT-PCR detection of seven Heamorrhagic fever viral agents [Ebola, Marburg, Machupo, Lassa, Junin, Rift Valley Fever, and Crimean Congo Heamorrhagic Fever viruses], Yellow Fever virus and one bacterial agent [*Rickettsia prowazekii*]. These signatures were designed by finding conserved and specific sequences belonging to each disease agent. They were then tested and validated using Taqman RT-PCR.

**Results:** Testing against near neighbors and over 2500 background samples eliminated potential cross-reactivity and increased specificity of the assay. Signatures that showed no cross reactivity were then tested for their Limit of Detection (LOD) value against their target virus.

**Conclusion:** These signatures will allow the specific and sensitive detection of Heamorrhagic fever agents essential for a timely response.

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**Type: Poster Presentation**

Usefulness of Montenegro skin test for the diagnosis of Mucosal Leishmaniasis

**B. Valencia**<sup>1</sup>, P. Diaz<sup>2</sup>, O. Ponce<sup>2</sup>, A. Ramos<sup>1</sup>, A. Llanos-Cuentas<sup>1</sup>

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**Background:** Mucosal leishmaniasis (ML) represents the hyperergic pole of American Tegumentary Leishmaniasis (ATL). This behavior makes its diagnosis difficult due to the scarce parasite load as a consequence of a strong local TH1 immune response. Only PCR has a good performance in comparison to traditional techniques like direct examination, culture and histopathology for the diagnosis of ML. Even when ATL is endemic in our country, only two centers can perform PCR for *Leishmania* detection in Peru. Since ML is a neglected disease, patients with mucosal involvement belong to poor, rural and remote areas. Montenegro Skin Test (MST) measures TH1 response, and theoretically ML patients have a longer skin reaction compared to patients with cutaneous leishmaniasis (CL).

**Methods:** A retrospective study was performed. Only medical records of patients with confirmed ML or CL, through scrapping, culture, PCR or an adequate clinical response, coming from jungle areas endemic in *L. (V) braziliensis*, and a well-defined intradermal reaction size were reviewed.

**Results:** We identified 358 medical records: 170(47%) of CL and 188(53%) of ML. Patients from both groups acquired the infection in Madre de Dios, Junin and Huanuco. Median age (IQR) of CL was 26 years (IQR:19-38) and 72% were male. Median age of ML patients was 30 years (IQR:21-75), 86% were male, and 99% had previous CL with 8 months (IQR:3-18) of active ulcerative lesion. ML patients reported CL 10 years ago (IQR:6-19): 40% received Pentavalent antimonials, 11% trivalent antimonials and 26% reported a spontaneous cure. The diagnosis of ML was done by PCR (9%), scrapping (22%), culture (38%), and/or an adequate clinical response (31%). MST was 15 mm (IQR:10-18) in ML and 8 mm (IQR:6-10) in CL. Using a MST diameter of 15mm as cut point for the diagnosis of ML, we found a sensitivity of 52%, specificity of 93%, positive predictive value of 90% and a negative predictive value of 63%.

**Conclusion:** Patients with lesions compatible with ML, previous CL, and an MST>15mm, have a high likelihood of ML; therefore, we can use this approach for diagnosis in low-resources settings where physicians have limitations in diagnosis and they need to provide a justified treatment.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Study of the efficient treatment of the influenza (S-OIV, A/H1N1)

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**Background:** Influenza, transmitted by contact with infected individuals, presents with fever, arthralgia, nasal discharge, etc. Although influenza kits are often used for the diagnosis of influenza, the reported percentage of S-OIV, A/H1N1 influenza cases in which the kit is useful for the diagnosis varies from 50% to 90%. This rate probably varies depending on the interval from symptom appearance to the test, and also the presenting symptoms. We recently investigated the efficient treatment of the influenza (S-OIV, A/H1N1).

**Methods:** The influenza kit (Hanaco Medical Co., Ltd.) was used for the diagnosis in 279 individuals presenting with influenza-like symptoms at the Tokyo Metropolitan Police Hospital between August 1 and October 6, 2009. The symptoms, history of contact with infected individuals, and interval from symptom appearance to the test (0-12 hours, 12-24 hours, 24-48 hours, and over 48 hours) were analyzed.

**Results:** Fever (over 38°C) was seen in 18%, sore throat in 26%, nasal discharge in 7% and arthralgia in 26% of all individuals testing positive with this kit. The positive rate did not differ significantly depending on the interval from symptom appearance to the test (0-12 hours, 19.6%; 1-24 hours, 26.1%; 24-48 hours, 34.1%; over 48 hours, 34.1%). The influenza positive rate was markedly high (83%) only in individuals having a positive history of contact.

**Conclusion:** The diagnostic rate with the kit was less than 50% even in individuals presenting with fever (the most characteristic symptom of influenza). Thus, a definitive diagnosis of influenza may not be possible with a kit alone. The positive rate did not differ significantly among different symptom groups, or depending on the interval from symptom appearance to the test. However, it was markedly high (83%) in individuals with a positive history of contact. Patients having a history of contact and presenting with influenza-like symptoms may be judged as having influenza without visiting a clinic and managed as home-care patients, by sending the antiviral agents to their homes. This approach would prevent massive influenza outbreaks and also have socioeconomic benefits.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Design of improved polymerase chain reaction (PCR) method containing internal positive control (IPC) for molecular detection of *Yersinia pestis*

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**Background:** Plague is one of the three epidemic diseases still subject to the international health regulation and notifiable to the WHO. *Yersinia pestis*, the causative agent of plague in natural foci transmitted between rodent and human via wild rodent fleas. So set up of suitable and sure to surveillance and prevention of the disease is necessary. The molecular methods such as polymerase chain reaction (PCR) has allowed improvement of detection methods currently used in laboratories, although not all of these methods include an Internal Positive Control (IPC) to monitor for false negative results. Therefore we improved a uniplex and multiplex PCR method with lower of limit of detection.

**Methods:** PCR reactions performed with primers which targeted of the *caf1* and *pla* genes located on the pFra and pPst plasmids and the *irp2* chromosomal gene located on the 'pathogenicity island. For acquired different size of these genes additional primers were designed. In each gene two parts required were ligated. The limit of detection determined by performing PCR reactions on serial dilutions of plasmids containing the corresponding inserts. For evaluating the specificity, PCR reactions were done for negative control bacteria.

**Results:** Assays were performed with genome of *Y. pestis* which produced three DNA fragments of the expected size 300, 400 and 520 base pairs (bp) corresponding to *irp2*, *caf1* and *pla* genes respectively. also, for IPC by the same primers the different fragment of the expected size 150, 500 and 300 base pairs (bp) corresponding to *irp2*, *caf1* and *pla* genes respectively were acquired. With The lower limit of detection was 370 copy numbers for *caf1* gene and 21 for *pla* gene. In PCR reactions for negative control bacteria detectable fragments were not observed.

**Conclusion:** Our method clearly discriminated *Y. pestis* DNA bacteria which were tested. The rapidity, specificity and sensitivity of this procedure with the use of IPC to monitor for false negative results can make this method suitable for diagnosis and suggest that it can serve as a useful alternative method for inoculation of laboratory animals or the use of specific culture media for routine plaque surveillance and outbreak investigations.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Development of a multiplex PCR system with application of IPC (internal positive control) for detecting *Bacillus anthracis* from environmental samples

**M. fallah raoufi**<sup>1</sup>, F. eini<sup>1</sup>, F. azarifard<sup>1</sup>, M. Soleimani<sup>2</sup>, E. jamshidiyan<sup>1</sup>, K. Majidzadeh<sup>2</sup>

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**Background:** *Bacillus anthracis*, the causative agent of anthrax is a major concern of a dangerous and zoonotic disease in medicine and veterinary medicine. The development of rapid, sensitive and simple techniques to detect *B.anthraxis* in suspicious specimens is the important aim of animals and subsequently public health. we described a multiplex PCR system with application of IPC (internal positive control) for detecting virulence markers in *B.anthraxis*.

**Methods:** In PCR assay *pag* and *cap* genes located on POX1 and POX2 plasmids were amplified by use of specific primers. In order to control of reaction conditions, we used from PCR products as a templet and designed IPC for *pag* and *cap* genes that their length was shorter than product segments. After cloning of PCR products, the sensitivity of the PCR test (limit of detection ) was also estimated. Control bacteria of defined species was confirmed by amplification of a fragment of the 16S rRNA gene by using two universal primers

**Results:** In post-PCR stage 591 and 173 bp fragments of DNA respectively to *cap* and *pag* genes recognized in agarose gel. Sensitivity determination assays revealed that this method can detect, 412 and 100 copies as LOD for *cap* and *pag* gene respectively in samples. Nonetheless, bands on the results with negative controls, these genes not present in these defined bacteria.

**Conclusion:** In this study, we designed and optimized a multiplex PCR assay for the detection and characterization of *B.anthraxis* from environmental samples. In addition to we used from IPC to prevention of false negative results and also to detect inhibitory effects of the sample matrix. The PCR system described here can be proposed as rapid, safe, diagnostic and confident method for detecting anthrax. The sensitive and specific nature of this assay provides a valuable tool that can be used for analysing clinical and field samples and for improving our understanding of the ecology and environmental prevalence of *B.anthraxis* in natural foci of disease.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

TB test-kit for rapid drug susceptibility testing of *M.tuberculosis*

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**Background:** Drug resistant tuberculosis is an increasing public health concern in many parts of the world, including Russia. Traditional drug susceptibility testing is either time-consuming (21-28 days for the absolute concentration method) or expensive (automatic BACTEC systems). In contrast, TB test-kit developed at SRCAMB (in the frame of BTEP/DHHS projects #2 and 86) allows for reducing the time of testing in 2.5 times, has low price and can be used in all the bacteriological labs.

**Methods:** Drug susceptibility testing with TB test-kit is based on the ability of *M.tuberculosis* to reduce nitrates to nitrites. The nitrites are detected with Griess reagent, which produces a color change. Sputum or suspension of *M.tuberculosis* strains inoculates into each a vial of the test-kit with syringe. Then vials of inoculated media incubate at (35±2) °C. In 8-13 days of incubation resistant isolates of *M.tuberculosis* produce color changes in the vial with an appropriate drug. Susceptible isolates does not produce a color change.

**Results:** TB test - kit is a kit of ready-for-use nutrient media with drugs or without them and reagent for reading results. The test-kit allows to determine susceptibility of *M.tuberculosis* to isoniazid, rifampicin, streptomycin, ethambutol and to perform primary identification of *M.tuberculosis*.

The SRCAMB TB test-kit have been tested in three clinical laboratories in Russia in comparison with the absolute concentration method and the automated BACTEC MGIT 960 using about one thousand *M.tuberculosis* isolates. Good agreement between the results of TB test kit and two techniques was found.

In 2008, the RF Ministry of Health approved the TB-test kit for production, sale and distribution at Russian Federation.

TB test-kit is currently being manufactured in SCRAMB and sold by small lots. The basic consumers of TB test - kit are mycobacteriological laboratories.

**Conclusion:** Performance of testing with the test-kit does not require special equipment and highly skilled personnel.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Production and standardization of *Brucella* national standard antiserum equivalent to OIEISS in Sudan

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**Background:** Brucellosis is a bacterial contagious zoonotic disease caused by one of the ninth species of the genus *Brucella*, including the three proposed new species *Brucella ceti*, *B.pinnipedis* (Foster *et al.*, 2002&2007) and *B.microti* (Scholz *et.al*, 2008a; 2008b).

The serological tests were rapid and applicable methods for Brucellosis diagnosis using validated test methods and standardized reagents.

For the control of brucellosis at the national or local level, the buffered *Brucella* antigen tests (Boats), i.e. the Rose Bengal test (RBT) and the buffered plate agglutination test (BPAT), as well as the ELISA and the FPA, are suitable screening tests. Positive reactions should be retested using a suitable confirmatory and/or complementary strategy (OIE manual, 2009).

The OIE reference standards are those against which all other standards are compared and calibrated. These reference standards are all available to national reference laboratories and should be used to establish secondary or national standards against which working standards can be prepared and used in the diagnostic laboratory for daily routine use. These sera have been developed and designated by the OIE as International Standard Sera. The use of these promotes international harmonisation of diagnostic testing and antigen standardisation.

**Methods:** Sudanese national standard antiserum equivalent to OIEISS was produced and standardised from naturally infected cows with a high titre of *Brucella* according to Veterinary laboratory agency-Webbridge Laboratory methods (Hendry *etal.*, 1985) and OIE manual (OIE, 2009). A panel of naturally infected cows sera were tested for *Brucella* Agglutinins by RBT, SAT and ELISA. The selected sera and OIEISS were titrated, tested and compared with previously mentioned serological tests.

**Results:** An sterile pooled high titre sera were successfully prepared. The serum was diluted perfectly with negative serum to match with the OIEISS titre which was 1000 IU.

**Conclusion:** This study was result in production of standardized National standard antiserum for the first time in Sudan, when checked locally using the appropriate methods of Veterinary laboratory agency-Webbridge, but needs further quality control check in the specialist OIE reference laboratories.

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**Session: Diagnostics**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Identification of novel microRNA biomarkers of viral infection

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**Background:** It has become clear in the recent years that predictive biomarkers are becoming an integral part of successful clinical management. Further, a lack of clear biomarkers has created a real challenge in licensing biodefense therapeutics and vaccines. The study presented seeks to develop tools for the discovery of new microRNA (miRNA) biomarkers for infectious diseases using advanced computational tools coupled with high density microarrays and animal test facilities to find novel miRNA markers of viral infection. The system is expected to be of utility to diagnostics and vaccine and therapeutics development.

**Methods:** Extant software developed for miRNA sequence mining coupled with supercomputers has been used to analyze the genomes of human, mouse, rhesus macaque, and all full-length viral genomes available to identify candidate miRNAs that may be of utility for detecting viral infections and other perturbances of normal metabolic function.

These sequences are being incorporated into high density microarrays that will be used to assess miRNA expression responses from humans, rhesus macaques, and mice for any infectious agent.

Initial tests will be conducted on healthy tissue and tissue infected with filoviruses to determine miRNA responses specific to filovirus infection. This system can also be used for any other viral infection as well as other metabolic disturbances not necessarily related to viral infection and for biomarker discovery related to therapeutics and vaccine efficacy testing. The research proposed also aims to apply, for the first time, an miRNA screening tool to discovery of miRNA biomarkers for filovirus infection.

**Results:** We have thus far identified 20442 viral pre-miRNA sequences, and 124292, 90054, and 104037 pre-miRNA sequences for humans, macaque, and mouse, respectively.

**Conclusion:** Given our preliminary results, we are confident that our experiments seeking to validate these predicted miRNAs will yield novel markers of infection.

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**Session: Diagnostics**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Procalcitonin as a diagnostic marker in differentiating bacterial from abacterial meningitis

**H. El-Shamy**<sup>1</sup>, A. Abbass<sup>1</sup>, Y. Omran<sup>2</sup>, A. Amine<sup>1</sup>

<sup>1</sup>High Institute of Public Health, Alexandria, Egypt, <sup>2</sup>Fever Hospital, Alexandria, Egypt

**Background:** Clinical differentiation of bacterial and abacterial meningitis is difficult even for experienced clinician, and current laboratory tests cannot distinguish between them accurately and rapidly. Therefore, we aimed to study the role of Procalcitonin (PCT) in differentiating bacterial from abacterial meningitis.

**Methods:** Patients suspected of having meningitis admitted to Alexandria Fever Hospital were included in the study. CSF and blood samples were collected. CSF samples were subjected to microscopic examination, and culture. Blood samples were used to measure serum PCT level.

**Results:** Out of the 75 examined cases; equal percentages (42.7 %) were definitely diagnosed as bacterial and abacterial meningitis cases. Elevated PCT level was found in (81.3%) of bacterial meningitis versus (9.4%) of abacterial meningitis. PCT was the least method of diagnosis to be affected by preadmission antibiotic intake. PCT serum level had a sensitivity of 80.6%, a specificity of 91.3%, a negative predictive value of 93.5%, and a positive predictive value of 75.0%.

**Conclusion:** Measurement of PCT blood level is a rapid, simple and specific test and may be recommended in the diagnosis of bacterial meningitis cases, especially those who received antibiotic therapy before hospitalization.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Western blotting studies of IgG antibodies, IgG avidity and IgG subclasses during the follow up of patients with pulmonary tuberculosis, under treatment

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**Background:** WHO in 2007, estimated that the incidence of tuberculosis in worldwide was about 8.8 million new cases per year. In attention to WHO recommendations serological tests are currently being studied and standardized for the diagnosis of the active and latent disease.

**Methods:** From clinical strain of *Mycobacterium tuberculosis*, maintained in our laboratory, the antigen was prepared and after SDS PAGE, the proteins were transferred to nitrocellulose membrane for Western Blotting analysis. For IgG antibodies detections we used monoclonal antibodies anti IgG labeled with peroxidase diluted 1/500, and for IgG subclasses we used anti-IgG1 and anti-IgG3 labeled with biotin diluted 1/100 and streptavidin-peroxidase diluted 1/100. The reactions were revealed with a solution of 4 chloro-alpha-naphtol. For IgG avidity, after the first incubation with serum samples, the strips were washed with 8M urea for 5 minutes.

**Results:** We tested serum samples from 5 patients, taken at the beginning and the end of treatment, after 6 months. The region of 30 kDa, related with metabolic activity of mycobacteriae, has been recognized in all samples for IgG antibodies, with intensity stronger in the last sample. There was the appearance of band in the region of 16 kDa in the first and in the last samples of two patients. In the others three patients this band appears only in the last sample, but after washing with urea less intensity of this band was observed. The region of 16 kDa is expressed by *M. tuberculosis* when it is maintained with low oxygen tension and it is associated with latent infection. The region of 6 kDa is better recognized in the last samples, and this region is not sensitive to urea treatment. With regard to IgG1, antibodies were most frequent in the region of 38 kDa, considered as specific protein of the *M. tuberculosis*; IgG3 antibody appears in serum samples from four patients reacting with bands in the regions of 30 kDa, 20 kDa and 6 kDa, at random.

**Conclusion:** The physiological pathological roles of antibodies reacting with the bands studied are being evaluated with a larger number of patients.

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**Type: Poster Presentation**

A multiplex real-time PCR method for presumptive identification of NAP1 clone of *Clostridium difficile* from stools

**P. Jayaratne**<sup>1</sup>, C. Lee<sup>1</sup>, C. Rutherford<sup>2</sup>

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**Background:** *Clostridium difficile* infection (CDI) is the leading cause of nosocomial diarrhea in adults and paediatric patients. There has been significant morbidity and mortality related to CDI due to the presence of hypervirulent strain (NAP1) associated with unregulated production of toxin. NAP1 has been responsible for a number of outbreaks in many countries. Rapid detection for the presence of NAP1 is essential for appropriate patient management and to minimize nosocomial transmission. Currently, pulsed-field gel electrophoresis (PFGE) and repetitive sequence-based (REP-PCR) methods which require bacterial culture have been used to identify NAP1. A 18bp deletion in the *tcdC* gene and the presence of *cdtA* gene (binary toxin) have been shown to be associated with NAP1. We describe a rapid multiplex real-time PCR method for presumptive identification of NAP1.

**Methods:** Ninety blinded frozen stool samples previously tested for toxin A/B by CD TOX A/B® II EIA (TechLab, Inverness Medical) were used. A fragment of *tcdC* flanking the 18bp deletion (surrogate marker for *tcdA/tcdB*), *cdtA*, and 16S rDNA (internal control) genes were amplified using Multiplex QuantiTech™ (Qiagen, Inc.) and detected by TaqMan probes. ProGastro™ (Prodesse, Inc.) kit that detects *tcdB* was used for comparison. DNA from stools were extracted using easyMAG (BioMerieux Inc) and tested according to manufacturers' specifications. Five µl of the same DNA was used for the in-house method. Both real-time PCR amplifications were done in a Rotor-Gene 6000 (Corbette, Inc.). PCR products from both *tcdC* and *cdtA* positive samples were separated on agarose gels to detect *tcdC* deletion.

**Results:** DNA from 85 specimens were positive for *tcdC* (toxigenic) and 53 of those showed the presence of *cdtA* gene (presumptive NAP1). DNA from none of the specimens were positive for *cdtA* only. The *tcdB* detection by ProGastroCDTM was concordant with the detection of *tcdC*. Gel electrophoresis of all *tcdC* and *cdtA* positive PCR products detected the 18 bp deletion in the *tcdC* amplicon and were confirmed as NAP1 by PFGE. All *cdtA* negative DNA samples were negative for 18bp deletion in the *tcdC*.

**Conclusion:** Detection of toxigenic strains by the in-house method is comparable to the commercial assay and it presumptively identified the presence of NAP1.

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**Session: Diagnostics**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Accuracy of an 'in-house' sputum polymerase chain reaction assay for rapid diagnosis of sputum smear negative pulmonary tuberculosis at Mulago hospital, Uganda

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**Background:** Accurate and early diagnosis of Tuberculosis (TB) is crucial for effective patient management and TB control yet adequate TB diagnosis is still a challenge. Mycobacterial nucleic amplification tests such as polymerase chain reaction (PCR) assays are promising rapid and accurate alternatives that can be done in developing countries. However, the performance of these assays in diagnosis of sputum smear negative pulmonary TB (PTB) has not been widely reviewed. We studied the accuracy of an 'in-house' sputum PCR assay based on detection of IS6110 sequence, in the diagnosis of TB among sputum smear negative PTB suspects.

**Methods:** A cross sectional study was conducted between September 2007 and February 2008 on the emergency medical ward of Mulago Hospital, Kampala, Uganda. After informed consent, we screened patients aged 13 years and above clinically suspected to have PTB by direct Z-N smear microscopy for acid fast bacilli (AFB). AFB sputum smear negative PTB suspects were recruited consecutively into the study and portions of processed sputum from these study participants were subjected to culture using Lowenstein-Jensen (LJ) media and IS6110-based 'in-house' sputum PCR. Using LJ culture as the gold standard, we analyzed for the diagnostic accuracy of the IS6110-based 'in-house' PCR by computing sensitivity, specificity, positive and negative predictive values.

**Results:** Overall, 320 PTB suspects were screened, 205 were AFB sputum smear negative and were included in the study. Of these 72/205 (35%) were positive for *Mycobacterium tuberculosis* on LJ culture while 128/205 (62.4%) were positive on the IS6110-based 'in-house' sputum PCR. Compared to LJ culture, the sensitivity and specificity of the 'in-house' sputum PCR were 75% and 35.9% respectively and the positive and negative predictive values were 39% and 72.4% respectively.

**Conclusion:** The IS6110-based 'in-house' sputum PCR showed poor sensitivity and specificity in detection of TB among AFB sputum smear negative PTB suspects and therefore may not be adopted as a rapid and accurate alternative to LJ culture. We recommend further evaluation of the IS6110-based 'in-house' sputum PCR in sputum smear negative PTB diagnosis using a more accurate gold standard than LJ culture.

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**Type: Poster Presentation**

Amplification of pre-membrane and non-structural region 5 for detection and typification of Dengue virus by real-time PCR using SYBR green I assay

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**Background:** Confirmation of Dengue virus [DV] infection by laboratory and serotyping is critical information for patient's management and to applying public health strategies to control the *Aedes aegyptis* vector. Molecular biology methods have largely displaced the classical cell culture techniques reducing the time of reporting. The aim of this study was standardized a Real-Time PCR assay for detection, serotypification and quantification of the DV using SYBR® green I RT-PCR technologies.

**Methods:** DV serotypes (DV1-4) were selected from the laboratory collection and human serum/blood specimens were collected from febrile patients clinically suspected of DV infection (WHO criteria), during the first three days of clinical symptoms. Total RNA were extracted from the patient samples using a QIAmp®RNA Viral/Blood Mini-Kit (QIAGEN®). Three different universal primers designed to amplified pre-membrane (CprM) and non-structural protein 5 (NS5) were used during the detection assay. Subsequently, the viral types were determined using the CprM forward primer and a specific reverse primer for each virus serotype [DV1-DV4]. The Reverse Transcription and amplification assays were performed in one step protocol using SYBR® Green-I RT-PCR kit (QIAGEN®) in a 38 cycle process.

**Results:** All viral types were amplified with the three universal assays. The average CT values for the three region and four-serotypes showed a CprM primer of 26,5; First NS5 region (3NC) 15,9, and finally for Second NS5 region (JMC) 20,9. When the specific reverse primers for DV1-4 were used, only the amplification occurs in the corresponding viral serotype. Validation assay was performed in two hundred-eight serum samples from febrile patients, 33.7% (70/208) of them were positive by RT-PCR. Genotyping showed DV2 (31,1%) as the most frequent genotype, followed by DV1, DV3 and DV4 and a significant difference was observed in viral load in patients with DHF and SSD.

**Conclusion:** We compare the efficiency of the amplification of three different regions of Dengue Virus using a Universal SYBR® green I RT-PCR assay. Amplification of First NS5 region (3NC) showed the best amplification efficiency in patients and controls. Serotyping assay showed the circulation of DV1-DV4 in our communities.

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**Type: Poster Presentation**

Cytokine response in severe sepsis – Predicting and modelling the course of illness

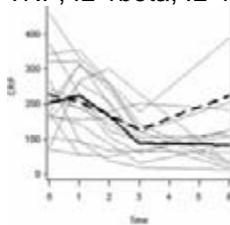
**J. Malaska<sup>1</sup>**, M. Kratochvil<sup>1</sup>, M. Kyr<sup>1</sup>, P. Jabandzjev<sup>1</sup>, F. Otevrel<sup>1</sup>, K. Muriova<sup>1</sup>, M. Fedora<sup>1</sup>, V. Sramek<sup>2</sup>, J. Michalek<sup>3</sup>, P. Sevcik<sup>1</sup>

<sup>1</sup>University Hospital Brno and Faculty of Medicine Masaryk University Brno, Brno, Czech Republic, <sup>2</sup>St. Anne's University Hospital Brno and Faculty of Medicine Masaryk University Brno, Brno, Czech Republic, <sup>3</sup>Faculty of Medicine, Masaryk University Brno, Brno, Czech Republic

**Background:** Severe sepsis remains one of the most threatening conditions in intensive care. During the progression of sepsis from early hit to multiorgan failure proinflammatory and anti-inflammatory cytokines are released. Cytokines can be used as a biomarkers to determine the specific patterns of sepsis progression and association with mortality (1). These biomarkers were successfully used as predictors in animal studies (2). Data from humans, especially comparison between children and adults, are limited. Hence, in this study we widely describe systemic cytokine response in this type of patient population. 1.Kellum JA et al. Arch Int Med. 2007;167(15):1655-63. 2.Osuchowski et al. CCM 2009;37(5):1567-73.

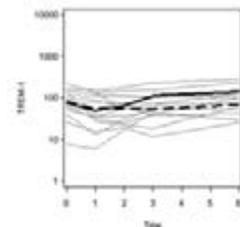
**Methods:** Prospective study of 37 subjects (20 children, 17 adults) hospitalized with severe sepsis in intensive care. We measured CRP, procalcitonin, TNF, IL-1beta, IL-4, IL-6, IL-8, IL-10, IL-12, TREM-1. ANOVA models were specified using Proc Mixed. Study was fully approved by an EC

**Results:** We identified a correlation of CRP levels with mortality or presence of shock. We found a distinct feature of CRP in adults with pronounced dynamic dichotomy in these subjects. Levels of IL-6 were significantly different in adult patients in the context of shock states. Highest risk of death was in adult patients associated with TREM-1 sustained high after 48 hours after sepsis onset. Otherwise, there was no correlation with death, shock states and SOFA score for PCT, TNF, IL-1beta, IL-4, IL-8, IL-10, and IL-12.



**SOFA adults: Solid line: SOFA 0-12, Dashed line: SOFA 13-24**

**Conclusion:** Response of circulating factors in patients with severe sepsis is heterogeneous in adults and children population and has some distinct features according to dynamics of CRP, IL-6 and TREM-1. We can find an evident discriminative feature of CRP and TREM-1 value dynamics. Sustained high levels of CRP and TREM-1 48 hours after sepsis onset were predictive of high risk of death. This finding could be related to the progression of illness to multiorgan failure. An activation of proinflammatory cytokines is associated with higher severity of sepsis and is probably related to severity of the initial hit. Supported in part by the Internal Grant Agency of the Ministry of Health NR 9297-3 and NR 9894-4



**Adults, Solid line: Exitus, Dashed line: survived**

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**Type: Poster Presentation**

Circulating antigens of *Mycobacterium tuberculosis*: Standardization of immunoenzymatic assay to detect circulating antigens related in the pathology of tuberculosis

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**Background:** In 2006, an estimated 9.2 million new cases of tuberculosis in worldwide (WHO 2008). WHO has been set goals for global TB control including early detection of infected individuals and the prompt treatment. For this end, it has been encouraged public-private partnerships to find solutions that would drive to the development of alternative diagnostics methods. In attention to WHO recommendations immunological tests are currently being studied and standardized for the diagnosis of the active and latent disease. Based on our laboratory expertise, we have been worked to standardize immunoassays to detect *Mycobacterium tuberculosis* antigens.

**Objective:** To standardize enzyme immunoassay to detect circulating antigens of *M. tuberculosis* in serum samples from patients with active pulmonary tuberculosis.

**Methods:** 6-kDa early secreted antigen target (ESAT-6), protein low molecular weight, secreted by *M. tuberculosis* and absent in BCG vaccine strain; 16kDa, heat shock protein, important in the persistence of the bacillus, antigen 85 complex is suggested to be involved in the binding of mycobacteria to specific surface receptors of macrophages, were the antigens studied. For this, plastic plates were coated with monoclonal antibodies anti-ESAT-6, 16kDa, Complex 85. In order to avoid false results we studied three block solutions (BSA 2%, 5% and 2% skim milk in PBS). Pool of normal human serum was spiked with different concentrations of proteins from H37Rv strain (500µg/mL to 100 µg /mL) to determine the ELISA analytical sensitivity. The immune reaction was detected using a rabbit anti-*M. tuberculosis* - peroxidase diluted to 1/500.

**Results:** Better results were obtained with plates coated with monoclonal anti-ESAT-6 and blocked with 2% BSA in PBS. Using these conditions our assay is able to detect concentrations of ESAT-6 from 0.1 to 1µg/mL.

**Conclusion:** Optimal conditions of the assay are being improved to increase the sensitivity to detect antigenic fractions that may be associated to other serological markers for global evaluation of patients looking for tuberculosis laboratory evidences. The sensitivity level achieved for ESAT-6 is being evaluated in patients with tuberculosis to determine its clinical applicability for TB diagnosis.

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**Type: Poster Presentation**

Variation in liver histopathology in chronic HBV-infected individuals with normal liver function tests correlates with HBV replication

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**Background:** Clinical and laboratory parameters may not reflect disease activity and imaging studies are insensitive indicators of fibrosis in the precirrhotic liver. The biopsy enables the pathologist and physician to define the extent of disease by grading the necroinflammatory activity and staging the fibrosis and thus determine the progression of liver disease. The aim of this study is to characterize the liver histopathological profiles and their correlation with hepatitis B virus (HBV) replication in chronic HBV-infected individuals with normal liver function tests (LFT).

**Methods:** We performed a percutaneous liver biopsy in 60 chronic HBV-infected individuals with normal LFT. The biopsied tissue was processed for histological examination including grade of disease activity and stage of fibrosis. HBV markers were detected with ELISA. Serum HBV DNA load was assessed with quantitative real-time polymerase chain reaction.

**Results:** The histological findings from mild to moderate grade of severity and stage of progression were the most common histological findings. Twenty five cases were with grade 1 stage 0 (G1S0), 25 cases with G1S1, 8 cases with G2S1 and 2 with G2S2. The patients with serum HBV DNA positive had significant severity in the grade of disease activity and stage of fibrosis in liver tissue than those in the patients with HBV DNA negative ( $P<0.05$ ). In the same way, there was a significant difference between the patients with HBeAg-positive and -negative groups ( $P<0.05$ ). The patients with the highest serum HBV viral load had the most severe necroinflammatory activity and fibrosis than those in the patients with lower viral load ( $P<0.05$ ). No significant differences were observed between sex groups and age groups ( $P>0.05$ , respectively).

**Conclusion:** The histological abnormal findings such as hepatic inflammation and fibrosis were common in chronic HBV-infected individuals with normal LFT. The severity of necroinflammatory activity and fibrosis correlates with HBV replication and viral load and HBeAg expression status.

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**Type: Poster Presentation**

Short primers for amplification of diverse virus strains

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**Background:** Due to the high range of viral diversity no universal, highly conserved primer set exists that is able to amplify all sequenced viral targets. We have performed a computational study that predicted that approximately 3,700 18-mers would be necessary to produce amplicons across the sequenced viral database. However, by decreasing the length of the primer from the traditional 18-mer to a 10-mer the number of primers necessary significantly decreases to approximately 1,000. Shortmers show promise for acting as universal primers that can discriminate both DNA and RNA viruses at the serotype level. As a demonstration of the specificity of shortmers for viral identification we designed 10 and 11-mers that were capable of amplifying three serotypes of Blue Tongue Virus in traditional Reverse Transcription PCR (RT-PCR).

**Methods:** An in-house program, the Multiplex Primer Prediction (MPP) algorithm, was used to identify a primer set capable of amplifying various serotypes of the Blue Tongue Virus (BTV). The RNA of BTV strains 2, 13 and 17 was extracted in-house and selected as the template for Reverse Transcription PCR (RT-PCR) reactions. The primer set was predicted to amplify a 231 bp product from BTV 2, 13 and 17.

**Results:** Using RT-PCR and gel electrophoresis we visually verified the presence of ~231 bp products from the singleplex reactions containing the shortmer primer set and individual templates BTV 2, 13 and 17. The target amplicons were then gel extracted and analyzed with using Sanger sequencing, using the forward 11-mer primer. Results indicated that the BTV2 amplicon was 95% homologous to its predicted amplicon, the BTV13 amplicon was 97% homologous for its predicted amplicon sequence and the BTV 17 amplicon was 97% homologous for its predicted amplicon. The percent homology of amplicon to predicted sequence was less than 100% due to gaps in the sequence reads.

**Conclusion:** We have successfully predicted short primers that are capable of serotype-level viral detection. As a streamlined version of this analysis, we are currently adapting this assay to run on Luminex platform.

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Evaluation of the *C. DIFFICILE TOXIN A/B ITM*, *C. DIFF CHEK-60TM*, and *PREMIER TOXIN A&BTM* with the cytotoxin assay for the detection of *Clostridium difficile* GDH antigen and toxins A and B in feces

**W. Greene**

PennState Hershey Medical Center, Hershey, PA, USA

**Background:** *C. difficile* causes about 25% of the cases of antibiotic-associated diarrhea and most cases of pseudomembranous colitis. Rapid assays have been developed to detect the toxins A and B and the enzyme glutamate dehydrogenase which is produced in high levels by all strains of the organism. Because this enzyme is present in both toxigenic and non-toxigenic strains of the organism, the assay can not confirm the presence of toxigenic organisms in a sample. However, the absence of this enzyme in a sample should be a good marker for the absence of the organism, making it a suitable screening assay to identify negative specimens.

**Methods:**

We compared two commercial EIA assays for detecting toxins A and B, and an EIA assay for detecting the GDH enzyme with our in-house cytotoxin assay as the "gold standard." All commercial assays were performed according to the manufacturer's instructions.

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**Results:**

Fecal samples (N=236) submitted for *C. difficile* cytotoxin assay were included in this study. The *C. DIFF CHEK-60TM*, detected all 10 of the cytotoxin positive specimens and was positive for 28 specimens where cytotoxin was not detected by any of the toxin assays. The *C. DIFFICILE TOXIN A/B ITM* detected all 10 of the cytotoxin positive specimens and gave no false-positive results. The *PREMIER TOXIN A&BTM* assay detected 9 cytotoxin positive specimens and had 2 positive results that were negative by all the other assays.

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**Conclusion:**

The GDH assay detected 100% of the cytotoxin positive specimens making it a useful assay for screening specimens for detecting *C. difficile*. This would allow for the rapid reporting of the negative specimens that made up 84% of the specimens examined in this study. GDH positive assays should then be tested with an assay specific for the toxins.

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**Type: Poster Presentation**

A highly sensitive reverse transcription polymerase chain reaction method for yellow fever virus detection

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**Background:** Diagnosis of YF is an important issue particularly in countries where it is endemic. Usual serological tests include IgM antibody capture by enzyme-linked immunosorbent assay (MAC-ELISA), hemagglutination inhibition (HI), complement fixation (CF) and neutralization (N). Nevertheless, antibody detection is feasible only 5 to 6 days after the offset of symptoms and the true usefulness is by evaluation of paired samples which are not easy to obtain. On the other hand, specific diagnosis during infection period is currently done by isolation of virus in mosquito cells or by genome detection through PCR based methods, while fatal cases are confirmed with immunohistochemistry with monoclonal antibodies over well conserved fixed tissues. Although viral isolation is a very specific technique, it takes at last 7 –10 days and sensitivity decrease when few virus particles are present in the sample or when toxic biliary salts present in serum inhibit cell growth. Detection of virus in the acute phase must be specific and sensible enough to confirm the diagnosis, and then start the surveillance measurements.

**Methods:** Twenty five human sera were processed with the QIAamp Viral RNA Minikit. Reverse Transcription (RT) was carried out following by Polymerase Chain Reaction (PCR) using specific primers designed to amplify 734bp from the prM/E junction. Negative samples were reamplified with internal primers (nested PCR) to obtain a 692bp fragment.

**Results:** The expected amplification product size was obtained in 20 of 25 samples analyzed. Positive reaction was obtained in 2 samples after first reaction, while the remaining 18 were positive after performing nested PCR.

**Conclusion:** This improved RT-PCR using primers designed in a conserved region of genome to amplify short fragments, not just ensure detection of all YFV genotypes, but also increase sensibility even in more difficult samples. Remarkable, this reaction allows rapid differential diagnosis between Dengue and Yellow Fever, which is absolutely necessary for an effective surveillance and opportune epidemiologic measures.

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**Type: Poster Presentation**

Molecular diagnosis of American tegumentary leishmaniasis with minimally invasive samples

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**Background:** American Tegumentary Leishmaniasis (ATL) is endemic in nearly 65% of Peru. Even when diagnosis is routinely done by direct examination, its sensitivity can be easily affected by several factors such as disease duration, superinfection and appropriate sampling. The last one is an important factor especially due to patient tolerability (particularly in facial lesions or children) and to an adequate training.

**Methods:** Diagnosis of ATL was evaluated in 40 lesions by 3 methods: direct examination, culture and PCR. PCR was performed in lymphatic secretion and dermal scrapings. Montenegro Skin Test (MST) was not performed in all cases. Lesions were cleaned with hydrogen peroxide and then lymphatic secretion was taken with microhematocrit capillary tubes. Dermal scrapings were taken with two sterile lancets: one for PCR and the other one for direct examination. Culture was done by the aspiration method. DNA was isolated by the phenol-chloroform-isoamyl alcohol method and precipitated with ethanol and 3M sodium acetate. The kinetoplast DNA (kDNA) PCR was performed with 0.5 U of HotStar Taq DNA Polymerase (QIAGEN), with a set of primers targeting the *Leishmania* kDNA minicircles: MP1-L (fwd) 5'-TACTCCCCGACATGCCTCTG-3' and MP3-H (rev) 5'-GAACGGGGTTTCTGTATGC-3'. Another set of primers targeting human beta-hemoglobin gene was used in the same reaction tube as an internal amplification control: HBBL (fwd) 5'-GGCAGACTTCTCCTCAGGAGTC-3' and HBBR (rev) 5'-CTTAGACCTCACCTGTGGAGC-3'.

**Results:** Of the 40 suspected ATL lesions, 39 were confirmed by at least one of the three conventional diagnostic methods used here. Only one lesion was negative to all methods, but MST was very strong, thereby supporting the diagnosis of ATL. The sensitivity of every method was as follows: direct examination 75%, culture 50%, PCR of dermal scrapings 95% and PCR of lymphatic secretion 90%. The DNA obtained from both PCR samples was enough for further *Leishmania* species identification. Human DNA obtained from dermal scrapings was more abundant in comparison to lymphatic samples.

**Conclusion:** PCR from dermal scrapings and lymphatic secretion have higher sensitivity in comparison to traditional procedures. Lymphatic secretion has less human DNA and this can improve the species typing process. We aim to show that diagnosis of ATL can be done without invasive procedures while keeping a high performance.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Multiprobes real-time PCR direct detection of MDR-TB related genes in pulmonary samples

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**Background:** Multidrug resistant *Mycobacterium tuberculosis* infection is a serious contagious disease that needs rapid detection to control the disease. Direct detection of the responsible genes using hybridization probes with melting temperature shift will confer information of the drugs resistance.

**Methods:** Primers and probes for *rpoB*, *kat G* and regulatory region of *inhA* genes were designed to perform in single tube real-time PCR of the LightCycler system. Three sets of hybridization probes were synthesized; probes cover codon 511-534 of *rpo B* gene were label with Red 640 and Red 705, codon 304-318 of *kat G* with Red 610 and -18 regulatory regions to codon 9 of *inhA* with Red 670. Reduction of melting temperature in the system analysis indicates mutation in the specific region. Specificity of the designed probes and primers were *in silico* tested with non-tuberculous mycobacterias and *in vitro* assessed with reference strains. The method was evaluated with 134 *M. tuberculosis* clinical isolates (41 susceptible, 18 INH resistant, 1 RMP resistant and 74 MDR), and 134 decontaminated tuberculosis pulmonary samples (75 smear-positive and 59 smear-negative) by comparing to proportion method results of their isolates.

**Results:** This multiprobes method shows 100% specificity to *M. tuberculosis* complex. All susceptible isolates showed absence of mutation by this method. Mutation in *rpo B* gene was detected in all of 75 resistances. Mutations of *katG* and *inhA* genes were detected in 79 and 13, respectively, of 93 INH resistances.

Among 134 decontaminated samples, susceptibility results of their culture were 104 susceptible, 23 INH resistances, 1 RMP resistance, and 6 MDR. This method could detect mutation in *rpoB* gene of all 7 RMP resistances. Mutation of *katG* or *inhA* genes were detected in 27/29 INH resistances. Nonsense mutation was found in the regulatory region of *inhA* gene in one susceptible sample. The specificity and sensitivity of the method for direct detection were 100% for RPM; and 99.0% and 93.1%, respectively, for INH.

**Conclusion:** This method is competent to detect mutation of the genes associated with RMP and INH resistant tuberculosis in pulmonary specimen in one tube reaction.

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Successful containment of an avian influenza outbreak through public health intervention in district Howrah, West Bengal, India, January '08

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**Background:** On 25.01.'08, Avian Influenza viruses were detected in blood samples of dead chickens of Nalpur Masjidpara village of Sankrail and Uttar Panchla village of Panchla blocks in district Howrah, West Bengal, India. Rapid Response Team (RRT) started investigating and containing the outbreak and also initiated surveillance for human cases with their management.

**Methods:** An observational study in the form of fever surveillance was conducted among all age groups between 26.01.'08 to 25.02.'08 within 10 km. radius of the epicentre of the outbreak [0-3 km. daily and 3-7 km twice a week]. Fever with Upper Respiratory Tract Infection (URTI) of the mentioned areas were considered as suspected H5N1 cases. They were observed, investigated and treated. Culling of poultry was done within 5 km radius by animal husbandry department. Cullers were checked twice daily before and after culling by Medical Officers and both were put on Oseltamivir prophylaxis. Daily report of surveillance and culling was prepared and transmitted and a 24 hours control room was functioning. Disinfection and extensive advocacy were conducted in the affected and adjacent areas.

**Results:** Out of 734 villages of 14 blocks of the district, two villages of two blocks were affected. Fever surveillance was conducted among 29,87,062 population of 186 villages in five blocks and nineteen wards of Uluberia Municipality with a result of 786 suspected cases. 418 cases attended in OPDs. Two suspected cases were admitted in IPD and later transferred to tertiary centre. H5N1 was excluded after laboratory investigation. Attack Rate of fever & URTI was 0.40/1000 without any case fatality. 1,60,264 birds, 1,013 eggs and 12,672 kg poultry feed were culled.

**Conclusion:** Outbreaks of Avian Influenza among birds occurred in two villages were contained by timely and successful public health intervention. Avian Influenza did not contract human. Recommendations were to continue surveillance on human fever & URTI and also on poultry and migratory birds; RRTs are to be extended up to block level and adequate medicines and logistics are to be procured to combat any future outbreak.

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How different is Chikungunya from Dengue?

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**Background:** Dengue fever has been endemic in Singapore since the 1960s. However, indigenous transmission of chikungunya fever has only been reported in 2008. As clinical features of chikungunya mimic that of dengue, it is important to identify key factors that can differentiate the two *Aedes* mosquito-borne infections at first presentation to hospital.

**Methods:** We conducted a case-control study on 117 patients confirmed with chikungunya infection on reverse transcription-polymerase chain reaction (RT-PCR) during the August 2008 outbreak, and hospitalised at the national infectious disease referral center in Singapore. These were compared to 917 PCR-confirmed dengue fever patients (including 55 with dengue haemorrhagic fever [DHF]) hospitalized at the center during the 2004 dengue outbreak.

**Results:** The mean ages of chikungunya and dengue patients were 39.7 years (SD 12.7 years) and 33.8 years (SD 12.2 years) ( $p=0.000$ ) respectively. At presentation to hospital, chikungunya patients were significantly more likely to have arthralgia/myalgia (OR 7.84, 95%CI 3.41-18.05,  $p=0.000$ ), rash (OR 1.83, 95%CI 1.24-2.69,  $p=0.002$ ), and conjunctivitis (OR 95.06, 95%CI 12.15-743.55,  $p=0.000$ ) than dengue patients, but less likely to present with anorexia (OR 0.17, 95%CI 0.09-0.31,  $p=0.000$ ), nausea (OR 0.21, 95%CI 0.12-0.35,  $p=0.000$ ), vomiting (OR 0.14, 95%CI 0.07-0.28,  $p=0.000$ ), abdominal pain (OR 0.21, 95%CI 0.08-0.56,  $p=0.001$ ) and diarrhoea (OR 0.11, 95%CI 0.04-0.31,  $p=0.000$ ). Chikungunya patients had significantly higher nadir leukocyte (median 5.1 vs 2.8  $\times 10^9/L$ ,  $p=0.000$ ) and platelet counts (median 192 vs 79  $\times 10^9/L$ ,  $p=0.000$ ), but lower peak alanine aminotransferase (median 28 vs 62 U/L,  $p=0.000$ ) and aspartate aminotransferase (median 34 vs 102 U/L,  $p=0.000$ ) levels, compared to dengue patients. Compared to chikungunya patients, DHF patients had an odds of 8.44 times (95%CI 2.22-32.10,  $p=0.001$ ) of having tachycardia (pulse  $>100/min$ ), 9.10 times (95%CI 0.99-83.43,  $p=0.037$ ) of hypotension (systolic blood pressure  $<90mmHg$ ) and 83.3 times (95%CI 17.9-333.3,  $p=0.000$ ) of gum bleeding, at presentation.

On multivariate analysis, the presence of arthralgia/myalgia, presence of rash, presence of conjunctivitis, the absence of gastrointestinal symptoms, and absence of thrombocytopenia were independent predictors of chikungunya.

**Conclusion:** Chikungunya patients can be differentiated from dengue patients at first presentation to hospital. They were significantly more likely to have arthralgia/myalgia, rash, and conjunctivitis, but less likely to present with gastrointestinal symptoms, and thrombocytopenia.

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Community-acquired *Staphylococcus aureus* infection in previously healthy neonates  
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**Background:** Community-Acquired *S aureus* (CA-Sa) resistance are changing in the last ten years in Argentina, increasing methicillin-resistant *S aureus* (MRSA) strains. First case in our neonatal unit was diagnosed in 2006.

**Objectives:** Identify clinical features, risk factors, sensibility and outcome of neonate with CA-Sa infection.

**Methods:** Prospective observational study. We included previously healthy patients ≤ 30 days of age admitted to the neonatal unit with CA-Sa infection from 2006 to 2009. We defined CA-Sa infection based on CDC guidelines.

**Results:** We included 9 healthy neonate with CA-Sa infection. Vaginal labor was present in 7/9. Mean gestational age 39 weeks, weight 3325 g, and < 3 birth hospital days. Of 9 *S aureus* infections, 5 (56%) were MRSA. Six of 9 were male. Median age at symptoms onset was 12 days (r:9-18). All infection involved skin and soft tissue (9/9) with pustulosis, omphalitis, mastitis, chest, facial/neck, inguinal and groin abscesses. Five of 9 had severe manifestation: sepsis, osteoarticular infection, orbital cellulitis and necrotizing pneumonia. Culture positive were obtained in 7 from soft tissue, 4 blood and 2 joint. All received systemic antibiotics. Four of 5 (80%) and 2/4 (50%) MRSA and MSSA infections respectively required surgical drainage. One patient died because of sepsis. Maternal skin/soft tissue infection history occurred with 3/4 MRSA versus none of MSSA infections.

**Conclusion:** Epidemiology of CA-Sa infection is changing even in the newborn period, with increasing MRSA strains. Male neonates 9-18 days of life were affected more often, Maternal infection and surgical drainage has a strong association with MRSA isolation.

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Etiologic aspects of 341 cases of acute undifferentiated febrile syndrome (AUFS) in southeast Brazil

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**Background:** The etiologic nature of the acute undifferentiated febrile syndromes (AUFS) has been a challenge everywhere. This last aspect has been overwhelming in developing countries like Brazil where limited information has been available. We systematically sought the etiology of AUFS in 341 in and outpatients seen from the city of Juiz de Fora, Minas Gerais State, Brazil, during a 5 years period.

**Methods:** Three hundred and forty one consecutive in and outpatients with AUFS were studied. Serological tests for rickettsial infections were performed by microimmunofluorescence with "in door" slides using antigens from CDC Atlanta USA: *Ehrlichia chaffeensis*, *Coxiella burnetii*, *Rickettsia typhi*, *Rickettsia rickettsii*, *Bartonella henselae* and *Bartonella quintana*. *Rickettsia felis* identification was done by PCR in the attacking fleas. Serological tests for salmonellosis, brucellosis, toxoplasmosis, leptospirosis, CMV, EBV, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, Lyme's disease, brucellosis, HIV and dengue fever were performed in referral commercial laboratories. Influenza antigens tests, *Toxocara canis* and hantavirus serologic tests were performed in patients with specific strong epidemiological and clinical evidence. Blood and other materials cultures were locally performed.

**Results:** Dengue fever was the most frequent diagnosis with 78 cases (22.9 %). Fifth-three rickettsial infections gave rise to 15.5 % of etiologies led by spotted fever group rickettsiosis with 19 cases (5.6%) 3 of then *Rickettsia felis* infections (0.9%). Q fever - 14 cases (4.1%), monocytotropic ehrlichiosis - 12 cases (3.5%), murine typhus - 5 cases (1.5 %) and *Bartonella* spp. infection - 3 cases (0.9%) were the other rickettsial causes. EBV infection were 20 cases (5.7 %). Parvovirus B19, CMV and *M. pneumoniae* infection, all with 13 cases (3.8% each) were also frequently diagnosed. Toxoplasmosis cases were 10 (2.9%) and *Chlamydia pneumoniae* 7 (2%). Influenza, leptospirosis and enteric fever were 6 cases each (1.7%), followed by yellow fever vaccine reactions (5 cases - 1.5%). Seventy-seven cases (22.6%) remained inconclusive and a myriad of other disorders was identified in 34 patients (10%).

**Conclusion:** This study emphasizes the need of investigating a wide array of etiological agents rather than simplistic syndromic approaches. High frequency of rickettsial infection and dengue fever were observed in this study.

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Tick-borne encephalitis virus in Mongolia

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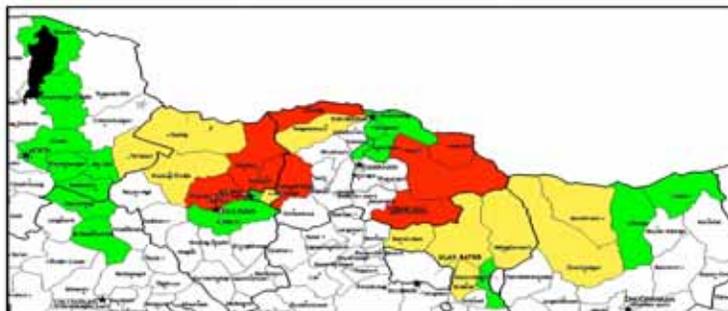
**Background:** In Mongolia, Tick-Borne Encephalitis virus (TBEV) now causes approximately 20 cases of disease annually. The disease is registered at national level from 2001 and 75 cases occurred from 2005 to 2008. We studied main ecological, epidemiological and genetic characteristics of TBEV in Mongolia

**Methods:** Questing ticks were collected in forested regions of Northern Mongolia by flagging. The amount of ticks per 1km was estimated and their species, gender and stage were identified by morphology. Ticks and human serum samples were analyzed by ELISA, RT-PCR and sequencing.

**Results:** The natural foci of TBE are spread in Northern part of Mongolia and linked to the Taiga Ticks *I. persulcatus* distribution. The average tick abundance vary from 0,2 tick per km in Huvsgol aimag to 43 ticks per km in Selenge aimag with the most numerous tick populations in Selenge and Bulgan aimags. The average TBEV infection rate in ticks in Selenge aimag is 5,5% and in Bulgan aimag - 4,4%. The rates of TBEV infection for Tuv, Hentii, Orkhon and Huvsgol aimags are not established so far due to low Taiga tick abundance in these territories. Several popular objects of pilgrimage, international tourism and recreation were studied to evaluate the risk of human infection with TBEV and Amarbayasgalant monastery (Selenge aimag) was found to be of high risk.

The serological survey among 965 residents of Selenge aimag revealed that average 6,2% of people have the specific antibodies against TBEV.

The genetic characteristics of Mongolian TBEV from ticks and patient indicate that Far Eastern and Siberian subtypes are common in Mongolia and viral populations are closely related to TBEV from neighbor regions of Russia – Buryat Republic and Irkutsk Region **Conclusion:** Thus, the highest risk of human infection with TBEV in Mongolia is the feature of Selenge and Bulgan aimags (Fig. 2) and intensive measures of prophylaxis are necessary both for residents and for tourists in those provinces. This project has been supported by Russian Fund for Basic Research, grant №08-04-90206-Mong\_a.



**Figure 2. Prevalence of TBEV in Northern Mongolia and risk of infection for humans**

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Bartonella spp infections diagnosed between 2005 and 2009 by the National Rickettsial Reference Laboratory in Rio de Janeiro, Brazil

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**Background:** *Bartonellae* are gram negative bacteria that parasitize erythrocytes and endothelium of several mammals, being widespread in nature. Its main source of infection to humans is the domestic cat. *Bartonella* infection is not notifiable, and no national data is available regarding its manifestations in Brazil. The aim of this study is to describe the cases referred with a possible diagnosis of bartonellosis to the National Rickettsial Reference Lab (LNHR) in Rio de Janeiro.

**Methods:** A Microsoft Excel® datasheet was designed for the study, so as to correlate the Brazilian Ministry of Health Notification requests received by LNHR (SINAN, for Brazilian Spotted Fever, notifiable since 2001) and the Lab's database. The diagnosis of bartonella infection was considered definite when paired samples showed a 4-fold difference in titers detected in the Indirect Immunofluorescence Assay for *B.henselae* IgG antibodies from Bion, USA, and/or the polymerase chain reaction (using primers CAT1/CAT2) was positive in biological samples.

**Results:** 54 patients had samples sent with the SINAN requests with the clinical suspicion of bartonellosis. Of these, 20 (37%) had definite infection by reactive IFA assay and/or positive PCR results (6 of 20). 19/20 patients were from Rio, 1 from Bahia. Mean age± standard deviation was 28,9±17,5 years, with median of 26. 12/20 patients were male. 14 referred contact with pets, mostly cats. Most prevalent signs and symptoms were fever and chills (11/15), adenopathy (11/12), hepatoesplenomegaly (7/13), abdominal pain (5/19), prostration (5/9), headache (5/10) and conjunctival injection (4/11). Six patients had neuroretinitis, one of which with associated meningoencephalitis. One of the 20 patients had aortic valve endocarditis which required surgery.

**Conclusion:** More human samples are gradually being sent for Bartonella testing in LNHR, possibly because of greater medical awareness of this infection. Although classically the agent of cat-scratch disease, severe syndromes such as neuroretinitis, disseminated disease (with fever and hepatosplenomegaly) and endocarditis were seen in this first documented series of cases from Brazil. This deserves public health attention and better information to health care providers. Supported by FAPERJ, Brazil

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Multiplexed diagnostic assays for detection of high consequence foreign and emerging animal disease

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**Background:** Due to the overwhelming number of foreign and emerging animal diseases a critical challenge to detection and response to outbreaks of animal disease is the availability of rapid, rigorously tested diagnostic assays able to detect multiple disease organisms. Methods currently used for veterinary diagnostics are generally single agent and can be time-consuming, labor-intensive, and difficult to scale up in the event of an outbreak. Multiplexed PCR detection capabilities provide many advantages over conventional single agent, time-consuming and labor intensive detection methodologies. Because of these advantages, coupled with their inherent adaptability and multiagent utility, multiplexed PCR assays are ideal for use in detection of foreign and emerging animal disease.

**Methods:** Our most promising work thus far has been the development of multiplexed RT-PCR nucleic acid assays that can detect multiple genome regions of pathogens in a single tube with a high degree of sensitivity and specificity.

**Results:** We have demonstrated the ability to simultaneously extract and amplify DNA and RNA targets from environmental and clinical samples with a high degree of efficiency. The FMD assay can detect 17 distinct genomic regions from a panel of foreign and domestic viruses that are clinically indistinguishable from Foot and Mouth Disease virus in cattle, sheep and pigs. While our deeply multiplexed avian influenza assay can simultaneously detect and subtype influenza A in a sample from poultry or wild birds and determine if the most common human (H1,2,3) and well as several avian subtypes (H3,5,7,9) are present. The avian influenza assay detect 37 distinct genomic regions located on segment 4 (hemagglutinin), segment 7 (matrix) and segment 8 (non structural) of influenza A. Of the 37, 34 are targeted exclusively to segment 4 to provide hemagglutinating subtyping for influenza A. The other three signatures provide universal detection of influenza A by targeting segment 7 and 8 in the assay.

**Conclusion:** These multiplex panels have been extensively tested and demonstrated to work with environmental and clinical samples at multiple public health and agricultural laboratories. It is hoped that these assays may be further developed to be acceptable for routine use for the detection of economically important animal diseases.

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Transmission of chikungunya in Singapore, 2008

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**Background:** Chikungunya is a vector-borne viral febrile disease that is not endemic to Singapore. We describe herein the epidemiology of 718 cases identified during the outbreak in 2008, including probable sources of infection, modes of transmission, and vector species responsible for this outbreak.

**Methods:** We investigated into all laboratory-confirmed chikungunya cases notified from the healthcare institutions between 30 Dec 2007 and 4 Jan 2009. Cases epidemiologically linked in space and time were defined into clusters and analysed. Vector operations at each cluster were carried out to identify the mosquito species responsible for transmission.

**Results:** A total of 718 cases of chikungunya were reported in 2008. The outbreak began with a cluster of cases at Little India in Jan–Feb. Sustained local transmission only occurred after a wave of imported cases in Jul. During the period from Jul-Dec, there were 693 cases, of which 173 (25%) were imported infections and 520 (75%) local infections. Among the 173 imported cases, 162 (94%) originated from Malaysia, mainly in people who developed the illness after visiting rural Johor where *Aedes albopictus* activity was reported. 152 (80%) of them were Singaporeans. Among the 520 local cases, majority were males (414, 80%) of working age (mean, 38 years) non-resident (327, 62.9%) labourers (277, 53.3%). Higher risks of infection were associated with landed property and temporary housing. Cases were distributed in the urban, sub-urban, industrial and agricultural areas of Singapore. Entomological surveys involving species identification of mosquito larva found in these areas implicated *Aedes albopictus* (31 out of 34 clusters with breeding) as the vector responsible for transmission.

**Conclusion:** Our findings showed that proximity to infective *Aedes albopictus* mosquito vectors, either through travel to rural Johor, or work/stay within local forested areas increased risks of acquiring the chikungunya virus infection.

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Escalation of Japanese encephalitis in India: Evidence from 2005 viral encephalitis outbreak and appraisal of nities

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**Background:** Viral encephalitis is a global emerging problem. North India faced a large epidemic of Japanese encephalitis (JE) in 2005. Therefore, the present study was planned to reconfirm the circulation of JE in the area and to get a better view of trend of the disease to slowdown the burden of JE.

**Methods:** Surveillance was carried out to identify patients with acute encephalitis. Blood and cerebrospinal fluid specimens from suspected cases underwent pathological, serological, demographic investigation and viral testing for evidence of Japanese encephalitis virus (JEV) infection; either by IgM capture ELISA/RT-PCR or both. To identify circulating JEV strain RT-PCR, sequencing and phylogenetic analysis was performed. Based on clinical cases reported between 1992-2008, trend of JE infection in the state was analyzed to examine the dynamics of infection.

**Results:** Our investigations (n=38) revealed that only 55.3% cases were positive for JE. Pathological examination revealed marked pleocytosis in CSF ( $90 \pm 76.9$  cells/mm<sup>3</sup>), and peripheral leucocytosis ( $64.7 \pm 8.86\%$  neutrophils) with mild anemia. Males were more susceptible than females with ratio of 1.63:1 and significant gender difference ( $P < 0.05$ ) was observed in patients below six years. In the patient group with age below six years, rate of infection per million was six fold higher ( $P < 0.005$ ) in males as compared to females. Our phylogenetic study suggests that circulating novel strain (JEV GP05; submitted in NCBI with FJ979830 accession number) during 2005 JE epidemic was close to GP78 and in future larger epidemic may occur.

**Conclusion:** The 2005 JE epidemic was possibly because of JEV GP05 (a close member of JEV GP78) and it is spreading in newer areas. The trend of JE (1992 – 2008) suggested that the problem in North India is escalating and larger epidemics may be envisaged in future. Considering possibility of larger epidemic in future, serious steps are necessary for combating JE, including development of more efficient surveillance methods and differential diagnosis.

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What is the current situation with plague in North Africa?

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**Background:** From Morocco to Egypt, North Africa was widely affected by plague throughout the first half of the twentieth century. Most of the countries had natural foci described. For unknown reasons, human plague disappeared in the 1950s (outside of very sporadic clusters in Libya, last occurring in 1984). However, plague has made an astonishing comeback in the last decade.

**Methods:** n/a

**Results:** After a silence of 50 years, an outbreak of bubonic plague suddenly occurred close to Oran in Algeria, in June 2003. Eighteen bubonic cases were identified, and *Yersinia pestis* was isolated from 6 patients. In July 2008, a new cluster was reported among nomads 300 km south of the first one. Four members of one family were affected and one died. The bacillus was isolated from one patient. No epidemiological association was identified between the two events. On June 2009, 25 years after the last occurrence in the country, Libya reported five confirmed cases of bubonic plague in the Tobruk area. *Y. pestis* was isolated from three patients.

In all these cases, further local ecological investigations confirmed the existence of a natural focus

The re-emergence of human plague in the region is not without international consequences. Two of the last concerned natural foci are close to an international port which raises the question of the potential exportation of infected rodents. Cross-border tensions, between "plague countries" and "plague-free countries" have been observed although the foci's limits are unknown as any systematic ecological investigation and surveillance is lacking.

Additionally, the potential weaponization of *Y.pestis* together with international political tensions feed a recurrent interest in plague in North Africa. False rumors of alleged military laboratory accidents or terrorist acts are routinely mentioned, although events could be first explained by the natural history of the disease.

**Conclusion:** In this context, and although the number of human cases has been very limited so far, the first priorities are to establish appropriate ecological surveillance and agree on a common plague control strategy for the region.

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Analysis and characterization of mouse monoclonal antibodies reactive to Chikungunya virus (CHIKV)

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**Background:** Chikungunya virus (CHIKV), an alphavirus belonging to the Togaviridae family, is transmitted to human by several species of mosquitoes, with *Aedes Aegypti* and *A. Albopictus* being the two main vectors. The virus is endemic in Africa, India, South-East Asia and recently in southern-Europe and is responsible for an acute infection of abrupt onset characterized by high fever, asthenia, headache, rash, myalgia and a painful polyarthralgia. Because the re-emergence of CHIKV fever, the development of anti-CHIKV monoclonal antibodies (MAbs) is critical for diagnosis and basic research on CHIKV infection. In this study we analyzed the reactivity of a panel of MAbs to CHIKV.

**Methods:** A panel of 10 MAbs has been tested for reactivity to CHIKV by indirect ELISA, for which a Maxisorp plate has been coated with purified CHIKV virions. All the MAbs that tested positive in ELISA, were further tested by indirect immunofluorescence assay (EUROIMMUN, Medizinische Labordiagnostika AG). Immunoblot analyses were carried out in order to identify the protein target towards the reactive Mabs are raised to. Furthermore, transient expression of E2 in COS7 cells, followed by fluorescence imaging, has been used for a better identification of MAbs reactive to E2 protein.

**Results:** Among 10 MAbs tested, 5 showed reactivity with CHIKV antigens in indirect ELISA; only 2 of them were further confirmed as reactive by immunofluorescence assay. Immunoblotting analyses, performed with purified CHIK virions, showed that these 2 MAbs had significant reactivity with envelope glycoprotein E1 or E2. Immunofluorescence studies performed on COS7 cells transiently expressing E2 protein, showed that both the anti-CHIKV Mabs targeted specifically viral glycoprotein E2.

**Conclusion:** In this study a panel of 10 Mabs were tested for their reactivity with CHIKV by indirect ELISA, Immunoblot and immunofluorescence assay on cells expressing E2 protein. 2 Mabs specifically targeted to viral glycoprotein E2 were identified. The 2 Mabs might be suitable for developing diagnostic tests other than be helpful for studying the biology of CHIKV and pathogenesis disease. For these 2 Mabs, the potential to neutralize CHIKV infection is under evaluation.

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Study of Hanta-viral hemorrhagic fever with renal syndrome (HFRS) in an endemic region of Bulgaria

**I. Baltadzhiev**

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**Background:** HFRS started registration in Bulgaria since 1953. The major epidemics were first described during 1957 and 1959 in Western Rhodopa region. In the 1953-1991 period 412 patients were registered and 67 of them with lethal exit.

The aim of the study was to describe the clinical and epidemiological characteristics of HFRS, and to discuss the professional implications of the disease, as well as its control and prevention.

**Methods:** The authors analyze clinical, laboratory and epidemiological data about 21 cases of HFRS for the period 1987-2009. The cases were etiologically proven in the National Reference Arboviral laboratory. Hantaan (37.5%) and Puumala (12.5%) viruses were identified.

**Results:** All cases were registered in typical season - early spring, summer and late autumn. Males (80.95%) present risk gender group for HFRS related to profession – military servants, construction workers in the endemic mountain regions etc. Mountaineer personnel are exposed to infection when working, digging, eating or sleeping in fields infested by infected rodents.

Patient age varies from 14 to 83 ( $38.52 \pm 4.49$ ) years and 57.14% of them were between 15-35 years old. Patients were febrile several days before admission, had dull and some of them severe abdominal pain, decreased urine output, diarrhoea (53%) visual troubles (48%) and arthromyalgiae. Bleeding was moderately expressed and rarely abundant (cavity organ hemorrhages in 30%). Jaundice was mild and not frequent.

Blood tests showed metabolic acidosis, increased serum creatinine ( $784 \pm 72$  mmol/l), blood urea nitrogen ( $37 \pm 5$  mmol/l), potassium ( $5.9 \pm 0.5$  mmol/l), ALT ( $67 \pm 8.1$  U/l), AST ( $89 \pm 7.4$  U/l) and decreased PLT ( $78 \pm 18 \times 10^9$ /l), haemoglobin ( $7.4 \pm 2.2$  mmol/l) and fibrinogen ( $1.98 \pm 0.34$  mmol/l) levels. Urinalysis showed considerable proteinuria, leukocyturia and degenerated renal epithelial cells with so-called fat corpuscles. Ultrasound: enlarged kidneys with acute parenchymal lesions. One of our patients with 8-fold increased antibody titer to Hanta-virus developed hemolytic anemia, decreased haptoglobin levels and exhibited transitional neurological signs, mimicking thrombocytopenic-thrombotic syndrome. In the last years lethal exit is very rare. Only one patient (4.76%), admitted in infectious shock state and abundant bleeding died four hours after admission.

**Conclusion:** Deep changes in the social-economic standards of last decades affected the spreading of HFRS and respective morbidity and mortality in this case.

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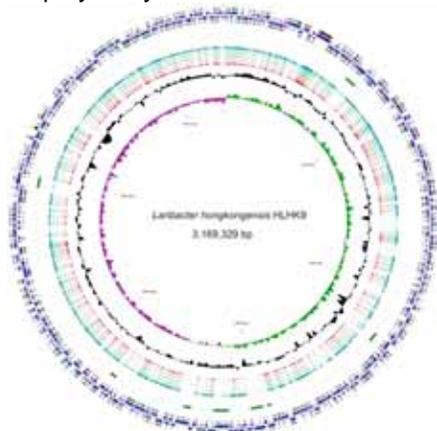
The complete genome sequence and analysis of the betaproteobacterium *Laribacter hongkongensis*

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The University of Hong Kong, Hong Kong, China

**Background:** *Laribacter hongkongensis* is a newly discovered, facultative anaerobic, Gram-negative, motile, sea gull-shaped bacillus of the *Neisseriaceae* family associated with freshwater fish-borne gastroenteritis and traveler's diarrhea.

**Methods:** The genome sequence of *L. hongkongensis* HLHK9 was determined with the whole-genome shotgun method. Shotgun sequences were assembled with Phrap. Gene prediction was performed by Glimmer version 3.02, and results post-processed using TICO for improving predictions of translation initiation sites.

**Results:** *L. hongkongensis* HLHK9 recovered from an immunocompetent patient with severe gastroenteritis, possesses a single chromosome of 3,169-kb chromosome with G+C content of 62.35%. Genome analysis reveals different mechanisms potentially important for its adaptation to diverse habitats of human and freshwater fish intestines and freshwater environments. The gene contents support its phenotypic properties and suggest that amino acids and fatty acids can be used as carbon sources. We identified extensive variety of transporters, including multidrug efflux and heavy metal transporters as well as genes involved in chemotaxis, which may make *L. hongkongensis* possible to survive in different environmental niches. A pool of genes encoding urease, bile salts efflux pump, adhesin, catalase, superoxide dismutase, and other putative virulence factors such as hemolysins, RTX toxins, patatin-like proteins, phospholipase A1, and collagenases are present. Proteomes of *L. hongkongensis* HLHK9 cultured at 37 degrees C (human body temperature) and 20 degrees C (freshwater habitat temperature) showed differential gene expression, including two homologous copies of *argB*, *argB-20*, and *argB-37*, which encode two isoenzymes of N-acetyl-L-glutamate kinase (NAGK)-NAGK-20 and NAGK-37- in the arginine biosynthesis pathway. NAGK-20 showed higher expression at 20 degrees C, whereas NAGK-37 showed higher expression at 37 degrees C. NAGK-20 also had a lower optimal temperature for enzymatic activities and was inhibited by arginine probably as negative-feedback control. Similar duplicated copies of *argB* are also observed in bacteria from hot springs such as *Thermus thermophilus*, *Deinococcus geothermalis*, *Deinococcus radiodurans*, and *Roseiflexus castenholzii*, suggesting that similar mechanisms for temperature adaptation may be employed by other bacteria.



**Conclusion:** The genome sequence and analysis of *L. hongkongensis* revealed novel mechanisms for adaptations to survival at different temperatures and habitats.

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The 2008 dengue epidemic on Curaçao: Correlation with climatological factors

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**Background:** Dengue fever is a common disease on Curaçao, a small Caribbean island close to Venezuela, with tens to hundreds of proven cases during the rain period each year. However, 2008 showed an unexpected and dramatic rise in incidence of dengue fever with almost 1200 serologically proven cases. We studied the correlation between climatological factors and the incidence of dengue fever.

**Methods:** The statistical correlation between the incidence of serologically proven cases of dengue fever and several climatological factors, such as rainfall, mean-, maximum- and minimum temperature, wind velocity, sun hours, cloud coverage, evaporation rate and air pressure in the past ten years was investigated by using the non-parametric Spearman's correlation test.

**Results:** Lower average temperature, lower absolute maximum temperature, lower absolute minimum temperature and lower average maximum temperature all showed a strong correlation with the incidence of dengue fever ( $r = -0.228$ ,  $p = 0.01$ ;  $r = -0.225$ ,  $p = 0.01$ ;  $r = -0.235$ ,  $p = 0.01$  and  $r = -0.268$ ,  $p = 0.003$  respectively). A less strong, but significant correlation with rain (positive correlation) and evaporation rate (negative correlation) could be observed ( $r = 0.192$ ,  $p = 0.04$  and  $r = -0.192$ ,  $p = 0.04$  respectively).

**Conclusion:** In 2008, an unexpected and dramatic peak in incidence of dengue fever in Curaçao was observed. In the same year, average- as well as absolute minimum and maximum temperature were significantly lower than in the previous years and were strongly correlated with dengue incidence. As expected from earlier studies, rainfall and evaporation rate were also significantly correlated to dengue incidence.

However, temperature changes showed a stronger correlation with the incidence of dengue fever than rainfall and evaporation rate, suggesting that lower temperatures are an important factor in the development of higher dengue incidence in Curaçao and not merely an epiphenomenon, concurring with the rain period. This is in contrast with results from earlier studies conducted in Mexico and Trinidad.

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Cross-reactive neutralizing antibody response to enterovirus 71 infection in Taiwanese young children

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**Background:** Enterovirus 71 (EV71) is causing life-threatening hand-foot-mouth disease (HFMD) involving neurological and cardiopulmonary complications in Asian children. Phylogenetically, EV71 viruses can be classified into 3 genogroups (A, B and C) and 11 genotypes (A, B1~B5 and C1~C5). In Taiwan, nationwide EV71 epidemics with different predominant genotypes occurred in 1998 (C2), 2000–2001 (B4), 2004–2005 (C4) and 2008 (B5) but the mechanism is not clear. In early 2007, genotype C5 viruses were isolated sporadically and EV71 epidemic did not begin until genotype B5 viruses were detected in late 2007. This study was conducted to measure cross-reactive neutralizing antibody response to EV 71 Infection in Taiwanese young children to explore the mechanism of the genotype replacement in Taiwan, which is critical to the selection of vaccine strains.

**Methods:** We prospectively conducted cohort study to follow up healthy neonates starting from June 2006 and sera were collected from participating children for measuring EV71 neutralizing antibody titers at birth and at 6, 12, 24, and 36 months of age. Throat swabs were collected from participating children developing herpangina or HFMD for virus isolation. Isolated EV71 viruses were genotyped using VP1 gene sequences.

**Results:** In 2008-09, 24 children developed EV71 neutralizing antibody seroconversion, including 11 symptomatic and 13 asymptomatic infections. Five EV71 viruses were isolated from the symptomatic cases and all belongs to genotype B5. These B5 viruses are phylogenetically related to B5 viruses circulating in the South-Eastern Asia recently. Thirty-one post-infection sera collected from the 24 seroconverted children were measured neutralizing antibody titers against genotype A, B4, B5, C2, and C4 viruses. Geometric mean (95% confidence intervals) of neutralizing antibody titers against these viruses were 52 (38-72), 150 (109-205), 234 (176-311), 114 (82-158), and 105 (76-144), respectively. Serological tests show that children infected with B5 viruses have lower neutralizing antibody titers against A, C2 and C4 viruses than B5 virus ( $p < 0.05$ , t-test).

**Conclusion:** Antigenic differences could be detected between enterovirus 71 viruses in different genogroups but not in the same genogroup using children post-infection sera. Significant antigenic differences between B5 and C4 viruses may explain the genogroup replacement occurring in the 2008 epidemic.

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'One World One Health' in practice: Integrating public health and veterinary curricula on emerging infectious diseases in Africa

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**Background:** Research indicates that up to 65% of emerging infectious diseases are zoonotic. This has contributed to the "One World One Health™" paradigm which highlights interactions between humans, domestic and wild animals in a shrinking environment. Though the OWOH theme has gained support within the scientific community, formal training integrating medical, veterinary and public health practices addressing emerging zoonotic disease is rare.

The USAID-funded Stamping Out Pandemic and Avian Influenza (STOP AI) global project is designed to prepare those responsible for the planning, surveillance, response to and prevention of highly pathogenic avian influenza (HPAI). Since inception, STOP AI has drawn upon veterinary and human medicine disciplines, public health, and the social sciences to design its training programs. Through practical field experience, STOP AI determined that effective training required the integration of veterinary, medical and public health aspects.

**Methods:** STOP AI training modules on HPAI epidemiology, risk assessment, surveillance, outbreak response, bio-safety and bio-security were subsequently integrated by teams of veterinary, medical, public health and socio-economics professionals to represent interaction between the disciplines at all stages of planning, surveillance and response to HPAI, e.g. outbreak investigation teams included individuals from both disciplines, trace back and contact tracing considered inter-species virus transmission, etc. Adult education specialists worked with the STOP AI technical team members to create clear messages and mechanisms to convey the information.

**Results:** The integrated STOP AI modules have repeatedly been field-tested with mixed groups of human and veterinary medical practitioners throughout sub-Saharan Africa. STOP AI monitored group response and uptake, and assessed comprehension of the new materials through observation and written evaluations. An integrated core curriculum on HPAI is being finalized which the University of Ghana's School of Public Health plans to offer as a Master's degree course elective in the spring of 2010.

**Conclusion:** The zoonotic nature of emerging diseases has brought the medical and veterinary communities into closer working relationships, but with limited impact on their respective training activities. The STOP AI project in Africa is a leading example of the type of innovative training programs needed to better prepare public health and veterinary practitioners to address emerging infectious diseases.

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Vector-based metagenomics for animal virus surveillance

**T. F. F. Ng**<sup>1</sup>, D. Willner<sup>2</sup>, C. Nilsson<sup>3</sup>, Y. W. Lim<sup>2</sup>, R. Schmieder<sup>2</sup>, B. Chau<sup>2</sup>, Y. Ruan<sup>3</sup>, F. Rohwer<sup>2</sup>, M. Breitbart<sup>1</sup>

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**Background:** Recent epidemics have underscored the importance of non-human viral reservoirs and the devastating impact of emerging viruses on human health. Broad understanding of the animal viruses circulating in a given region is crucial for monitoring emerging diseases, but is hindered by the inability to sample enough individuals and by the difficulty of characterizing previously undescribed viruses. As mosquitoes draw blood from animals and humans and are known to carry a number of viruses, metagenomic sequencing of viral particles purified from mosquitoes enables surveillance of the animal viruses circulating in a given region.

**Methods:** To characterize actively circulating viruses and provide a baseline for detection of emerging viruses, viral metagenomics was performed on mosquitoes captured from San Diego, California. Virus particles were purified from mosquitoes through filtration, chloroform, and nuclease treatment. Nucleic acids were then extracted from the purified viral particles, and subjected to metagenomic sequencing followed by bioinformatic analysis.

**Results:** This study identified a number of previously unknown DNA and RNA viruses from the mosquito virome. Highly diverse novel single-stranded DNA viruses were discovered, with limited amino acid identity (40-63%) to known human anellovirus and animal circovirus genomes in Genbank. One of the circoviruses showed a unique genomic organization with characteristics of several ssDNA viral types. Several closely related virus genomes were recovered from individual samples; however, viruses from different samples varied widely, indicating distinct virus profiles. A number of partial sequences from novel RNA animal viruses were also identified. New RNA viruses characterized from the mosquitoes had only extremely limited amino acid identity to members of the *Bunyaviridae* and *Rhabdoviridae* families.

**Conclusion:** In summary, this study has identified a diversity of novel DNA and RNA viruses, successfully demonstrating that numerous unknown viral species are circulating in mosquitoes. Through the use of vector-based viral metagenomics, the animal viruses present in a given region can be characterized and used as a baseline to monitor emerging viruses of relevance to human and animal health.

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Epidemiological, clinical and zoonotic evidences for the existence of Lyme disease in Central of Mexico

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**Background:** Lyme borreliosis, caused by *Borrelia burgdorferi*, is the most commonly reported vector-borne disease in the United States. Infection is transmitted to humans by *Ixodes* tick-bite, and causes a disease affecting skin, nervous system, heart, and joints. Recent studies in Mexico documented cutaneous clinical cases infected with *B. burgdorferi* and ticks from the Northeast of the country infected with *B. burgdorferi* sensu stricto. Aim: To document *Borrelia* infection in patients with suggestive Lyme disease in Central of Mexico, and to search for the presence of *B. burgdorferi*-infected competent vectors and reservoirs.

**Methods:** We studied 800 patients suggestive of Lyme disease in one Pediatric and one General hospital. Sera, biopsies, and cerebrospinal fluids were tested for infection using immunological, microbiological, and molecular test. Ticks and mice were collected in forest areas, which patients visited, and infection studied by culture and molecular tests.



#### **MEXICAN PATIENT WITH EM**

**Results:** From 800/136 cases (17%) fulfilled the CDC criteria confirming *B. burgdorferi* infection, 88 adults (64.5%) and 48 children (35.5%). Eighty-one presented neurological manifestation (59.6%), 45 cutaneous lesions (33%), and 10 arthritis (7.4%), and 68 reported previous tick exposure (50%). Thirty-six *Peromyscus* sp. and 17 *Neotomodon alstoni* mice were collected in forest areas; five *P. melanotis* were found infested with *Ixodes spinipalpis* ticks. In 16 mice, samples from bladder and articulation were tested; five were culture+ and seven PCR+ for *fla* and *ospA* genes. Amplified *fla* and *ospA* genes from clinical, rodent and tick samples were sequenced and confirmed as *Borrelia burgdorferi* sensu stricto genes.



#### ***Ixodes spinipalpis* tick infesting *Peromyscus* mouse**

**Conclusion:** These studies confirm for the first time the existence of this emerging zoonosis in Latin America, and leaves open the intriguing possibility that this represent an example of the epidemiological transition due to world climate changes.

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Population incidence estimates for hepatitis C virus infection in Egypt

**F. D. Miller**

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**Background:** Egypt has the highest prevalence of antibodies to Hepatitis C virus (HCV) in the world, estimated to be 14.7% by the first national study completed by the Egyptian Demographic Health Survey. Numerous seroprevalence studies dating back to 1993, published various estimates from different communities, mostly rural, had strongly suggested that, Egypt, relative to the other nations of the world, has a HCV epidemic. More importantly, the new national data base provided an opportunity to apply established epidemiologic models to estimate incidence, from the prevalence of anti-HCV antibodies.

**Methods:** Validated mathematical models for estimating incidence from age specific prevalence were used. We reviewed all previous seroprevalence studies and estimated incidence from each study that provided sufficient age specific prevalence required by the models.

**Results:** On review, it was not possible to establish a benchmark for HCV prevalence or incidence prior to the 2008 national study. The modeled incidence from the national study and collectively, from the modeled incidence from the previous seroprevalence studies were, 6/1000 per person per year and 6.57/1000 per person per year, respectively. Projected to the age structure of the Egyptian population, there are and estimated 535,093 new HCV infections per year. An estimated 4,458,691 persons are positive for HCV RNA and infectious.

**Conclusion:** The study demonstrates the urgency to reduce HCV transmission in Egypt.

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Prioritizing US Dengue Fever interventions utilizing remote sensing and predictive modeling

**F. Grant**

Northrop Grumman Corporation, Atlanta, GA, USA

**Background:** Dengue Fever is a mosquito-borne, acute febrile syndrome prevalent in tropical and subtropical areas. Based upon disease surveillance and an examination of remote sensing data from climate satellites, Dengue Fever should be considered a significant emerging global threat and a threat for the continental United States. Planning effective public health interventions around mosquito-borne diseases such as Dengue Fever can be a slow and highly manual process, requiring many hours of time, requiring specialized tools and knowledge not always readily available to local public health organizations. Additionally, it is difficult for low resource environments to identify and prioritize the use of limited public health resources.

**Methods:** An interdisciplinary team of climate and public health scientists, explored the use of remote sensing data, climate modeling downscaling, GIS, and predictive modeling as adjunctive tools for effective public health planning. Ensemble modeling – multiple runs of regional downscaled climate models – combined with probabilistic climate data interpretation and population health data were utilized as variables for identifying potential public health intervention priorities.

**Results:** The outcome was a GIS visualization which suggested priorities for public health intervention planning. The visualization suggested climate and age-stratified population health priorities to make best use of limited public health resources in vector monitoring, case surveillance and reporting, and for public information alerts regarding Dengue Fever disease hazards.

**Conclusion:** Although many fundamental aspects of public health action have been identified, there is a gap in the ability to translate the global information developed from remote sensing data into specific, effective, locally actionable knowledge. Addressing the connections between global information and local public health intervention can be accelerated by integrating remote sensing information, climate downscaling data, and models. This investigation supported the theoretical value of interdisciplinary collaboration of Regional Climate Knowledge Integration Centers (CKICs) as an adjunctive resource to local public health. Additional prototypes and decision aids are needed to begin exploring how to best integrate and use remote sensing data to benefit local public health practice needs.

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Prevalence and presentation of Lassa fever in Nigerian children

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**Background:** Although up to 6% of febrile hospital patients in endemic areas in Nigeria may have Lassa fever (LF), and there may be variations in the presentation and outcome from that in other West African countries due to multiple viral strains, there is a paucity of reports on the clinical manifestations and few, if any reports on LF in children. This study highlights the prevalence, presentation and outcome in Nigerian children.

**Methods:** Review of consecutive cases of RT-PCR confirmed LF admitted from 09/01/2008 – 10/31/2009 at the CHER, Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria.

**Results:** 22 (1.6%) of 1380 admissions had LF. Eight were <2 yrs of age, 5 >2-5 yrs and 9 >5 yrs. The M:F ratio in children > 2 yrs of age was higher (11M:3F versus 2M:6F in younger children;  $p = 0.024$ ). The duration of fever on admission was 1-3 days in 5 children, 4-7 in 8, 8-14 in 8 and >14 in 1. The other common symptoms included jaundice 11, vomiting 10, convulsions and/or loss of consciousness (coma) 8, pain 5, bleeding 5, passage of coke-coloured urine 4 and cough 4 while the common signs were a toxic appearance 12, hepatomegaly 18 (with splenomegaly in 7), jaundice and pallor 10, respiratory distress 9 and coma 5. LF was the provisional admission diagnosis and differential in 5 and 7 children, respectively. 15 children (71%) survived and 6 (29%) died while 1 was discharged against advice. Convulsions or coma were the only presentations which significantly increased the case fatality rate (5/8 with versus 1/13 without convulsions or coma died;  $p = 0.015$ , Risk Ratio (95% CI) = 8.12 (91.15, 57.56)).

**Conclusion:** LF is an important problem in febrile Nigerian children and jaundice/pallor, convulsions or coma and hepatosplenomegaly are common clinical manifestations unlike in other series. Presentations with acute fever (<7 days) may be more frequent than with prolonged fever and convulsions or coma are associated with a poor outcome. Although these results should be validated in a prospective study, they have important implications for the evaluation and treatment of febrile children in endemic areas.

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New lessons from a case series review of Lassa fever in pregnancy

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**Background:** In endemic communities, 25% of maternal mortality is due to Lassa fever (LF) with CFRs of 30-75% for the mother and 95% for the fetus. The extra challenges in Management are to differentiate the features of LF from those of pregnancy and its complications as well as address the presence and the effect of the fetus. The erstwhile recommendation that uterine evacuation is required to improve maternal outcome raises ethical issues when the fetus is alive. The management in such circumstances therefore needs further review particularly as some reports indicate that outcome may be independent of the modality adopted. This review was done to provide further guidance in management.

**Methods:** This was a descriptive case-series study of the presentation and outcome of 7 cases of RT-PCR confirmed LF in pregnancy managed at the Irrua Specialist Teaching Hospital, Irrua, Nigeria between February 2008 and August 2009.

**Results:** The patients were aged 23-28 years with a parity of 0-4 and fetuses of gestational ages 7-34 weeks. Abdominal pain and bleeding from multiple sites were present in 5 patients and retrosternal pain, sore throat, difficulty in swallowing and seizures in 4. Other symptoms were oliguria in 3, breast pain in 2 and preterm contractions in 2 while one had bilateral deafness. There were 4 maternal deaths, all with IUFDs as well. The average duration of symptoms before presentation was 4 days in survivors and 8 days in those who died. All 4 mothers with seizures died. All the three survivors had live fetuses at discharge; two subsequently had normal term deliveries after 8 and 12 weeks respectively, while the third who did not receive treatment with ribavirin while on admission had an IUFD 8 weeks later.

**Conclusion:** The occurrence of breast pain and tenderness, preterm contractions and bleeding may be distinctive features of LF in pregnancy. The clinical finding of a viable fetus may be predictive of improved maternal and fetal outcome. Evacuation of the uterus in such circumstances should not be a first option in management. Rather conservative management with ribavirin treatment may be worthwhile. These observations need to be confirmed prospectively.

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Neuroimmunological findings from the first report of *Angiostrongylus cantonensis* outbreak in Ecuador

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**Background:** *Angiostrongylus cantonensis* meningoencephalitis was recently reported in South America. The aim of this paper is to present a neuroimmunological study of the first report of two outbreaks in subtropical regions from Ecuador.

**Methods:** Eight young adults from two different outbreaks were studied. Simultaneous blood and cerebrospinal fluid samples were taken. IgA, IgM, IgG and albumin were quantified by radial immunodiffusion. Corresponding reibergrams were employed. The diagnosis of *A. cantonensis* meningoencephalitis was based on a previous antecedent of raw snail consumption, its symptoms and the cerebrospinal fluid characteristics.

**Results:** A moderate elevation of protein content in cerebrospinal fluid with 7, 7 and 26% of mean eosinophilia in serum and cerebrospinal fluid were respectively observed. Three immunoglobulins synthesis pattern was the most frequent one and IgM was presented in all the different patterns.

**Conclusion:** The reported patterns may be useful epidemiologically. The neuroimmunological findings contribute to improve and confirm the diagnosis of this disease now spreading over South America

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Pericarditis caused by spatype t011 MRSA in a butcher; an occupational hazard

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**Background:** A 39 year old man from Turkish descent was admitted to our cardiology ward for evaluation of general malaise, fever and pericardial calcification with effusion. Diagnostic pericardiocentesis was performed and fluid was sent to the microbiology laboratory. Cultures revealed growth of methicillin resistant Staphylococcus aureus (MRSA) spatype t011, indicating veterinarian MRSA origin. Additionally Mycobacterium tuberculosis was cultured from the same material. Vancomycin treatment was started and the patient was transferred to the pulmonology department for further evaluation of his mycobacterial infection and for anti-tuberculosis treatment.

**Methods:** MRSA of veterinarian origin is an emerging pathogen in the Netherlands causing a wide variety of infections. The majority of pigs and calves in our country is MRSA colonized; a quarter of the farmers is also MRSA positive. About eighty percent of meat from pigs and calves is MRSA colonized. Veterinarian MRSA strains almost all have spatype t011. Our patient was working in a large abattoir; his job was to carry large heaps of meat, resting on his chest, from one place to another.

**Results:** Our hypothesis is that our patient was MRSA colonized due to his profession. During pericardiocentesis the cardiologist perceived a suctioning sound. Tuberculous pericarditis causes pericardial calcification. During systolic contractions a vacuum will form in the pericardial space caused by non-compliance of the pericardium due to calcification. The vacuum causes inward airflow transporting airborne microorganisms to the pericardial space. Despite skin disinfection airflow during pericardiocentesis probably introduced MRSA from the patient chest. Nosocomial MRSA acquisition was ruled out; none of the healthcare workers involved was MRSA positive. Furthermore human to human spread of veterinarian MRSA is extremely rare.

**Conclusion:** We present a case of occupational MRSA acquisition which led to MRSA pericarditis by pericardiocentesis in a patient with preexisting tuberculous pericardial calcification.

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Skin and soft-tissue infections caused by community-associated methicillin-resistant *Staphylococcus aureus*

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**Background:** Community-associated methicillin-resistant *Staphylococcus aureus*(CA-MRSA) is an increasingly common cause of substantial morbidity worldwide. In several Latin American countries-including our own- the incidence of CA-MRSA infections has dramatically increased although it remains underdiagnosed in many cases. Skin and soft-tissue involvement are the most common, accounting for 85 to 95% of infections caused by this microorganism.

The aim was to describe the epidemiological, clinical and prognostic features of skin and soft-tissue infections caused by CA-MRSA treated in general hospital.

**Methods:** We included all patients with forunculosis and skin abscess by CA-MRSA treated in the infectology service between may of 2008 and september of 2009. CA-MRSA association was defined by epidemiological (community arousalment) and microbiological features (oxacillin resistant isolates). The following variables of interest were analysed age, sex, risk factors, number of lesions, surgical drainage required, recurrence, presence of familiar forunculosis, empirical and post-isolation treatment, and CA-MRSA nasal carriers.

**Results:** 23 patients were included. Men: 56%. Mean age: 32 years (3-69). Only one patient had classic risk factors (health care center worker). 52% (n=12) had a sole lesion, while the others presented between 2 and 5. Only 6 cases required surgical drainage. Recurrence occurred in 21% (n=5) and the majority, 74% (n=17), had a history of familiar forunculosis. Empirical treatment had been given to 20 patients; being inadequate in 16 (80%) (first generation cephalosporin or amoxicillin plus clavulanic). Antibiotic treatment after isolation was: Trimethoprim/Sulfamethoxazole (n=18), clindamycin (n=4) y Vancomycin plus rifampicin (n=1). Nasal sample was taken in 11 of 23, being positive only in 3 of them (27%).

**Conclusion:** Patients with forunculosis CA-MRSA related were generally men of adult age and lacked risk factors. There was a large number of family outbreaks and nasal carriers were scarce, suggesting infection was due to interpersonal contact rather than state of carrier. Inadequate empirical treatment was very common.

**Comment:** It is of paramount importante to bear in mind CA-MRSA as a posible cause of skin and soft-tissue infections in the community -even when risk factors are not present and particularly in a family outbreak- in order to start adequate empirical treatment and drain surgically if needed.

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The emergence of *Clostridium difficile* NAP1 hypervirulent strain in Latin America

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<sup>1</sup>University of Costa Rica, San José, Costa Rica, <sup>2</sup>National Microbiology Laboratory, Winnipeg, MB, Canada

**Background:** The NAP1 strain of *Clostridium difficile* have been linked to nosocomial outbreaks of antibiotic-associated diarrhea (AAD) in North American and European countries. We found these strains, together with seven additional PFGE patterns, among 37 isolates recently recovered from patients with AAD in a Costa Rican hospital

**Methods:** The isolates were recovered by inoculating a loopful of diarrheic stool samples onto CCFA agar plates (Oxoid®). They were identified with the rapid ID32A (bioMérieux®) and a PCR reaction targeting the *tpi*. We typed the isolates using pulsed-field gel electrophoresis (PFGE) with *Sma*I and amplified fragments of the *tcdA*, *tcdB*, *tcdC*, and *cdtB* genes using PCR. Minimal inhibitory concentrations (MICs) of clindamycin, metronidazole, vancomycin, moxifloxacin, ciprofloxacin, and amoxicillin-clavulanate were determined using E-test strips.

**Results:** Nine different PFGE types were identified. All isolates were positive for *tcdA* and *tcdB* and susceptible to vancomycin and metronidazole, which are the first antibiotics to be prescribed for this type of infections. They were also susceptible to amoxicillin/clavulanate.

More than half the isolates exhibited the macro-restriction pattern of the NAP1 strain (54%). Ten NAP1 were intermediate (50%) and another nine resistant to clindamycin (45%). One clindamycin-resistant NAP1 strain had a MIC of >256 µg-ml<sup>-1</sup>. All NAP1 isolates were highly resistant to the two fluoroquinolones (>32 µg-ml<sup>-1</sup>).

The 13 isolates with novel PFGE patterns 447, 448, 449, and 452 clustered together. These isolates lacked the binary toxin but had a deletion in *tcdC*. They were without exception categorized as highly resistant to clindamycin, moxifloxacin and ciprofloxacin. All the NAP1 isolates had the *cdtB* and a deletion in *tcdC*. One isolate with the pattern 447, and the isolates with the patterns 449 and 452 exhibited the highest MICs to vancomycin (2-4 µg-ml<sup>-1</sup>).

The isolates with the PFGE patterns 100, 450, 451 (NAP2) and 247 (NAP9) were negative for *cdtB* and did not have deletions in *tcdC*. The strains with the patterns 100, 450, and 247 (NAP9) were resistant to clindamycin, moxifloxacin and ciprofloxacin.

**Conclusion:** The finding of the NAP1 strain in Latin American countries is novel and deserves attention from infectious disease specialists and epidemiologists to prevent its dissemination.

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Differential patterns of tuberculosis disease between immigrant and native Spanish patients  
**P. Merino**, A. Vanegas, R. Cias, I. Gestoso, J. Picazo  
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**Background:** The increase of the tuberculosis (TB) cases in Spain has been observed in the last years respect to the European Union. This fact correlates with the high immigrants waves coming from countries with high-incidence of the disease. The knowledge of the possible differential characteristics of the disease depending on its origin could be useful for the correct diagnosis and treatment.

**Methods:** Patients newly identified with *M.tuberculosis* culture positive in January 2004 to July 2009 were included. Drug susceptibility testing was performed on at least one isolate per patient by using a radiometric BACTEC 460 system (Becton Dickinson Diagnostic Systems).

**Results:** 193 (57,1%) out of 338 patients with positive culture were from Spain and the remaining 145 (42,9%) were immigrants. Taking into account the whole group, the resistance to isoniazid, and rifampicin was 4,9% and 1,9%, respectively and the multiresistance (isoniazid and rifampicin) was 1,9%. These percentages totally correlate with the immigrant group. Pulmonar TB was the most frequent form of the disease (80%) independently of the patient origin. Miliar TB was more frequent in Spanish patients (statistically non significant). Renal failure and haematology diseases are the differential risk factors in developing TB in Spaniards respect to the immigrant group.

**Conclusion:** This study demonstrates that the immigrants presented more resistance than the Spanish group and they had less risk factors to develop TB. Therefore, the origin of the patients is important in the diagnosis and treatment of the disease.

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Imported cases of Chikungunya and Dengue fever in Emilia Romagna region, Italy

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**Background:** Chikungunya virus (CHIKV) was introduced in Emilia-Romagna, Italy, in 2007, when a man highly viraemic came back from India. A local transmission occurred involving 205 cases and CHIKV RNA was detected in human blood and in *Ae. albopictus* mosquitoes. *Ae. Albopictus*, which is well-established in the region, is competent also for the transmission of Dengue virus (DENV) and the prevention of CHIKV and DENV was based on mosquitoes' control and on early detection of human cases. A patient with a clinical suspicion and positive epidemiological criteria (travelling to an endemic or epidemic area for these diseases) was considered a suspected case and the laboratory diagnosis was performed. From April to November 2008 eight patients resulted serologically positive and one was viraemic for CHIKV, over 157 cases. About DENV, NS1 antigen was detected in three patients and six were serologically positive, over 50 suspected cases. Because of these data the surveillance system was carried on in order to identify each imported case.

**Methods:** From December 2008 to October 2009, 35 suspected cases of CHIKV were analyzed; 22 were tested only for the detection of IgM/IgG antibodies by immunofluorescence, 3 for CHIKV RNA by Real Time RT-PCR and 10 samples were evaluated by both tests. Moreover, 47 suspected cases of Dengue were collected, 23 sera were analyzed by immunofluorescence for the detection of antibody response, 5 were tested for NS1 antigen detection and 19 samples were evaluated by both tests.

**Results:** About CHIKV, one patient was IgM/IgG positive and two patients IgG positive; none was positive in PCR. About DENV, in the sera of 6 patients NS1 antigen was detected. Two samples NS1 negative were respectively IgM/IgG positive and IgG positive.

**Conclusion:** From December 2008 to October 2009 a lower number of suspected and positive cases of Chikungunya and Dengue fever were observed in comparison to the last year. Four of the six DENV viraemic patients were collected on January and February when *Ae. Albopictus* in Emilia Romagna is not active. In conclusion, we observed a decrease of Dengue fever imported cases which could be a risk for a local outbreak.

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Importance of the animal/human interface in events of international concern for the Americas

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**Background:** Diseases have increased their potential to cross geopolitical boundaries through international travel and trade. Zoonoses represent 75% of human pathogens emerging during the past decade. The International Health Regulations (IHR) are a binding global legal instrument that includes all the World Health Organization (WHO) Member States, which aim to ensure public health through prevention and respond to acute public health risks. The objective of this study is to understand the importance of diseases common to man and animals in events of public health importance reported to, or identified for follow up by the Organization, in an effort to reduce the risk of infectious diseases at the human/animal interface.

**Methods:** This study analyzes events recorded in the databases for the Americas region from the implementation of the IHR (June 2007) until the end of 2008. The main source of data was the WHO Event Management System (EMS). The criterion for inclusion in human/animal interface was, "considered a zoonoses or communicable disease common to man and animals," using the third edition of the book, *Zoonoses and Communicable Diseases Common to Man and Animals* published by the Pan American Health Organization. Subgroups were also created and percentages were calculated.

**Results:** During the period studied, 110 events were recorded in the EMS for the region of the Americas. 86/110 were communicable diseases. Among these, 77/110 (70.0%) were zoonoses and communicable diseases common to man and animals (human/animal interface); 9/110 (8.2%) were considered not common to animals; 16/110 (14.5%) were syndromes with unknown etiologies; 8/110 (7.3%) were related to products. Among the 77 events considered human/animal interface related, 48 were designated "substantiated" at time of event closure.

**Conclusion:** This analysis demonstrates approximately 70% of events are either zoonoses or communicable diseases common to man and animals, confirming previous publications and the importance of the animal/human interface. Analysis also supports "One World, One Health" framework, and the necessity of collaboration between science and other sectors. An early warning system that quickly identifies events that pose a risk to international public health may also support the countries in identifying events that are exceeding expected numbers and require an emergency response.

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Dengue fever in a Southeastern region of Brazil. Ten years period (1997-2007) clinical and epidemiological retrospective study.

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**Background:** Dengue is viral endemic/epidemic mosquito-borne disease, with worldwide distribution, and with increasing incidence in many tropical countries. Clinically, ranges from asymptomatic or mild cases to severe forms. It is estimated that annually more than 50 millions of infections, 500.000 cases of dengue hemorrhagic fever (DHF), and 22.000 deaths occurs worldwide. In Brazil, where more than 60% of notified cases of dengue in the American continent occurs and there are circulating the serotypes 1 (DEN1), 2 (DEN2), and 3 (DEN3), there have been seen annually successive epidemics and an increasing number of hemorrhagic dengue fever cases.

**Methods:** Retrospective study based on data collected from epidemiological files of confirmed dengue cases notified during epidemics occurred in the municipality of Sumaré, São Paulo State, Brazil, in the period between 1997 and 2007.

**Results:** There were analyzed a total of 4818 cases (56% female; median age 31 years [range 0-89 years]); of them, 4028 (83.6%) were notified during an epidemic in 2007. A reduced number of studied cases were distributed in the other years: 1997 (1.1%); 2002 (5.2%); 2003(2.3%); 2005(0.8%); 2006(6.9%). The most frequent clinical signs were fever (97%), headache(94%), myalgia(87%), malaise(85%), ocular pain(78%), nausea/vomiting(69%), arthralgia(68%), rash(42%), diarrhea(36%). Petechiae(10%), epistaxis(6%), gingival hemorrhage(3%), hematuria(1%), vaginal bleeding(1%), gastrointestinal bleeding(0,37%) were the spontaneous hemorrhagic phenomena observed; tourniquet test was positive in 9% of examined cases. Among the warning signs associated with severity, abdominal pain(33%), arterial hypotension(4%), hepatomegaly with pain(2%), and neurological manifestations(2%) were the most observed. The majority of cases (99.17%) were classified as dengue classic; while a small proportion of dengue hemorrhagic fever [DHF] (0.13%) and dengue with complications (0.13%), there was not observed dengue shock syndrome [SSD] cases.

**Conclusion:** Since its reemergence in the beginning of 1980's, dengue remains one of the most important public health problem in Brazil. In the present study the number of DHF was not significant. One possible explanation could be associated to the difficulty of fulfill the WHO criteria. As observed in the present study, during an epidemic as occurred in 2007 the increased number of symptomatic suspected cases usually impacts markedly in the whole health system.

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The potential of social networks for early warning and outbreak detection systems: the swine flu Twitter study

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**Background:** The recent swine flu outbreak in April-May 2009 truly demonstrated the potential of these media for early warning systems. Web 2.0 has generated a great interest recently as a possible media for early warning system for outbreak detection and epidemic intelligence (EI). Traditional systems such as GPHIN, Medisys are well established and used by ECDC/WHO on a daily bases, however, there has been recent interest in the ability to estimate flu activity via aggregating online search queries for keywords relating to flu and its symptoms by commercial companies like Google. However, the search data remain proprietary and therefore not useful for research. However, content of social networks such as twitter are in public domain and therefore available.

**Methods:** Twitter, a micro-blogging service that allows people to post and read other users' 140 character messages currently has over 15 million unique users per month. Twitter allow third parties to search user messages using an open source API and return the text along with information from the poster's profile, such as their location, in a format that can be easily stored and analysed.

We searched and collected over 1 million tweets in the period from May until August 2009 and carry on collecting them on a minute bases to understand public concerns, keywords used and the profile of users who discuss these topics on the web.

Word	Frequency	Word	Frequency
Flu	138,260	New	7,668
Swine	99,179	News	6,498
Have	13,534	Confirmed	6,456
Cases	13,300	Just	6,373
H1N1	9,134	People	5,820
Has	8,010	Case	5,647

### Frequencies of the most popular words found in all tweets

**Results:** We found over 1 million tweets reporting flu related illnesses and symptoms via Twitter in this period. Most popular words in tweets were these (frequency in brackets): flu (138, 260), Swine (99, 179), Have (13, 534), Cases (13, 300), H1N1 (9, 134), Has (8, 010) and others.

The actual sentence "I have swine flu" appeared 2, 888 times and "I have flu" 1,530 times. Further evaluation of the collected tweets, semantic relationship of keywords, geo-location of posters is underway and will be presented at the conference.

I word to the left		I word to the right	
Word	Frequency	Word	Frequency
Swine	96,651	Http	6,598
The	5,701	Cases	6,194
H1n1	5,225	Case	2,210
Bird	1,425	Death	2,001
New	1,304	Virus	1,411
Pig	1,164	Outbreak	1,321
Man	720	H1n1	1,147
Stomach	510	Spreads	927
Regular	426	Lol	924
Flu-bird	319	Deaths	912

### Collocation of words, one word to the right and the left of the word "flu"

**Conclusion:** The potential of social networking system for early warning systems and for better understanding public concerns about their health is enormous, however, further research is required to reveal the underlying principles and implement adequate integration with existing healthcare services.

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The meanings of resistance in a Brazilian dengue fever epidemic

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**Background:** The wave of dengue fever in Rio de Janeiro, Brazil in 2008 was "calamitous" according to locals, a comment on the epidemic's alarming and unprecedented proportions: over 300,000 people were stricken with the virus in Rio state alone, overwhelming hospitals and community clinics, and exposing urban and public health infrastructural deficiencies. Some observers said that local community resistance to government interventions impeded control of the outbreak. Public health agents, for their part, were said to resist carrying out their charges when they refused to work in dangerous, drug-lord controlled "war-zone" *favelas*, or shantytowns. Meanwhile, medical and epidemiological understandings of resistance foregrounded the role of immunology and were fraught with contested racial-genetic complexities.

This paper poses the anthropological question of what resistance means in the Brazilian dengue context, and how various interpretations of resistance affect projects to reconceive dengue prevention as a horizontally-integrated enterprise.

**Methods:** The paper employs ethnographic methods, including participant-observation and informal and structured informant interviews, both among Ministry of Health surveillance employees and local resident groups. Media narrative analysis is also used to frame the larger discourse that shaped public understandings.

**Results:** Results of the study showed that the widely circulated images of local resistance to dengue control served as a major justification for launching nascent programs that rely less on government dispatches and more on the enlistment of local community leaders in quotidian prevention. On the other hand, prevailing notions about active non-compliance in poor neighborhoods were not supported by the study's findings: resistance to state-conducted control measures was in fact more frequently observed in affluent condominium neighborhoods. Finally, opinions about racial-genetic theories of resistance were considered to be more useful in vaccine research than in community-based interventions.

**Conclusion:** The paper concludes that the variously interpreted concepts of resistance related to dengue in Brazil in 2008 obscured an overarching social resistance to the enactment of just policies that would allow for a more equitable distribution of infrastructural resources in Brazil. If brought into dialogue, competing notions of resistance could become isomorphic to the ultimate goal of improving the social and ecological circumstances that underlie dengue transmission dynamics.

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Assessment of prognosis of tetanus and its related factors among Albanian adults

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**Background:** Tetanus diagnosis is mainly a clinical one. Identification of prognostic factors is very important to institute effective and prompt treatment of this disease. The aim of this study is to assess the associated factors with prognosis of tetanus among the adult population of Albania.

**Methods:** All reported cases of tetanus (N=64) hospitalized in the regional hospitals of Tirana (the Albanian capital), Shkodra (main district in north Albania) and Korca (one of the main districts in Southeast Albania) for the period 1984-2004. Four patients were excluded from the analysis due to missing information on prognosis. Data on prognosis-related factors included age, sex, incubation period, onset period, as well as location and type of wounds. Multivariable-adjusted binary logistic regression was used to assess the independent association of prognosis (survival vs. death) with covariates.

**Results:** The overall case-fatality rate was 38.6%. It was higher among older individuals ( $P < 0.01$ ), and in those with a shorter incubation period ( $< 11$  days;  $P < 0.01$ ) and with infected wounds ( $P = 0.03$ ). There was no association, however, with location of wounds. Upon simultaneous adjustment for all covariates, age ( $> 50$  years vs.  $< 50$  years) and a shorter incubation period ( $< 11$  days vs. 11-20 days) were positively related to case-fatality (OR=3.4, 95%CI=1.23-5.13 and OR=2.41, 95%CI=1.19-4.37, respectively), whereas the association with infected wounds was not statistically significant any further ( $P = 0.13$ ).

**Conclusion:** Age, incubation period and type of wound are important factors related to prognosis of tetanus in Albanian adults. Health care practitioners should actively seek information on prognostic characteristics of patients with tetanus in order to institute effective and prompt treatment of this disease.

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Thrombocytopenia in murine typhus: A study of 161 cases  
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<sup>1</sup>CHANIA, Greece, <sup>2</sup>SAINT GEORGE GENERAL HOSPITAL CHANIA GREECE, CHANIA, Greece

**Background:** According to the international literature thrombocytopenia is frequent in murine typhus.

**Methods:** One hundred and sixty- one patients with compatible clinical status of murine typhus and high serological titers of antibodies against Rickettsia typhi, were studied from our team, during a period of time between January 1993 and December 2007. Three blood samples were obtained from each patient for the study of their thrombocytopenia. The first sample was obtained on admission, approximately 9 days after the onset of the disease. The second sample approximately two weeks after the first. The third sample, taken from the one third of the patients, was obtained one month after the second. On admission (first sample) 88/161 patients (54.6%) presented thrombocytopenia and the median value of platelets was 141x10<sup>3</sup> /ml respectively. Two weeks later (second sample) 23/147 patients (15.3%) presented thrombocytopenia. The median value of platelets was 247x10<sup>3</sup> /ml respectively. One month later (third sample) 2/42 patients (4.8%) presented thrombocytopenia and the median value of platelets was 224.5x10<sup>3</sup> /ml respectively.

**Results:** Our study showed that thrombocytopenia is frequent in murine typhus on admission of the patients to the hospitals. The number of platelets, according to our results, returns to normal value after almost a month of the onset of the infection.

**Conclusion:** Thrombocytopenia returns to normal values after a month of the onset of the infection.

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Prevalence of neonatal conjunctivitis due to *Chlamydia trachomatis* in two hospitals in Iran  
**S. rafiei tabatabaei**, S. A. Afjeiee , A. Karimi , F. Fallah, A. Tavakkoly Fard, N. Tahami Zanjani ,  
R. Radmanesh ahsani, A. R. Shamshiri  
Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of

**Background:** One of the most common bacterial infections causing ophthalmia neonatorum is *Chlamydia trachomatis*. Very few studies have been done in Iran to determine the prevalence of *Chlamydia trachomatis* causing ophthalmia neonatorum using cell culture and polymerase chain reaction methods. This study aimed to evaluate the prevalence of neonatal chlamydial conjunctivitis by these methods, in two hospitals, Tehran, Iran.

**Methods:** From March 2008 to May 2009, of the 2253 neonates, 241 (10.7%) with clinical findings of conjunctivitis were included in this study. A total of 241 conjunctival swabs were investigated by cell culture (as the gold standard test), polymerase chain reaction and Giemsa staining.

**Results:** Cell cultures were positive for *Chlamydia trachomatis* in 31 (12.9%) neonates. Also *Chlamydia trachomatis* was positive in 40(16.6%) and 18(7.5%) neonates by polymerase chain reaction and Giemsa staining respectively. The sensitivities of polymerase chain reaction and Giemsa staining were 100% and 58.1% respectively.



**Conclusion:** Regarding to high prevalence of neonatal chlamydial conjunctivitis by cell culture, and high sensitivity and specificity (100% and 95.7% respectively) of polymerase chain reaction in the present study, polymerase chain reaction can be considered as a proper diagnostic method for detection of *Chlamydia trachomatis*.

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Invasive *Streptococcus pneumoniae* serotypes associated with in-patient and out-patient isolates from the United States

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**Background:** *Streptococcus pneumoniae* (SPN) is a major cause of invasive diseases and upper respiratory tract infections. Introduction of the pneumococcal conjugate 7-valent vaccine (PV7) into the US childhood vaccine schedule in 2000 has significantly reduced invasive pneumococcal disease in children and adults, with concurrent reduction in the seven vaccine serotypes. Consistent monitoring of possible replacement serotypes is essential to determine possible antibiotic resistance patterns as certain serotypes are more closely associated with antibiotic resistance, as well as to determine targets for future vaccines. In this study we evaluate the serotypes of invasive SPN isolates from in-patients and out-patients from 2004.

**Methods:** The capsular serotypes of 275 invasive SPN isolates collected in the US through the Tigecycline Evaluation Surveillance Trial were determined using sequential multiplex PCR and confirmed using the Quellung reaction. Invasive isolates were defined as those from normally sterile sites, such as blood, CSF and other body fluid.

**Results:** In the following table.

	Total N	PV7 types (%)	Non-PV7 types
			Serotypes (% total non-PV7)
In-patient	163	31 (19)	19A(23), 7(5),33(5),3(5),22F(5),6A(4), 12(4) ,others(42) ,nt*(7)
Out-patient	111	24 (22)	19A(13),3(7),22F(7),11(6),15(6),35(6), 4(5), 7(5),12(5),others(40)

\*nt = non-typeable

**Conclusion:** While PV7 serotypes have declined since 2000, in 2004 approximately 20% of the invasive isolates from this study were from the seven vaccine types (4, 6B, 9V, 14, 18C, 19F, 23F). In-patients and out-patients were equally likely to carry PV7 serotypes. Serotype 19A was the most common serotype in both in- and out-patients, which is cause for concern as this serotype is often non-susceptible to penicillin and erythromycin. In-patients were more likely to carry non-typeable isolates, while equal percentages of isolates from both groups were non-encapsulated. Continued monitoring of post-vaccine serotype trends will be vital to the management of pneumococcal disease.

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Clinical and laboratory test follow up of patients with severe leptospirosis, after hospital discharge  
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**Background:** Although the acute phase disease is well described in leptospirosis, few studies have focused on the follow up after Hospital discharge. Thus, our knowledge is still insufficient on how frequent are some late complications or what is the rate of remission of laboratory markers of disease such kidney function tests, platelet count or liver enzymes. We investigated the frequency of clinical manifestations and the laboratory test abnormalities in patients who had severe leptospirosis at the ambulatory care level, after discharge.

**Methods:** Patients who had severe disease requiring inpatient were asked to return to the reference Hospital Emilio Ribas after discharge. The mean interval between Hospital discharge and the ambulatory visit was  $24.7 \pm 11.1$  days (mean  $\pm$  SD). Clinical and laboratory data were collected.

**Results:** Thirty-nine consecutive patients were enrolled. Most patients were males (85%, 35/39) and the mean age was  $35.5 \pm 17.6$  years. The mean period of hospitalization was  $11.5 \pm 7.0$  days. During hospitalization, the patients presented the following clinical manifestations or complications: pulmonary involvement (26%, 10/39), jaundice (59%, 23/39), renal failure (44%, 17/39, six requiring dialysis), and shock (8%, 3/39). Twelve (31%) required ICU support. The following abnormal laboratory test results were obtained in the acute phase disease: serum creatinine  $> 3$  mg/dl (36%, 14/39), Bilirubin  $> 6$  mg/dl (41%, 16/39), platelet count  $< 70,000$  units per mm<sup>3</sup> (21%, 8/39), aspartate aminotransferase  $> 100$  UI/L (29%, 9/31) and alanine aminotransferase  $> 100$  UI/L (26%, 8/31). At the ambulatory visit, only minor complaints were reported: weakness (n=7), headache (n=3), myalgia (n=1) while jaundice persisted three cases. Uveitis was not detected. No patient had platelet count lower than 150,000 units/mm<sup>3</sup>. No patient had serum creatinine  $> 3$  mg/dl or serum Bilirubin  $> 6$  mg/dl at the ambulatory visit. Serum Alanine aminotransferase  $> 100$  UI/L persisted in two patients.

**Conclusion:** Our results confirm the general concept that leptospirosis usually resolves without long lasting effects. Further studies are recommended to evaluate if elevation of liver enzymes, a laboratory test abnormality already associated with poor prognosis in previous studies, improves in a lower rate when compared to other manifestations.

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The detection of antibodies directed against specific antigens of *Borrelia burgdorferi sensu lato* (*B. burgdorferi* s.s., *B. afzelii*, *B. garinii*, *B. spielmanii*) in patients with borreliosis in Eastern Poland

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**Background:** The most common *Borrelia* genospecies involved in the etiology of Lyme disease in Eastern Europe are: *B. burgdorferi* s.s., *B. garinii*, and *B. afzelii*. However, the role of other genospecies including *B. spielmanii* has also been suggested.

In view of a paucity of epidemiologic data concerning the role of *B. spielmanii* as the potential causative agent of Lyme disease in Poland, investigation towards identification of patients with serologic evidence of infection caused by this *Borrelia* genospecies was undertaken.

**Methods:** The study group comprised 41 patients from Eastern Poland with borreliosis (age range: 19-65 yrs). Detection of IgG and IgM antibodies to *B. burgdorferi* s.l. was performed using test (Mikrogen) which included the following antigens for IgM and IgG: OspC and p18 (*B. burgdorferi* s.s., *B. afzelii*, *B. garinii*, *B. spielmanii*), VlsE, p100, p58, p41, p39, and OspA.

**Results:** The results of the study indicate that the simultaneous presence of IgM antibodies against OspC protein characteristic of all four genospecies (*B. burgdorferi* s.s., *B. afzelii*, *B. garinii*, and *B. spielmanii*) was detected in 36 patients with clinical manifestations of borreliosis. The presence of IgM antibodies to OspC antigen of *B. spielmanii* accompanied by negative results for the presence of antibodies against antigens of the remaining genospecies was detected in one patient.

The IgG antibodies against *B. burgdorferi* s.l. were observed with varying frequency:

- anti- VlsE in 22 patients,
- anti-OspC for four genospecies: *B. burgdorferi* s.s., *B. afzelii*, *B. garinii* and *B. spielmanii* in 9 patients,
- anti-p18 for single genospecies, more often for *B. afzelii* (4 patients) and *B. spielmanii* (5 patients).

**Conclusion:** The results of the study suggest the possibility of co-infections with several genospecies of *B. burgdorferi* s.l. evidenced by the presence of IgM antibodies to OspC of *B. burgdorferi* s.s., *B. afzelii*, *B. garinii*, and *B. spielmanii*.

The detection of antibodies directed against specific *B. spielmanii* antigens suggests that this microorganism may be responsible for triggering borreliosis both as a single etiologic agent and in co-infection with other *Borrelia* genospecies.

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Enterococcal bone and joint infections: A working group experience along 17 years

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**Background:** Enterococcal bone and joint infections (EBJI) are an uncommon disease and the optimal treatment and outcome have been poorly defined.

The objective was to analyze the outcome of EBJI episodes (ep.) according to patients' characteristics and microbiological features, considering type of surgery implemented and antibiotic treatment chosen.

**Methods:** We performed a retrospective cohort study of patients with EBJI, treated by our working group from 1/91 to 3/09. Categorical variables are presented as percentages and continuous variables as median and interquartile range (IQR). Differences were considered statistically significant at  $p \leq 0.05$ .

**Results:** We evaluated 35 EBJI ep. occurred in 34 patients accounting for 3.3% of all BJI (35/1071) seen in the period considered.

Median age 63 years old (range IQR 40-72), 63% female patients.

Comorbidities (diabetes, peripheral vascular disease, cancer) were found in 31.4%.

Twenty eight ep. (80%) were implants associated infections (IAI) (15 prosthetic joint, 13 fractures with internal fixation) and 7 (20%) osteomyelitis (OM; 86% chronic).

Five ep. (14.3%) had polymicrobial cultures (4 *P. aeruginosa*, 1 MRSA).

All patients receive antibiotic therapy (35 aminopenicillins, 5 glycopeptides, 1 carbapenems)

during a median of 13 weeks (IQR 8-24); 13 (37,1%) were treated with combined

aminoglycosides and 22 (62,8%) without them. Fifteen ep. (42,8%) underwent prolonged suppressive therapy for a median of 16 weeks (IQR 4-32).

Surgery was performed in 19 ep. (67,8%) of IAI (52,6% with debridement and retention of the implant) and 4 ep. (57,1%) of osteomyelitis.

Follow up: 15 months (median; IQR 10-24). The overall outcome was favorable in 30 ep. (85,7%); there were 5 relapses (14,3%) and no failures.

Using an univariate analysis, we evaluated the outcome according to these variables: age, gender, comorbidities, microbiology, implant, surgery performed and its modality, use of aminoglycosides and length of antibiotic treatment. None of them were related to a better or worse outcome.

**Conclusion:** EBJI are an uncommon disease mostly associated to implants. Treatment must include surgery and antibiotics. In our series, outcome was not modified by patients' characteristics, presence of an implant, type of surgery performed, length of antibiotic treatment and use of combined therapy with aminoglycosides.

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*Bacillus cereus* bacteremia in hospital setting

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**Background:** Since *Bacillus cereus* is rarely associated actual infections, single positive blood culture is often considered as contamination. However, it can cause number of nosocomial infections such as catheter-associated blood stream infection. We investigated the bacteremic patients to assess the clinical impact of this organism.

**Methods:** We performed a retrospective analysis of bloodstream infections due to *B. cereus* at a tertiary care hospital in Kobe, Japan from October 2008 to September 2009. Cases were identified via microbiology laboratory reports, and relevant clinical data were collected from the electronic medical record of each patient. The case with at least 2 set positive blood cultures were defined as real infection.

**Results:** Nine cases of catheter related blood stream infections (CRBSI) caused by *B. cereus* were identified. Only one case had the presence of an indwelling central venous catheter (CVC). Three cases (33%) were associated with previous antibiotic therapy. Two cases (2/9, 22%) were associated with solid organ malignancies whereas no case with hematological malignancy. One patient was on continuous hemodialysis. The most common characteristics (7/9, 77%) were the use of peripheral parenteral nutrition. These 7 cases had use of solutions containing 7.5% glucose and 3.0% amino acid. For 8 cases vancomycin was used with success. No patient died. All isolates were sensitive for vancomycin whereas resistant to beta-lactams. Two isolates were sensitive for clindamycin.

**Conclusion:** *Bacillus cereus* can cause CRBSI. Indwelling CVC may not increase *Bacillus cereus* CRBSI whereas peripheral parenteral nutrition with higher amino acid concentration can be a risk factor. Vancomycin may be appropriate antibiotic for empiric therapy. Further studies are necessary to further determine the risk of *Bacillus cereus* infection.

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Prognostic markers and out comes in severe leptospirosis in a tertiary care hospital from south India

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**Background:** Leptospirosis is a common zoonotic disease causing epidemics all over the world. Severe leptospirosis with multiple organ involvement causing high mortality is common along costal India. APACHE III is the most recent version of the scoring system developed by Knaus and colleagues. So far no studies are available using APACHE III in severe leptospirosis in Indian setting. Develop simple scoring in leprospirosis to predict outcome.

Prospective clinical study of APACHE III score and other independent variables as a predictor of mortality in patients with severe Leptospirosis.

To develop simple scoring system to predict outcome measure

**Methods:** 61 patients with diagnosis of severe Leptospirosis admitted in Kasturba Hospital Manipal, tertiary care hospital in south India during the period of May 2006 to June 2008.

**Results:** During 2 year study period (May 2006- June 2008) 61 patients with Severe Leptospirosis were admitted in different ICUs of Kasturba Hospital, of whom 13 were females and 48 were males. 21 patients succumbed to their illness and 40 survived. Mean age was  $48.38 \pm 18.31$  years among those who succumbed to Leptospirosis. All patients in dead group had hypotension in comparison to 25(65%) patients out of 40 patients in alive group. 20 out of 21 patients who succumbed had acute lung injury or acute respiratory distress syndrome. Artificial ventilation was associated with increased mortality ( $P < 0.001$ ). Alterations in renal functions such as raised creatinine or raised blood urea nitrogen had no correlation with mortality, however low urine output had a significant correlation ( $p < 0.001$ ). Alterations in liver function tests such as low albumin, high total bilirubin, AST, ALT, ALP or a prothrombin time had no correlation with outcome. Hematological alterations such as low hematocrit, high counts or low platelets were not associated with increased mortality. Mean APACHE III scoring among alive patients was  $56.32 \pm 31.0$  and among dead patients was  $100.33 \pm 26.37$ . The scores were compared by using Independent T test and the scores were highly statistically significant ( $p < 0.005$ )

**Conclusion:** 1. APACHE III is very good prognostic marker in predicting mortality in severe leptospirosis

2. Older age, hypotension, oliguria and ARDS predict independently high mortality and need for ICU monitoring and aggressive treatment.

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Multilocus sequence typing of *Enterococcus faecalis* clinical isolates in Cuba

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**Background:** *Enterococcus faecalis* has recently emerged as an important multidrug-resistant nosocomial pathogen. However, studies on population structure and global epidemiology of this species are limited. We carried out the first study in Cuba by multilocus sequence typing (MLST) of *E. faecalis* clinical isolates and to link the molecular typing data with the presence of virulence determinants and the antibiotic resistance genes.

**Methods:** A total of 23 *E. faecalis* isolates recovered from several clinic sources and geographic areas of Cuba during a period between 2000 and 2005 were typed by MLST. Different antibiotic resistance genes [*aac(6')*-*aph(2'')*, *aph(3')*, *ant(6)*, *ant(3'')*(9), *aph(2'')*-*Id*, *aph(2'')*-*Ic*, *erm(B)*, *erm(A)*, *erm(C)*, *mef(A)*, *tet(M)*, *tet(L)*] and virulence genes (*agg*, *gelE*, *cylA*, *esp*, *ccf* and *efaAfs*) were searched by PCR in the selected isolates.

**Results:** The MLST data revealed 13 sequence types (STs) of which the five STs were novel type. The most frequently types were ST 64 (CC8), ST 6 (CC2), ST 21(CC21), and ST 16. The ST16 showed a great dissemination and persistence among Cuban hospitals and community. Sixty seven percent of STs corresponded to STs reported previously in Spain, Poland and Netherland, and other STs (ST115, ST64, ST6 and ST40) were close to those detected in USA. Aminoglycoside resistance gene *aac(6')*-*le-aph(2'')*-*Ia*, *aph(3')*, *ant(6)*, *ant(3'')*(9) were more frequently detected in ST6, ST16, ST23, ST64 and ST115. The multidrug resistance was distributed to all the clonal lineages detected, except ST117 and singleton ST225. The *ccf* and the *efa* genes were carried by all *E. faecalis* isolates, while 61.5%, 46%, 38.5% and 23% of isolates carried the *esp*, *gel*, *agg* and *cyl* genes, respectively. The presence of *cyl* gene was specifically linked to the CC64 and ST16. Presence of the *esp*, *gel* and *agg* genes was not specific to any particular ST.

**Conclusion:** This research provided the first insight into the population structure of *E. faecalis* in Cuba. Most Cuban strains are related to European strains, and others strains are related to US strains. The CC2, CC21 and CC8, three of the biggest CCs in the world are evident in Cuba circulating with multidrug resistance and virulence traits.

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Analysis and correlates of atypical lymphocytosis in leptospirosis in the cohort of patients at an infectious diseases unit, University Hospital, from 1996 to 2009, in Rio de Janeiro, Brazil

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**Background:** Laboratory findings associated with dengue fever include a lymphocytosis often marked by atypical lymphocytes while in leptospirosis, peripheral-blood smear will indicate bacterial infection. There is no case report in medical literature associating leptospirosis and atypical lymphocytes. The role of cell-mediated immunity in host defense to *Leptospira* spp. remains poorly understood in both animal models and human disease. Whereas Leptospirosis related to atypical lymphocytes should be cause for scientific investigation.

**Methods:** This study was developed at the Pedro Ernesto University Hospital, over a period of 13 years (1996-2009) with suspected leptospirosis. Leptospirosis was defined in accordance with the World Health Organization criteria. We describe the demographic, epidemiological, clinical and laboratory characteristics of 33 patients. A value of  $P < 0.05$  was considered significant. For proportional analysis  $\chi^2$  test was used.

**Results:** Mean age was 34 years, and there was no case of leptospirosis among adolescent females in our cohort. 82% had epidemiological factor positive. The mean hospital stay was 11 days and only 78,7 % received antibiotic treatment. Fever was recorded in 100% of the cases, myalgia in 96,9% and 90.9% had jaundice. Complications: 62.5% with bleeding, 60.6% progressed to renal failure, 21.2% respiratory failure and 18.0% were admitted in the ICU. After comparing the groups with atypical and without atypical lymphocytes, we found that the first group had less renal failure ( $p < 0,04$ ). There were two cases in the first group where we had the opportunity to study the lymphocytes in the cytometry flow. We observed a predominance of gd lymphocytes. There were no deaths in the group of atypical lymphocytes and the mortality in our cohort in Rio de Janeiro was of 9.1%.

**Conclusion:** In Brazil, most cases of leptospirosis and dengue fever were reported in the tropical rain season. We observed an expansion of  $\gamma\delta$  T cells in peripheral blood in two patients with Leptospirosis. We found lower morbidity in patients with atypical lymphocytosis in our cohort.

Leptospirosis is an emerging infectious disease where atypical lymphocytes may be observed. Therefore, the infectious diseases physician must be aware of this new data.

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Knowledge of Leptospirosis among community-based rural residents in Kandy, Sri Lanka:  
Implications for intervention

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**Background:** Leptospirosis is a zoonotic disease that has emerged as an important cause of morbidity and mortality among impoverished populations, such as those in the rural communities in Kandy, Sri Lanka. Poor knowledge has contributed to an increase in number of rural residents who have died as a result of acquiring Leptospirosis. In 2008 alone, 207 local deaths were reported, but the number may be underreported. To address the incidence of Leptospirosis, a community-based intervention is being planned.

**Methods:** A survey involving 159 randomly-selected community-based rural residents in Kandy was carried out to provide baseline data for the intervention program. The study examined Leptospirosis-related knowledge, attitudes and practices. This paper reports on the knowledge part of the study in which respondents' knowledge of the transmission routes (TR), reservoirs (R), sources (So), symptoms (Sy) and prevention (P) of Leptospirosis were determined using the yes/no question format. Knowledge responses were recoded and constructed into high and low categories. Using SPSS Version 14, knowledge data were analyzed using descriptive statistics and were cross-tabulated with respondents' socio-demographic characteristics.

**Results:** Respondents were: of varying ages; mostly men and married; of differing educational levels; primarily farmers and of low-income category; and were residing in the rural community for diverse periods. Across four knowledge items, far greater numbers of respondents had low than high knowledge level of Leptospirosis. Specifically, two-thirds had low TR knowledge; three-fourths had low R knowledge; 60% had low So knowledge; and most (83%) had low P knowledge. However, in the Sy knowledge item, respondents were about equally divided into those with high (50%) and those with low knowledge level (46%). Cross-tabulation results between socio-demographic characteristics and Sy knowledge item revealed a significant association between income and knowledge. In particular, those with low income had a lower Sy knowledge level than those with high income.

**Conclusion:** Rural residents of Kandy, Sri Lanka, across socio-demographic categories, are at a continuing risk for Leptospirosis because of their poor knowledge. Appropriate interventions are needed.

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*Bacillus cereus* as a pathogen and retention of medical devices are risk factors for *Bacillus* bacteremia

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**Background:** *Bacillus* species are ubiquitous in environment. Positivity of blood cultures with these bacteria usually are recognized as contamination, although several cases have been reported as definite bacteremia. The aim of this study is to evaluate the incidence, risk factors, antibiotic susceptibilities and prognosis in *Bacillus* bacteremia.

**Methods:** From April 2003 to March 2008, all the adult inpatients from whose blood culture was isolated *Bacillus* species in the University of Tokyo Hospital were enrolled. Clinical characteristics were retrospectively reviewed from medical records. Definite bacteremia was defined as the following criteria; 1) two or more positive blood cultures collected at the same day, 2) one positive blood culture and positive cultures sampled from other sites, or 3) positive blood cultures in continuous days. Other cases of positive blood cultures with *Bacillus* species were defined as indefinite cases. Microbiological data including antibiotic susceptibilities were also collected. Primary endpoint was defined as 30-day mortality.

**Results:** In all the 3094 cases of positive blood cultures in the study period, 94 cases (3.0%) were due to *Bacillus* species. Of the 94 positive blood culture cases with *Bacillus* species, definite bacteremia were 22 cases (23.4%) and indefinite cases were 72 cases (76.6%). As for clinical characteristics and microbiological data, only two significant factors were higher in definite bacteremia cases than in indefinite cases; retention of medical device other than central venous catheters and *Bacillus cereus* as a pathogen (45.5% vs. 13.2%;  $p=0.001$ , 36.4% vs. 12.5%;  $p=0.010$ , respectively). In multivariate analysis, these factors were significant for *Bacillus* bacteremia. (Odds ratio (OR) 3.78; Confidence Interval (CI) 1.10-13.0, OR 4.42; CI 1.42-13.8, respectively). No statistical differences were shown between the two groups in antibiotic susceptibilities, the rate of the initial use of susceptible antibiotics and 30-days mortality

**Conclusion:** In cases of positive blood cultures with *Bacillus* species, the rate of definite bacteremia was 23.4%. *Bacillus cereus* as a pathogen and retention of medical devices were two significant risk factors for *Bacillus* bacteremia. Antibiotic susceptibilities and prognosis were similar between definite bacteremia cases and indefinite cases.

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Concordance between Vir and protein M gene (*emm*) types of *Streptococcus pyogenes* strains isolated from patients with invasive and non-invasive infections of Mexico

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**Background:** Identification of the *emm* types of Group A *Streptococcus* (GAS) strains associated to invasive infections and acute pharyngitis from Mexico by the protein M gene (*emm*) typing is crucial to evaluate the potential usefulness of the 26-valent candidate GAS vaccine developed in the U.S.A. In the same way, RFLP patterns of other method to type GAS strains such as Vir typing has been described to have a high concordance with specific *emm* types, but consistency has not been proven.

**Methods:** Cross sectional study. We studied 101 GAS strains isolated of patients with invasive (n = 32) and non-invasive (n = 69) infections from three cities of Mexico, during the 1999-2008 period. RFLP of the Vir regulon (Vir typing) was performed using *Hinf*I restriction enzyme according with the technique described by Gardiner et al. The M protein gene (*emm*) typing of GAS was performed according with the CDC protocol (<http://www.cdc.gov/ncidod/biotech/strep/protocols.html>) described by Beall B.

**Results:** All the GAS strains were typeable by the M protein gene (*emm*) typing method. Analysis of the 101 GAS strains with both techniques resulted in 33 Vir RFLP patterns and 13 M protein gene (*emm*) types. Seven *emm* types (*emm*12, *emm*1, *emm*4, *emm*6, *emm*22, *emm*3, and *emm*59) accounted for 79% of the isolates. Other *emm* types (*emm*28, *emm*11, *emm*49, *emm*75, *emm*77 and *emm*89) were isolated less frequently. In some cases, a single protein M *emm* type resulted in several RFLP patterns by the Vir typing method. All but one (*emm*4) M protein *emm* gene types isolated were included in the 26-valent M protein-based vaccine described by Hu, M and Dale J et al.

**Conclusion:** Although the number of GAS strains studied is small, the M protein gene (*emm*) typing resulted in the best technique for studying and comparing the genotypes of GAS strains from this study and elsewhere. The fact that all the GAS strains studied was able to be typed by the M protein gene (*emm*) method, and that all but one belonged to the *emm* types included in the 26-valent M protein-based vaccine, seems to be promising for the potential of this vaccine in Mexico.

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Clinical aspects of neuroborreliosis in Bydgoszcz, Poland

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**Background:** The aim of the study was to assess the clinical, radiological and laboratory findings of neuroborreliosis.

**Methods:** Patients with diagnosed neuroborreliosis from 2006 through 2007 at Department of Infectious Diseases in Bydgoszcz were analyzed. The diagnosis was based on the presence of neurological symptoms with serologic evidence to exposure to *Borrelia burgdorferi* and/or erythema migrans (EM) in the medical history.

**Results:** In the study period 37 (female - 70.3% mean age: 48 years) cases of the neuroborreliosis were recognized.

Acute neuroborreliosis was recognized in six cases with the incubation period from 3 to 10 weeks. Clinical presentation included: facial nerve palsy (3), six cranial nerve lesion (1), six cranial nerve lesion and the retrobulbar neuritis (1), meningitis (1). 5 from 6 patients were diagnosed with the Bannawarth's syndrome with meningitis and painful radiculitis. Symptoms occurred concomitantly with erythema migrans (EM). In all cases CFS abnormalities were noted: lymphocytic pleocytosis (713-2883 cells/mL), elevated proteins (213 to 450 mg/dL) and normal CFS/serum glucosae ratio.

The late neuroborreliosis (LNB) was diagnosed in 31 patients. The group of 28/31 patients had history of tick bite, 25 had history of untreated EM - 2-7 years before neurological impairment. Clinical presentation: 12 - painful polyradiculoneuropathy with numbness and paraesthesia, 5 - paraplegia, 5 - reflex asymmetry; 1- Guillain-Barre-like syndrome, 3- disseminated neurological symptoms: paraplegia, cerebellar ataxia and mental deterioration, 5- memory loss, headaches. CFS examination was performed in all patients. 20/31 had CFS abnormalities: 6 - elevated proteins, 15 - lymphocytic pleocytosis, 1- lymphocytic pleocytosis and elevated proteins. In all cases specific antibodies to *Borrelia burgdorferi* were detected in CFS and in serum. MRI was performed for 2/6 patients with early borreliosis and in 13/31 patients with late NB. Abnormalities in white matter with multifocal hyperintense lesions in T2-weighted and FLAIR scans of brain were detected in 5 patients with LNB.

All patients were treated with ceftioxone 2g/day. In all cases with early neuroborreliosis clinical outcome was good, all symptoms resolved whereas neurologic deficits from LNB improved slowly and have not subsided completely.

**Conclusion:** This study gives an overview of serologically confirmed neuroborreliosis. We have shown large spectrum of clinical symptoms in the late stage of disease with not satisfactory outcome in most of them.

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Diagnosis and treatment challenges in patients with chronic tick Associated Poly-organic Syndrome (TAPOS) - Case series

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**Background:** Chronic Lyme disease is often considered in case of long lasting miscellaneous symptoms after tick bites. Despite new codified diagnosis algorithm and treatment persistent signs and symptoms frequently occur. The aim of the study was to assess the diagnosis of Tick Associated Poly-organic Syndrome (TAPOS) and to evaluate the treatment's efficacy.

**Methods:** A consecutive case series of patients referred to the Cluj-Napoca Teaching Hospital of Infectious Diseases (January 2006 -October 2009) revealed 52 patients with TAPOS. Inclusion criteria were: more than 18 years of age, chronic symptomatology, positive *Borrelia burgdorferi* serology and/or tick-bite. Data was collected through chart review and medical observation. We used two clinical scores classifying the diagnosis of TAPOS. All patients were seropositive for IgM and/or IgG antibodies to *Borrelia burgdorferi* (EIA and western blotting). Treatment regimen was established according to the literature data. They received intravenous ceftriaxone, 2 g daily for 21-28 days and doxycycline 200 mg daily for 21 days. Patients with persistent symptoms were retreated with the same regimen.

**Results:** The baseline assessment documented that the most frequently reported symptoms were neuropsychological (90%), systemic (98%) and articular (23%). The sex ratio was 0.26 (41 women, 11 men), the average age was 43.2 ±12.6 years. Only 7 patients experienced erythema migrans, 57% had tick exposures and *Borrelia burgdorferi* serology was 92% positive [44 (84%) IgM positive, 19 (36%) IgM and IgG positive]. According to both clinical scores all patients were classified as "very probable" or "probable". All patients were evaluated at 3 months showing a decrease in the number and intensity of signs and symptoms and the same serologic pattern. Ten patients were 2-3 times retreated due to persistent clinical picture, all presenting mood disorders or depression. No case of clinical aggravation or serious adverse events was reported and Jarish-Herxheimer syndrome was observed just in two cases. Most of the patients remained with at least one neuropsychological complain.

**Conclusion:** Diagnosis of miscellaneous *Borrelia burgdorferi* chronic infection is challenging but should be always considered if prolonged symptomatology or tick related. Treatment regimens are not standardized, we appreciate as reasonable shorter 6 week regimens.

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*Corynebacterium striatum*: an under recognised cause of diabetic foot osteomyelitis.

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**Background:** In common with other coryneform species, *Corynebacterium striatum* colonizes the skin and mucous membranes of normal hosts, and is one of the more frequent corynebacteria isolated in the clinical laboratory. However, its clinical significance is often unclear as it can be difficult to distinguish between colonization and infection. Most reported cases of *C. striatum* infection are of endocarditis, pulmonary infection or are associated with prosthetic devices. We observed a number of cases of diabetic foot osteomyelitis in our hospital from which a heavy, pure growth of *C. striatum* was isolated from tissue or fluid samples.

**Methods:** A review of the medical literature was carried out and did not suggest *C. striatum* was a recognised cause of diabetic foot infection. We identified all pure cultures of *C. striatum* grown in our laboratory from tissue and fluid samples. We carried out a review of the patients' medical notes recording details of past medical history, antimicrobial history including changes made upon isolation of *C. striatum* and the clinical outcomes following appropriate therapy.

**Results:** Three tissue cultures and one fluid culture of *C. striatum* were identified. The three tissue cultures were from patients with diabetic foot osteomyelitis. The fluid sample came from a deep washout of a diabetic foot ulcer with underlying osteomyelitis, and the growth was both heavy and pure. The four patients were male, and had a mean age of 76 years, and all had a diagnosis of insulin dependent diabetes. They were all treated successfully with targeted antimicrobial chemotherapy, and followed up for at least eight months post-treatment, with no evidence of disease recurrence.

**Conclusion:** We report 4 cases of *C. striatum* osteomyelitis of the foot in patients with diabetes mellitus. We suggest that where *C. striatum* is isolated in pure culture from tissue samples from diabetic feet, it should not be discounted as contaminating flora but considered as a genuine cause of infection.

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The rising concern of community-acquired methicillin-resistant *Staphylococcus aureus* central nervous system infections: 2 case reports

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**Background:** The increasing incidence of community-acquired methicillin-resistant *Staphylococcus Aureus* (CA-MRSA) central nervous system (CNS) infections in healthcare settings is concerning and may warrant a reappraisal of current treatment guidelines.

**Methods:** Case 1: A 64 year old man with no significant past medical history was admitted with new-onset seizures. Besides recurrent headaches, there were no associated symptoms. Physical examination and labs were normal. CT scan of the brain showed a hypodense left parietal lobe mass. However once seizures controlled, he insisted on early discharge. He was readmitted 12 days later with recurrent seizures. Repeat imaging showed a significant increase in the size of brain mass. The patient was taken to OR for emergent excision of the mass which revealed a brain abscess which grew methicillin-resistant *Staphylococcus aureus* (MRSA) with antibiotic susceptibility consistent with a CA-MRSA. The patient was treated postoperatively with intravenous vancomycin on which he recovered completely with resolution of the abscess.

Case 2: A 67 year old man was admitted with fever, facial swelling, altered mental status, hypotension and tachypnea needing pressers and intubation. He had presented to ER 5 days earlier with facial swelling and was discharged with impression of angioedema. Admission labs showed leukocytosis, Brain CT scan revealed cavernous sinus thrombosis and CSF analysis showed leukocytosis, high protein and low glucose. His Blood and CSF cultures grew MRSA and Pulse Field Gel Electrophoresis showing Type USA 300. The patient on treatment had a complicated and prolonged hospital course.

**Conclusion:** Community-acquired MRSA (CA-MRSA) is now firmly established as a worldwide, community prevalent infection. It's now increasingly recognized as an organism with invasive potential causing an ever-increasing spectrum of diseases.

Only three previous cases of CA-MRSA brain abscess and CA-MRSA meningitis with Cavernous sinus thrombosis have been reported respectively. Both our patient's had a benign initial presentation. In contrast to all fatalities in previous brain abscess cases, our patient had a complete recovery on treatment.

The increasing appearance of life threatening CA-MRSA, CNS infections in healthcare-associated settings and the difficulties in readily identifying risk factors warrants measures to increase awareness and a reappraisal of current empiric treatment guidelines for Community Acquired Brain Abscess.

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A scoring system for severity of disease associated with mortality for *Clostridium difficile* associated disease

**P. Ko**, R. Jayasingam, C. Modi, R. Hallit, N. Nguyen, S. Daniel, T. Soleymani, J. Fallon, L. Booth, G. Perez, J. Slim, J. Sensakovic, J. Boghossian  
St. Michael's Medical Center, Newark, NJ, USA

**Background:** *Clostridium difficile* associated disease (CDAD) has been an increasing problem and a burden in our healthcare system which is estimated to be over a billion dollars per year in the United States. The increasing severity of disease has been described in previous literature attributed to the BI/NAP1 gene or ribotype 027. There have been several scoring indices used for CDAD studies such as Modified Horn's Index and APACHE II score. We designed a study to stratify by severity of disease over a period of time and develop a new scoring system.

**Methods:** We reviewed patients with CDAD from January 2005 to May 2008 from a 357-bed regional tertiary center, St. Michael's Medical Center in Newark, NJ. We analyzed the data to arrive at a scoring system based on age, albumin, creatinine, and WBC. The criterias for the scoring system were 0 to 1 mild; 2 to 3 moderate; 4 to 5 severe; 6 to 7 extremely severe. One point is given for age >60; albumin level <2.5mg/dL is given 2 points, albumin between 2.5 to 3.5mg/dL is given one point; creatinine level of >2.0mg/dL is given 2 points; creatinine between 1.0 to 2.0mg/dL is given 1 point; WBC count of >20,000cells/mL is given 2 points; between 10,000 to 20,000cells/mL is given one point.

**Results:** The total number of patients with CDAD were 629 from January 2005 through May 2008. The mean age for 2005, 2006, 2007, and 2008 were 69.1, 64.7, 68.6, and 70.1 respectively. The mean for the lowest albumin levels were 2.05, 2.04, 2.05, and 2.09mg/dL respectively. The mean for the highest creatinine levels were 2.25, 2.57, 2.58, and 2.24mg/dL. The mean for the highest WBC counts were 18,800, 19,400, 18,800, and 21,100cells/mL. The recurrence rates were 5.68%, 4.28%, 16.1%, and 8.86%, respectively. The mortalities for 2006, 2007, and 2008 were 13.25%, 12.75%, and 18.99% respectively (p-value <0.34). The mean score for those who were alive were 4.50, 4.73, and 4.87 respectively. The mean score for those who expired were 5.68, 5.45, and 5.50 respectively which showed high correlation with patients with mortality (p-value <0.0001).

**Conclusion:** There was an increase in mortality for 2008 suggesting virulent strain of *C. difficile*. The use of the scoring system will identify these patients with likelihood of high mortality. Therefore clinicians will be able to observe these patients with closer supervision and treat more aggressively.

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Disseminated nocardiosis

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**Background:** The Nocardia is described as an infectious agent of systemic involvement especially in immunocompromised patients. From what we wanted to describe the case of a patient with type-2 diabetes mellitus developed a severe systemic infection with fatal outcome.

**Methods:** We describe the case of a farmer of 62 years from the lower Cauca Colombia, who presented a clinical that begins with 2 months whitish lesions in the mouth, associated with persistent fever and gradual onset of cough, hemoptysis, dyspnea, cyanosis and generalized pustule skin lesions with budding tree display and cavitary lesions in lung and liver abscess only, with no echocardiographic vegetations and negative blood cultures. In skin culture displayed filamentous gram-positive bacteria compatible with Nocardia.

**Results:** The patient has an unfavorable clinical course with eventual development of multiple organ failure and death.

**Conclusion:** Nocardiosis should be taken into account as an etiologic agent in immunocompromised patients from rural areas with systemic manifestations and progressive clinical deterioration.

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Effectiveness of linezolid versus vancomycin treatment for MRSA skin and soft tissue infections  
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Dartmouth College, Hanover, NH, USA

**Background:** Methicillin-resistant Staphylococcus aureus (MRSA) infections of skin and soft tissues are an increasing concern in hospital and community settings. Whether newer agents like linezolid offer efficacy, safety or cost advantages over traditional first line treatment with vancomycin is not known. We conducted a systematic review and meta-analysis to assess the safety and efficacy of linezolid compared to vancomycin in hospitalized patients with presumed MRSA infections of skin or soft tissue.

**Methods:** In October 2009, we searched MEDLINE (1950-2009), Web of Science (1900-2009), both the Cochrane Database of Systematic Reviews and CENTRAL (Issue 4, 2009), ClinicalTrials.gov, relevant conference proceedings and reference lists using no limits. We selected randomized controlled trials comparing linezolid (intravenous or oral) versus vancomycin (intravenous) in non-surgical hospitalized patients with MRSA-infected or suspected skin or soft tissue infections who were followed for a minimum of 7 days. Two independent reviewers extracted data from each qualifying report using a standard data collection form. We used Cochrane Collaboration's risk of bias tool to assess methodological quality and data was summarized in RevMan using random effects models.

**Results:** Of 455 reports identified, 7 studies qualified for inclusion. Linezolid was favored over vancomycin for clinical cure rate (RR 0.79; 95% CI 0.67, 0.94; P = 0.007) and microbiological eradication rate (RR 0.80; 95% CI 0.62, 1.04; P = 0.10). Analysis of adverse events revealed no difference between the two agents (RR 0.98; 95% CI 0.76, 1.27; P = 0.90). Results for length of stay were inconclusive.

**Conclusion:** Our results suggest that linezolid is more effective than vancomycin for achieving clinical cure in hospitalized patients with MRSA skin and soft tissue infections while adverse events are no different. However, other important benefits and tradeoffs such as length of stay, resistance, and cost would be important to explore before changing current practice.

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**Type: Poster Presentation**

Follow-up of Gambian 9-valent pneumococcal conjugate vaccine trial participants who experienced acute lower respiratory infection aged less than 2.5 years

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<sup>1</sup>Medical Research Council (UK) The Gambia, Fajara, Gambia, <sup>2</sup>London School of Hygiene and Tropical Medicine, London, United Kingdom, <sup>3</sup>Bill and Melinda Gates Foundation, Seattle, wa, USA

**Background:** The long-term outcome following ALRI at a young age is poorly described. Hypothesizing that ALRI with X-ray diagnosed pneumonia is associated with worse outcome, we described the mortality experience until 6 years of age of a sample of participants in a pneumococcal vaccine trial.

**Methods:** Trial follow-up ended at age 2.5 years. We followed 3920 trial survivors. Five groups were defined by exposures during the trial: 1. Severe ALRI with X-ray end-point consolidation (SALRI-EPC), 2. Severe ALRI without EPC (SALRI-no EPC), 3. Mild ALRI with EPC (MALRI-EPC), 4. Mild ALRI without EPC (MALRI-no EPC), and 5. Controls without ALRI or admission. We calculated incidence proportion of mortality from trial end until follow-up.

**Results:** In 2008-9, 3367 (85.9%) participants were traced. Complete data were available for 3162 (80.7%). Mortality was greater among those with SALRI-EPC compared to controls and greater among those with past SALRI compared to MALRI, irrespective of radiological findings (see table). Confounding by vaccination status was not evident as the crude relative risk of mortality for SALRI-EPC compared to no ALRI (3.72; 90% CI 2.82, 4.92) was similar to analysis stratified by vaccination status, 3.70; 90% CI 2.80, 4.89.

**Follow-up groups: number of deaths, proportion vaccinated & deaths by vaccine allocation**

Participant category	Number traced/sampled	Sex (% Male)	Incidence proportion	Proportion vaccinated	Deaths vaccine	Deaths placebo
SALRI-EPC	423/499 (84.8%)	242 (57.2%)	66 (15.6%)	176 (41.6%)	25/176 (14.2%)	41/247 (16.6%)
SALRI-no EPC	390/480 (81.3%)	212 (54.4%)	55 (14.1%)	195 (50.0%)	32/195 (16.4%)	23/195 (11.8%)
MALRI-EPC	851/1020 (83.4%)	451 (53.0%)	45 (5.3%)	424 (49.8%)	23/424 (5.4%)	22/427 (5.2%)
MALRI-no EPC	397/503 (78.9%)	199 (50.1%)	21 (5.3%)	179 (45.1%)	7/179 (3.9%)	14/218 (6.4%)
No ALRI	1101/1418 (77.6%)	568 (51.6%)	44 (4.0%)	579 (52.6%)	21/579 (3.6%)	23/522 (4.4%)
Total	3162/3920 (80.7%)	1672 (52.9%)	231 (7.3%)	1553 (49.1%)	108/1553 (7.0%)	123/1609 (7.6%)

**Conclusion:** ALRI-EPC <2.5 years of age was associated with 3.7 times the risk of mortality until 6 years of age, while compared to MALRI or no ALRI, SALRI was also associated with increased mortality. SALRI at a young age may be associated with a predisposition to life threatening illness.

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Tetanus: Clinical and epidemiological characteristics. Review of 11 years

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**Background:** Tetanus is an infection disease, caused by the neurological toxicity of tetanoplasmina, exotoxin produced by *Clostridium tetani*. Although it is a preventable disease through vaccination, there are about 100 cases annually.

**Methods:** A retrospective study and analysis of patients with tetanus, made in the Rawson Hospital in Córdoba city, during the period covered between May 1, 1998 and October 30, 2009.

Inclusion criteria: patients with age > 15 years, clinical diagnosis of tetanus.

**Results:** The incidence was 0.33 per 100,000; average 1.4 cases per year. Of the 12 cases analyzed, 58.3% were men, average age 68.5 years. 50% had incomplete immunization and 50% had no immunization records. 100% of the patients presented as generalized tetanus. Port of entry was determinate in 75%: skin lesions predominate in the lower limbs. Incubation period of less than 7 days and a period of invasion less than 48 hours in 66%. Symptoms more common: trismus 100%, dysphagia 91%, generalized muscle contracture 83%, muscle spasms 58%. Complications: Ventilator-associated pneumonia 50%, atelectasis 33%, scarring 25%. Treatment: 42% received penicillin G sodium, 42% metronidazole and 16% both antimicrobials. Mortality 75%.

**Conclusion:** Lower incidence. Predominantly found in elderly men. Lack of verifiable previous vaccination. Predominates generalized tetanus and infectious complications. High mortality

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Characterization of community-acquired *Staphylococcus aureus* methicillin-resistant (CA-MRSA) vs methicillin susceptible *Staphylococcus aureus* (CA-MSSA) infection (Inf) in Argentinean children

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**Background:** CA-MRSA inf are increased worldwide and few data are published from Latin America. Objective: to define the prevalence of CA-MRSA vs CA-MSSA in hospitalized children in our setting; to characterize the demoepidemiological, clinical and laboratory data between groups

**Methods:** Restrospective study Jan`05 – March`08, at Hospital de Niños, Buenos Aires. CA *Staphylococcus aureus* inf: positive culture ≤72 hs of admission consistent with CDC´s ABC criteria. Exclusion: patients with health care associated infection. Likert scale for parent´s education and frequency of bath score was performed. Chi2, *t* test and regression logistic model (LRM) were used.

**Results:** A total of 302 *Staph* inf were studied: CA-MRSA:153 (51%) and CA-MSSA:149 (49%). CA-MRSA increased frequency from 39.8% in 2005 to 60% in 2008, (*p*=0.01). No difference between groups was observed in: age: 70.3(±61.5), median:51 mo; gender: male 187/302 (61.9%); underlying disease: 74/302 (24.5%); crowding: 125/302 (41.4%). CA-MRSA was related to lower parent´s education and less frequency of bath, *p*=0.009, respectively. Clinical and laboratory features:

Infection	CA-MRSA	CA-MSSA	p
Skin and soft tissue	135/153 (88.2%)	199/149 (67.1%)	<0.001
Two or more skin sites affected	50/135 (37%)	15/100 (15%)	0.0002
Subcutaneous Abscess	107 /135 (79.2%)	56/100 (56%)	<0.001
Piomyositis	14/135 (10.4%)	2/100 (2%)	0.01
Lower Resp Tract Infection	18/153 (11.7%)	14 /149 (9.4%)	0.5
Meningitis	4/153 (2.6%)	5/149 (3.3%)	0.7
Sepsis	19/153 (12.4%)	14/149 (9.4%)	0.4
Osteomyelitis	32/153 (20.9%)	54/149 (36.2%)	0.003
Bacteremia	34/153 (22.2%)	48/149 (32.2%)	0.005
Invasive Disease (total)	68/153 (44.4%)	92/149 (61.7%)	0.002
WB count (/mm3)	17,226 (±6,328)	14,715 (±5,602)	0.0003
Duration of Hospitalization	11.5 (±10.2) ds	9.1 (±8.9)ds	0.13
Mortality	1/153 (0.7%)	1/149 (0.7%)	-

CA-MRSA resistance: GEN (10 (7.5%); CLI: 7 (4.5%); ERY: 7(4.5%); RIF and SXT: 1 (0.65%).

- Conclusion:**
1. Prevalence of CA-MRSA inf increased during last years in our setting (*p*=0.01)
  2. Independent risk factors associated to CA-MRRSA in RLM were: subcutaneous abscesses (*p*<0.0001), two o more skin sites affected (*p*=0.002) and use of antimicrobials during the last year (*p*=0.0001)
  3. CA-MSSA was statistically associated to invasive disease (*p*=0.002)

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Diphtheria *tox* gene polymorphism in *C. diphtheriae* strains isolated in Russia during 2002-2009  
**S. Kombarova**, I. Mazurova, O. Borisova, N. Gadua, T. Kornienko  
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**Background:** Diphtheria *tox* gene is responsible for diphtheria toxin synthesis by diphtheria causative agent. We studied diphtheria *tox* gene structure in *C. diphtheriae* strains isolated in Russia over the period of decreased and sporadic incidence of diphtheria (2002-2009).

**Methods:** 16 toxigenic strains from various regions of Russia (North-Western region, i.e. St.Petersburg; Central region, i.e. Moscow, Smolensk, and Tula; from Siberian region, i.e. Omsk) were used to determine the full nucleotide sequence of their *tox* gene. 13 of the strains were of the *gravis* biovar, the other 3 – of the *mitis* biovar. 2 strains were isolated from patients, 14 strains – from passive carriers. Also 115 nontoxigenic *C. diphtheriae* strains isolated between 2006 and 2009 were observed to identify nontoxigenic strains carrying a "silent" *tox* gene.

**Results:** 12 point mutations in *tox* gene were registered between 2002-2009. In comparison, we have studied 40 strains circulating before and during diphtheria epidemic in Russia (1985 – 1993) and only 5 point mutations were revealed. By now 13 point mutations have been registered among strains isolated in 1985-1993 and 2002-2009. Majority of mutations did not lead to substitutions in amino acid sequence of diphtheria toxin. Over the period of decreased and sporadic incidence of the disease two point mutations corresponding to substitutions on amino acid level were registered. Such changes affected 4 out of 16 strains.

Further research was done to identify nontoxigenic *C. diphtheriae* strains carrying a "silent" *tox* gene. No nontoxigenic strains carrying a *tox* gene were identified. Comparing these results with the data collected over many years of monitoring showed that the percentage of nontoxigenic *tox* gene carrying strains in the strain population was different in different years. Thus, at the time of sporadic incidence of the 1980s and during the period of epidemic rise (1990-1995) such strains were either registered at a very insignificant level (2-4%) or not registered at all. In the period of low diphtheria incidence following an epidemic rise (1996-2004), the concentration of nontoxigenic *tox* gene carrying strains considerably increased (up to 17%).

**Conclusion:** Polymorphism of *tox* gene can create wide adaptation possibilities of diphtheria causative agent.

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*Staphylococcus lentus*: The troublemaker

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**Background:** *Staphylococcus lentus* has been associated with infections in animals, however *S. lentus* has rarely been reported as a pathogen in humans. Here we report 72 cases involving *S. lentus* at our institution over a period of 9 years. Review of the literature revealed only one case of a human infection, and to our knowledge, this is the largest series of *S. lentus* infection in humans reported so far.

**Methods:** Cases were defined as clinically significant if there was evidence of a positive culture, in addition to signs and symptoms of pain, tenderness, swelling, fever or leukocytosis.

**Results:** Of the 72 cases, 20 involved only *S. lentus*, 50 had associated microbes, and 2 cases were unknown. Of the 20 cases involving solely *S. lentus*, 9 were from urine, 1 from peritoneal fluid, 7 from blood, 1 from CSF and 2 from wound cultures. All patients with culture positive *S. lentus* (both with and without associated microbes) had clinical signs of infection including leukocytosis, fever, pain, tenderness, swelling, infection noted by physician or improvement with antibiotic therapy. Analysis of demographic data revealed no particular patterns. Forty-four of the 72 cases were culture positive within 1-3 days of culture or admission. Antibiotic sensitivities revealed 83% sensitivity to Vancomycin and 29% sensitivity to Oxacillin

**Conclusion:** Little is known about *S. lentus* and only one report of human infection exists. Our case study involved 72 cases of *S. lentus* positive cultures at our institution with evidence of clinical infection in all 72 cases. Based on our experience, *S. lentus* is a true pathogen that deserves attention. We feel, however, that it will require clinical sense to decide if infection with *S. lentus* is significant when analyzed on a case-by-case basis.

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Staphylococcus lugdunensis – A wolf in sheep's clothing

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**Background:** The coagulase negative staphylococci are generally much less virulent than *Staphylococcus aureus*, but there are increasing reports of *Staphylococcus lugdunensis* behaving aggressively. We analyzed our teaching hospital's experience with this pathogen.

**Methods:** Retrospective chart review in an 808 bed tertiary care medical center. All 70 initial isolates were reviewed, and clinical and microbiologic data compiled.

**Results:** There were 70 unique patient isolates; 57 were from inpatients. 21 had positive blood cultures; 5 of them met Duke's criteria or had autopsy proven endocarditis. Three endocarditis patients underwent valve replacement and survived. Two patients died, including a 26 year old healthy man who died within 48 hours of admission due to refractory shock; his autopsy revealed a ventricular wall abscess and large aortic valve vegetations. There were 13 urinary isolates; the others were primarily from skin, soft tissue and bone sites, including 6 from breast abscesses. 3 had prosthetic joint infections, and 1 had postoperative meningitis. Two deaths were attributable to *S. lugdunensis* infection. Only 12% of isolates were penicillin sensitive, and 9% were oxacillin resistant.

**Conclusion:** *S. lugdunensis* is a virulent pathogen, capable of causing life threatening infection, including both native valve and prosthetic endocarditis. Sensitivity to penicillin and oxacillin cannot be assumed. The clinical laboratory should identify to the species level all clinically important coagulase negative staphylococci.

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**Type: Poster Presentation**

Association of breast milk *Lactobacilli* and *Staphylococcus aureus* in women with mastitis using quantitative PCR

U. srinivasan, N. Shrivastwa, S. Ponnaluri, J. Debusscher, C. Barbossa-Cesnik, C. F. Marrs, **B. Foxman**

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**Background:** *Staphylococcus aureus* is the most common causative agent of lactation mastitis; an infection of the breast that affects up to 30% of lactating women and often requires treatment with antibiotics. Commensal breast milk *Lactobacilli* inhibit pathogenic *Staphylococcus* growth *in vitro*; whether this is true *in vivo* is not known. We will test *Staphylococcus* and *Lactobacillus* levels in mastitis and healthy breast milk collected from a study of lactating mothers in Brazil and determine the association of *Staphylococcus* and *Lactobacilli* levels in breast milk.

**Methods:** Breast milk samples were collected from 72 healthy mothers and mothers suffering from mastitis in Brazil. Bacterial genomic DNA from breast milk was purified from breast milk samples and quantitative PCR assays were designed to measure the amounts of *Staphylococcus*, *Lactobacilli* and total bacteria. Primers for *Staphylococcus aureus* assays were based on the *Staphylococcus aureus* specific *nuc* gene, while *Lactobacilli* specific 16S ribosomal gene regions were used to design primers specific to *Lactobacillus* genus. Total bacteria were determined in quantitative PCR assays using universal bacterial primers based on conserved regions of the 16S ribosomal gene.

**Results:** Preliminary quantitative PCR results of 24 samples indicate that *Staphylococcus aureus* was present in roughly 47% (n=11) of milk and *Lactobacilli* was present in 82% (n=19) of milk samples. *Lactobacilli* levels varied between 70 - 103 copies / ul while *Staphylococcus aureus* levels were higher (102-106 copies/ul).

**Conclusion:** Our results suggest that *Lactobacilli* are more common in breast milk than previously reported in literature. We will investigate whether *Lactobacilli* levels in breast milk are related to *Staphylococcus aureus* levels in healthy and mastitis milk. If the levels of commensal *Lactobacilli* and pathogenic *Staphylococcus aureus* are related, this suggests that microbial milk composition is an important determinant of breast milk health and indicates the need to investigate whether probiotic supplementation with *Lactobacilli* can help reduce the risk of mastitis in women.

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*Streptococcus pneumoniae* treatment with catecholamines affects growth based on strain isolation site with a lack of effect on biofilm formation

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**Background:** It is known that bacterial physiological responses are affected by catecholamines (norepinephrine, epinephrine) *in vitro*. Considering that catecholamines participate in stimulating growth in bacteria such as *E. coli* and development of biofilms with *Salmonella*. Along with observations of the effect of catecholamines on oral *Streptococcal* species, not including *Streptococcus pneumoniae*, revealing a varied affect on growth and biofilm formation between species. *S. pneumoniae* is the most common cause of bacterial pneumonia worldwide and is known to develop biofilms in otitis media infections. We aim to determine if norepinephrine stimulates *S. pneumoniae* growth and biofilm formation *in vitro*, and if the clinical isolation history of the bacteria is a factor in this response. The gathered data will provide information on catecholamine participation on pathogenesis.

**Methods:** Four clinical strains of *S. pneumoniae* of which two were isolated from nasal swabs and two from blood samples were cultured in Todd Hewitt broth with yeast extract; treated culture included 10<sup>-4</sup>M of norepinephrine or epinephrine. Biofilm experiments utilized cell-culture microtiter plates in which control and treated bacteria were added to respective wells and incubated for 24 hrs. Changes in growth and biofilm formation were determined by measurement of optical density.

**Results:** The data revealed a 4 to 6 fold increase in growth in catecholamine treated *S. pneumoniae* collected from blood samples, while a 3 to 4 fold increase in nasal swab samples as compared to non treated cultures. Catecholamine treatment of *S. pneumoniae* revealed no significant difference in the formation of biofilms.

**Conclusion:** The results demonstrate that *S. pneumoniae* exposed to catecholamines increases growth which could allow for increased colonization of the bacteria in the respiratory tract thereby increasing the chances of infection. The difference in the magnitude of fold increase in blood samples versus nasal swab samples could indicate that cultures vary their responses to catecholamines as infection enhances. Biofilm data shows that there is no significant difference in biofilm formation 24hrs post treatment versus control indicating that catecholamines have no affect on stimulating biofilms in *S. pneumoniae in vitro*.

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Chronic pain: Crucial symptom in a case of neuroborreliosis analysis of three cases

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**Background:** Neuroborreliosis is a one of the form of Lyme disease, which involving nervous system. The symptomatology results from disease's localization in any part of nervous system.

**Methods:** Retrospective review of the patients from pain outpatient Clinic, with untreatable, chronic pain.

**Results:** Case 1

A 41 years old woman presented with pain and sense disturbances of superior and inferior extremities. The pain intensification was estimated in 6 points, numerical rating score (NRS). The patient denied tick bite and erythema migrans (EM). Physical examination showed sense abnormalities corresponding to patient's complaints. EMG showed the signs of peripheral neuronal damage involving both motor and sensory fibers. Analgesic treatment was ineffective. The antibodies against *Borrelia burgdorferi* (B.b.) were detected in serum, confirmed by Western blot assay (W.b.).

Polineuropathy in course of neuroborreliosis was diagnosed.

Case2

A 55 years old woman after surgery for chondrofibroma of vertebral canal, reported severe pain involving the whole length of spine, with bilateral radiation to intercostals spaces and pelvis, accompanied by muscle and joint pain. Pain intensity was 10 points according to NCS. She did not notice tick bite, but EM was recognized. Analgesic treatment was ineffective. MRI studies showed low grade discopathy which did not explain patient complaints. The antibodies against B.b. were detected in serum, confirmed by W.b. assay. CSF examination showed a lymphocytic pleocytosis, a moderately elevated protein level. Antibodies against B.b. were found in CSF. Generalized borreliosis with CNS and joints involvement was diagnosed.

Case 3

A 31 years old woman presented severe pain NCS=8 points in anterior femoral region. No tick bite was noticed. EM was recognized. Analgesic treatment did not cause any alleviation of pain. Physical examination showed skin paresthesia corresponding to region of pain. The antibodies against B.b. were detected in serum, confirmed by W.b. assay.

Femoral nerve radiculopathy in course of neuroborreliosis was diagnosed.

All patients were treated with antibiotics: ceftriaxone.

**Conclusion:** In cases, when pathomechanism of pain is difficult to understand, neuroborreliosis should be included as one of differential diagnosis.

The real diagnosis and treatment result in complete withdrawal of pain and other clinical symptoms.

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The emerging of non-AIDS related neoplasms in the era of combined antiretroviral therapy

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**Background:** The introduction of combined antiretroviral therapy (cART) since the year 1996, contributed to a rapid, significant drop of frequency of all AIDS-defining opportunistic infections and some selected AIDS-related tumors (like Kaposi'sarcoma), with a consequent, remarkable reduction of both morbidity and mortality rates associated with these disease complications.

**Methods:** Our cohort of over 1,700 HIV-infected patients followed in two connected outpatient centres by the same physician staff were prospectively followed since the year 2000 (9 years), with special interest focused on the diagnosis, treatment and outcome of non-AIDS related malignancies.

**Results:** Among hematological malignancies other than non-Hodgkin's lymphoma and primary central nervous system lymphoma, we observed three cases of acute myelogenous leukaemia and 4 episodes of Hodgkin's lymphoma. A greater number of solid tumors involved different organs and sites: laryngeal cancer (8 cases, with 6 episodes of papillomatous laryngeal cancer), rhinopharyngeal squamous carcinoma (4 cases), adenocarcinoma of the lung (6 cases), gastric adenocarcinoma (3 episodes), esophageal carcinoma (2 patients), prostate cancer (4 cases), bladder adnecarcinoma (3 episodes) pancreatic adenocarcinoma (2 cases), and squamos anal carcinoma (2 episodes). Some of these malignancies have been reported with extremely rare frequency until now (usually as single-case anecdotal reports), in particular before the cART era. The patient's age ranged from 34 to 67 years, the mortality rate of these episodes was very elevated (over 80%), and occurred 3-41 months after diagnosis, despite appropriate surgical and/or cytotoxic chemotherapy and/or radiotherapy.

**Conclusion:** The significantly increased life expectancy of HIV infected patients in the cART era was characterized by a proportionally increase of non-AIDS-defining tumors, which may depend on the advanced mean patients' age, their prolonged exposure to risk factor, the persisting functional immune system imbalance, and probably some direct oncogenic property of HIV itself, even when a "quantitative" recovery of CD4+ lymphocyte count has been achieved thanks to cART. The differential diagnosis of non-AIDS-associated tumors may be delayed by the low clinical suspicion, and their frequency to mimick and/or overlap infectious complications. Further epidemiological and clinical investigation is strongly warranted, to increase the awareness of this emerging phenomenon.

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**Type: Poster Presentation**

The role of the tuberculosis in outcome and treatment failure in HIV-infected Cambodian children  
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**Background:** Tuberculosis and AIDS are considered to be a “deadly synergy” among HIV-infected children in Southeast Asia and sub-Saharan Africa.

**Methods:** We have assessed a cohort of 118 HIV-infected children, 72 of them receive antiretroviral therapy (HAART) for an average of 38 months (12-63 months), for the prevalence of tuberculosis, anti-tuberculosis therapy, immune response to HAART and coincidence of other opportunistic infections. Table 1 shows a cohort of 40 HIV-infected children with tuberculosis (36 pulmonary and 4 extrapulmonary) compared to 32 HIV –infected children without tuberculosis but with AIDS and other opportunistic infections.

**Results:** Children with tuberculosis were significantly younger ( $P<0.01$ ) (7.8 vs. 12.8 years) and had significantly lower CD4 absolute count (180 vs. 302) in comparison to HIV-infected children without tuberculosis. Immune category 3 (82.5% vs. 59.4%,  $P<0.05$ ) and CDC category C (45% vs. 9.4%,  $P<0.001$ ) were also more frequently observed among HIV-infected children with tuberculosis.

Proportion of deaths (3 children within 5 years within 72 children on HAART) or treatment failure on 1-st line HAART (6 children) was similar in both groups of children (with tuberculosis and without tuberculosis). Frequency of opportunistic infections (more than 5 per year) among tuberculosis children cohort was also higher among tuberculosis HIV-infected children (70% vs. 21.9%,  $P<0.001$ ). Otitis media (55% vs. 31.1%  $P<0.05$ ), oropharyngeal candidiasis (45% vs. 21.9%  $P<0.04$ ), Immune reconstruction syndrome (IRS) (40% vs. 12.5%,  $P<0.001$ ) occurred significantly more frequently in HIV-infected children with tuberculosis in comparison to children without tuberculosis. Anti tuberculosis treatment (Isoniazide + Rifampin + Pyrazinamid) was given 4-8 weeks before onset of HAART (lamivudine + didanosin + efavirens), to decrease the probability of IRS. Most children received 6 months treatment with Isoniazide + Rifampin + Pyrazinamid (60 %) or Ethambutol (40 % children older than 10 years), 7-9 months received 30 % of children and 10 – 12 months 20 % of children (extrapulmonary tuberculosis or treatment failure).

**Conclusion:** In conclusion, occurrence of several opportunistic infections decreased absolute CD4 count, immune reconstruction syndrome, younger age, severe anaemia, more than 5 opportunistic infections within 6 months, CDC category C and immune stage 3 were significantly associated with tuberculosis in HIV-infected children.

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AIDS-associated mycobacterial infections in a large urban hospital during the HAART era (2003-2006)

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**Background:** Mycobacterial infections in HIV are well recognized. The aim of this study is to describe mycobacterial infections in HIV positive patients in a large urban setting with a large immigrant population in the HAART era by assessing mycobacterial species prevalence, clinical parameters, radiological findings, and survival rates.

**Methods:** All mycobacterial cultures reported positive at Los Angeles County-University of Southern California Hospital from 2003 to 2006 were reviewed and their respective clinical charts analyzed. This descriptive retrospective study was approved by our local Institutional Review Board.

**Results:** From 2003 to 2006, 965 mycobacterial isolates were obtained from 814 patients. Of these, 201 (25%) of the patients were HIV positive and accounted for 262 (27%) of the isolates. The average age of the patients was 42 years and the male:female ratio was 166:35. 65% of the patients' country of origin was outside the United States. The mean CD4 count was 137 cells/mm<sup>3</sup> and approximately 25% of patients were receiving antiretrovirals at the time of mycobacterial diagnosis. Of the HIV positive isolates, 30% were *Mycobacterium tuberculosis* and 70% were non-tuberculosis mycobacterial infections [*Mycobacterium avium* (39%), *M. flavescens* (1%), *M. fortuitum* (11%), *M. goodii* (12%), *M. kansasii* (3%), *M. simiae* (2%), *M. terrae* (0.8%), and *M. xenopi* (0.4%)]. For the *Mycobacterium tuberculosis* and the non-tuberculous mycobacteria, the body sites from which the isolates were obtained included pulmonary (64% and 80%), gastrointestinal (10% and 7%), skin/soft tissue (18% and 4%), hematologic (1% and 8%), central nervous system (5% and 0.5%) and genitourinary (1% and 0.5%) respectively. 83% of all patients had abnormal chest radiographs. 3% of the *M. tuberculosis* isolates were multi-drug resistant. The survival rate of patients was 91% at 6 months, and 49 of the patients were lost to follow up.

**Conclusion:** Unlike previous studies that report *M. tuberculosis* species as the predominate strain in AIDS-associated infections our survey demonstrated that *Mycobacterium avium* infections are the most common. This has clinical implications when treating AIDS patients with a positive acid-fast culture as the identification of the specimen is made.

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A prospective cohort study of immunologic and virologic outcomes in patients with HIV/AIDS and hepatitis virus co-infection in Jos, Nigeria

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**Background:** In the era of highly active antiretroviral therapy (HAART), hepatitis B and C virus (HBV and HCV) co-infection have emerged as significant co-morbid conditions. Local reports indicate that co-infection is not uncommon in Nigeria as in other sub-Saharan African countries. Whether treatment outcomes of HIV mono-infected patients differ from those with co-infection remains largely unknown. We hypothesised that co-infected patients will have lower CD4+ count recovery and viral load reduction following HAART.

**Methods:** A cohort study in antiretroviral therapy-naïve HIV-infected adults involving 150 cases (HIV and hepatitis co-infection) and 150 controls (HIV infection only). Patients' care was according to the National guidelines and patients received first line therapy mostly comprising Lamivudine, Stavudine and Nevirapine. Additionally, abdominal ultrasound and prothrombin time tests were done when necessary. Medication adherence was monitored using pharmacy log system, and CD4+ cell counts and HIV viral load (VL) were compared at baseline, 3 and 6 months of therapy.

**Results:** There were 98(65.3%) and 96(64%) female cases and controls (p=0.79) respectively. The mean ages of cases and controls were 38±8.4 and 37±8.9 years (p=0.20) respectively. Cases comprised 73(48%) HBV, 70(47%) HCV and 7(5%) with HBV plus HCV infection. Medication adherence was >95% in both arms. Attrition rate was 2.7%(8); seven of these were co-infected. Five cases (3.3%) compared to zero controls developed clinical hepatitis. The proportions of patients with CD4+ count <200 cells/µl among cases and controls were 111(74%) and 109(72%), p=0.36 at baseline; 66(45.5%) and 64(42.7%), p=0.21 at 3 months; 60(42%) and 56(37.6%), p=0.40 at 6 months respectively. Significantly, more controls (60.7%) had CD4+ increases ≥50cells/µl at 3 months compared to 37(54.5%) HCV+ cases (p=0.03). No significant difference in CD4+ counts between controls and cases at 6 months. The baseline median VL for cases and controls were log<sub>10</sub>104.95 and log<sub>10</sub>104.83 (p=0.17) respectively. The proportions of cases and controls with undetectable VL at 3 and 6 months were 96(66.2%) and 97(65.5%); p=0.74, and 116(81.1%) and 97(79.3%); p=0.10 respectively.

**Conclusion:** Co-infection has limited impact on immunologic and virologic outcomes, but may be an important cause of hepatotoxicity.

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Pulmonary microbiology of HIV positive subjects with community-acquired pneumonia (CAP) with special emphasis on *Mycoplasma pneumoniae*

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**Background:** A great deal of effort has been devoted to understanding the role of AIDS-associated mycoplasmas in recent years. However, the role of *Mycoplasma pneumoniae* in HIV disease remains unclear.

**Methods:** We studied 300 adult HIV infected persons (200 with community-acquired pneumonia (CAP) and 100 with no respiratory illness) and 75 HIV uninfected persons with CAP and analysed the prevalence of respiratory pathogens.

**Results:** Prevalence of *M. pneumoniae* was 17% by induced sputum and 11.3% by throat swab culture in HIV positive subjects. Seroprevalence of anti-*M. pneumoniae* IgM was 11.7% by ELISA and 14.3% by gelatin microparticle agglutination test. Prevalence of *M. pneumoniae* among HIV negative cases was relatively low. *Streptococcus pneumoniae* was predominant (28%) among subjects with lower respiratory disease, whereas *Staphylococcus aureus* (15%) was more common among cases with upper respiratory illness. Rales (P=0.001), pharyngeal erythema (P=0.02), cervical adenopathy (P=0.004) and crepitations (P=0.001) showed significance in relation to *M. pneumoniae* positivity. Statistical significance was observed with regard to total lymphocyte count (P=0.02) and erythrocyte sedimentation rate (P=0.04), and *M. pneumoniae* positivity.

**Conclusion:** The prevalence of *M. pneumoniae* in HIV infected subjects was relatively higher than HIV uninfected subjects with respiratory disease.

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Kaposi's sarcoma (KS) and AIDS. Survival study

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**Background:** To evaluate the factors associated with survival time of AIDS patients with KS.

**Methods:** We studied the medical records of patients with AIDS / KS, adults of both sexes admitted for clinical follow-up at the Infectious Disease Unit from 1985 to 2008. We used a protocol with demographic data, CD4 cell count, extension of the lesion ( localized or disseminated ) and survival time of each patient. The EPI-INFO 3.4.3 was used to analyse the results.

**Results:** Among 136 patients with AIDS and cancer we found anal carcinoma 4( 2.9%) Hodgkin's lymphoma 7 ( 5.1% ),non-Hodgkin's lymphoma 26 ( 19.1% ), KS 90 ( 66.2%), other 9 ( 6.6% ). Among the cases of KS, 86 men and 4 women, disseminated disease 62 ( 68.9%), 3 women ( 4.8% ), 59 men ( 95.2% ); localized 28 ( 31.1% ), 1 woman ( 3.6% ), 27 men ( 96.4% ). Average age of 37.27 ± 9.09 SK, localized 38.92 ± 10.06, disseminated 36.5 ± 8.59 ANOVA p=0.25. Median CD4 localized 315 cells/mm<sup>3</sup> ( 27-1088 ), disseminated 297 cells/mm<sup>3</sup> ( 11-778 ) Kruskal-Wallis p=0.41, no statistical difference. Survival time of KS total median 304 days ( 0-4090 ), localized median 270 ( 0-4090 ), disseminated 330 ( 10.0-3105 ) Kruskal-Wallis p=0.96, no statistical difference. The median survival time of those on antiretroviral treatment was 721 days ( 0.0-4090 ) and without antiretroviral treatment 120 days ( 2.0-2555 ) Kruskal-Wallis p=0.0000.

**Conclusion:** The use of antiretrovirals drugs was the only determinant of improved survival time among patients with AIDS and KS.

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Opportunistic infections in central nervous system and associated neurologic disorders in HIV infected patients in a Western Hospital of Venezuela, 2007-2009

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**Background:** HIV-related central nervous system (CNS) disorders and infections represent substantial personal, economic, and societal problems. However, its understanding is still lacking in areas of some developing countries where HIV programs are still limited.

**Methods:** Herein we describe epidemiology, clinical features and diagnosis of opportunistic infections in CNS and associated neurologic disorders in HIV infected patients in a western hospital of Venezuela, January 2007 to September 2009. Monthly records of confirmed HIV cases from the epidemiological archives were collected. All the patients with opportunistic infections in CNS or associated neurologic disorders were selected. All the clinical relevant variables were considered in this analysis.

**Results:** A total of 228 patients with HIV infection were treated during the study period; thirteen of them (5%) presented neuropathology. Twelve (92%) were male, mean age was 35 years-old, previous HIV was diagnosed in nine patients (69%) with a mean of 9 months, just 4 (30%) were receiving antiretroviral treatment. The most common presented symptom was fever 6/13 (46%), followed by muscular strength loss and headache 5/13 (38%), convulsions and conscience alteration also had an important incidence 4/13 (30%) among other findings. Neurological diagnoses were: 7/13 (53%) Cerebral Toxoplasmosis, 3/13 (23%) Central nervous system *Cryptococcus neoformans* infection, 2/13 (15%) Cerebral Tuberculosis and 1/13 motor-sensitive neuropathy (7%). Most of them (84%) also had associated infections or diagnoses: pancytopenia (46%), *Pneumocystis jirovecii* pneumonia (38%) and candidiasis (30%); Kaposi sarcoma, diabetes, pulmonary tuberculosis and chronic diarrhea were also presented. CD4 count mean was 120 cells/mm<sup>3</sup>, CD8 count mean was 571 cells/mm<sup>3</sup>, and viral load was 12,120 copies/mm<sup>3</sup> log 3.22 . CFR was 15%.

**Conclusion:** In this study neuroinfection was the first cause of hospitalization in HIV infected patients with neurologic symptoms. Toxoplasmosis was the most common opportunistic parenchymal brain lesion. The majority of patients had associated other opportunistic diagnosis. Proper studies to determinate the real country prevalence and characteristics of these neuropathologies are necessary. Early diagnosis is important in order to prevent potential complications and sequels. Even more, clinical follow-up studies of these infected patients are also needed in order to evaluate the evolution of disease and development of associated conditions.

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**Type: Poster Presentation**

HIV and opportunistic infections in a western hospital of Venezuela, 2007-2009

**A. Herrera-Martínez**<sup>1</sup>, Y. Herrera<sup>1</sup>, A. M. Pineda<sup>2</sup>

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**Background:** Numerous opportunistic infections occur in HIV infected patients, particularly in those without access to antiretroviral therapy. In Venezuela it is known a high relationship between TB, PJP and candidiasis in HIV infected patients.

**Methods:** We describe epidemiology, diagnosis and clinical features in HIV infected patients admitted to the Central Hospital of Barquisimeto, Western Venezuela, from January 2007 to September 2009. Monthly records of confirmed HIV cases from the epidemiological archives were collected. Only patients with opportunistic infections were selected. All the clinical relevant variables were considered in this analysis.

**Results:** A total of 228 patients with HIV infection were treated in this medical center during the study period; Forty three (84%) were male; mean age was 36 years 51 (22%) had associated opportunistic infections.; Most cases due to sexual transmission (96%), 56% were heterosexual, 75% promiscuous, with a mean of four couples, beginning sexual relations at 17 years old. Fourteen (27%) patients had previous HIV diagnosis, with a mean of 25 months, just 8 (15%) were receiving treatment; 2/51 (4%) also had other sexual transmission diseases. Most common presented symptoms were fever 21/51 (41%), cough 19/51 (37%), weight loss, vomits and dyspnea had also an important incidence among others. Half patients had anemia. Pneumonia was the most common diagnosis 23/51 (45%), 65% caused by *Pneumocystis jirovecii*; followed by wasting syndrome 18/51 (35%), candidiasis (mucocutaneous, tracheal, esophagic), pancytopenia 14/51 (27%), and chronic diarrhea 11/51 (21%) among other diagnosis. TB had a low incidence during the study period (11%). Patients, in which lymphocytic count was available, had a CD4 count mean of 168 cells/mm<sup>3</sup> and CD8 count of 813 cells/mm<sup>3</sup> and a viral load of 62,913 copies/mm<sup>3</sup> log 3.06. CFR was 15%.

**Conclusion:** A significant incidence of pneumonia due to *Pneumocystis jirovecii* was observed. Since the introduction of HAART in Venezuela, a considerable reduction of opportunistic infections was observed but HIV diagnosis usually occurs after opportunistic infections presentation, then, proper diagnosis and prevention campaigns are for outmost important not only in Venezuela, also in other developing countries in order to reduce the incidence of complications and to increase the quality of life of HIV infected patients.

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Prevalence of occult hepatitis B infection among HIV infected patients at an inner city clinic  
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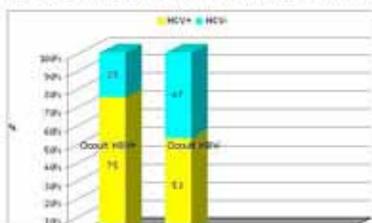
**Background:** Morbidity and mortality is higher in HIV/HBV coinfectd than HIV monoinfected patients. Occult HBV infection i.e. isolated positive Hepatitis B core antibody (anti HBc) but Hepatitis B surface antigen (HBs Ag) and surface antibody (HBsAb) negative with HBV DNA is a form of chronic HBV infection that impacts HIV management.

**Objective:** To determine the prevalence and patient characteristics of occult hepatitis B infection in HIV infected patients attending an outpatient HIV clinic in New York.

**Methods:** An electronic database of 630 HIV positive patients at NYC Harlem Hospital Center's Infectious Diseases clinic between June 2007 and January 2009 was reviewed. Total of 82 patients with HBV serology showing HBsAg-, HBsAb -, anti HBc+ were identified. Of these 57 (69.5 %) had HBV DNA testing information. Information on Demographic variables, Hepatitis C antibody (HCV Ab), antiretroviral therapy (ARV), CD4 and HIV viral load were collected.

**Results:** Among the final study population, majority were males (n=36, 63.2%), African American (n=34, 59.6%), mean age of  $48.7 \pm 6.2$  yrs, on ARV (n=39, 70.2%), had CD4 > 200 (n=44, 77.2%), VL < 50 (n=30, 52.6%) and 47.4% (n=27) tested positive for HCV. Approximately 7% (n=4) of study population met the definition of occult HBV infection. 22% of patients not on ARV (n=18) had positive HBV DNA, whereas none of patients on ARV (0%, n=39) had detectable HBV DNA (p=0.007). All patients on ARV (n=39) were on a regimen with anti HBV activity. Of those with occult HBV infection, 50% (n=2) were males, 100% (n=4) were African American, mean CD4 count was  $408 \pm 280$ , 75% (n=3) had HIV viral load > 50, 100% were not on ARV and 75 % tested positive for HCV.

Prevalence of HCV by Occult HBV status



#### Prevalence of HCV by Occult HBV status

**Conclusion:** Though the overall prevalence of occult HBV (7%) in our community clinic is lower than historical prevalence of 11%, among those not on ARV the prevalence was much higher (22%). Further, high proportion of patients with isolated anti HBc also tested positive for HCV ab (47.4%). Therefore clinicians should have a high index of suspicion for occult HBV among HIV infected patients whose hepatitis panel is positive only for core antibody. Clinicians should have a high index of suspicion for occult HBV among HIV infected patients whose hepatitis panel is positive only for core antibody particularly those not on ARV and with HCV coinfection.

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Predictors for hepatic carcinoma surveillance screening in a cohort of hepatitis B and HIV co-infected patients in a large urban HIV clinic

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**Background:** Co-infection with hepatitis B virus (HBV) and HIV is common. Approximately 70-90% of HIV-infected individuals have evidence of past or active infection with HBV. It is unclear what predicts the likelihood that patient with active HBV/HIV infection will receive appropriate surveillance screening for hepatic carcinoma. We hypothesized that patients who have successful HIV treatment are likely to have successful hepatic carcinoma surveillance screening.

**Methods:** To evaluate predictors for surveillance screening of hepatic carcinoma within the period of July 2008 to June 2009. A retrospective review of medical records of patients with active HBV and HIV co-infection for hepatic ultrasound, alpha fetoprotein (AFP), liver biopsy, HBV viral load, HBV e Ag screenings and gastroenterologist (GI) referral.

**Results:** Of 77 HIV infected patients whose charts were randomly selected, 55.8% (n =43) obtained care during the study period. The demographic was 58% male (n=25). 7 of 43 patients (n =16.3%) had active HBV infection. All 7 patients were referred for hepatic ultrasound, only 43% (n =3) had the procedure performed with 66% (n =2) abnormal. All 7 patients had AFP screening, hepatitis B DNA measurement, hepatitis B e Ag screening and GI referral. 1of 7 patients had an elevated AFP while all 7 had detectable HBV DNA and 43% (n =3) had positive hepatitis B e antigen. 71% (n =5) had an initial GI evaluation while only 28% (n =2) had >1 GI follow up. 28% (n =2) were no show. None of the 7 patients had liver biopsy.

In patients who did not obtain an abdominal ultrasound or who did not follow up with GI or no show, there was no significant difference in the CD4 count range when compared to those who obtained ultrasound or follow with GI. 57% (n =4) of patients had issues with adherence as documented in the chart. All 4 patients did not obtain abdominal ultrasound, 3 did not follow up with GI and 1 was no show.

**Conclusion:** 16.3% of our cohort of HBV/HIV co-infected patients had active HBV infection. Non-adherence to follow up plan is the reason in patients who did not obtain abdominal ultrasound or GI evaluation. CD4 count did not appear to be a predictor of probability to obtain hepatic carcinoma screening. Strategies that improve adherence will likely improve the success of hepatic carcinoma surveillance screening.

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Virological and biochemical evolution of HIV-HBV co-infected patients treated with tenofovir  
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**Background:** Tenofovir (TDF) is a nucleotide with dual activity against HIV and hepatitis B (HBV) viruses. Thus, it has become the antiviral of choice for HIV-HBV co-infected patients when treatment for both viral infections is indicated.

**Methods:** We reviewed 19 medical records of patients who received tenofovir as part of the antiviral treatment. Five patients had previously received 3TC (lamivudine) as part of HAART. Immunological parameters were recorded for HIV infection (CD4/mm<sup>3</sup>), AST, ALT and viral loads for HIV and HBV were followed over an average period of 27 months (range, 3-44m).

**Results:** All patients were male and the median age was 42 years (range, 29-65). Concomitant antiviral medications were 1 NRTI +1 NNRTI (3TC/FTC) in 13 patients, 1 ritonavir-boosted PI + 1 NNRTI in 1 patient, 1 PI + 1 NRTI (3TC or FTC) in 4 patients and 1 IP ritonavir-boosted + 2NRTI (AZT + 3TC) in 1 patient. The mean HBV DNA at baseline was: 6.53 log. Six patients presented at baseline with normal ALT and 13 a mean elevation of AST/ALT x 3,8/x 5. The results of liver biopsy were available for 6 patients: 1 had cirrhosis, 3 mild chronic hepatitis (stage 1), 2 moderate chronic hepatitis (stage 2). 17 patients were HBeAg positive, and two were HBeAg negative at baseline. Seven out of 17 patients underwent HBeAg loss and seroconversion to anti e. Loss of HBsAg was documented in 1 patient. HBV-DNA follow up was available in 17/19 patients (2/19 pending). 13/17 achieved HBV-DNA undetectability (mean 58 weeks). 4/17 reduced their HBV-DNA by a mean of 4.75 log (range: 3.1 to 6.2 log) with a mean follow up of 33 weeks. Among 13 patients who had abnormal ALT at baseline, 9 normalized during the follow up. Among patients previously experienced with 3TC: 4 achieved undetectable HBV DNA and the other decreased 6.2 log from baseline.

**Conclusion:** As pointed out by the international literature, we found a high virological and biochemical efficacy of TDF in HIV-HBV co-infected patients, in terms of HBV antiviral inhibition in both previously treated with 3TC and naive patients.

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An assessment of the seroprevalence of hepatitis A antibody and vaccination among HIV patients in an inner city HIV clinic

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New York, NY, USA

**Background:** Routine hepatitis A virus (HAV) screening and vaccination is recommended in the guidelines for the management of high risk persons infected with the human immunodeficiency virus (HIV). Scientific evidence shows that patients with no immunity against HAV suffer prolonged clinical course and hepatitis related liver abnormalities if infected with acute hepatitis when compared to those who have immunity.

The objective of this study is to assess the seroprevalence of anti-HAV and hepatitis A vaccination among high risk patients in the outpatient HIV clinic.

**Methods:** A retrospective review of medical records of high risk adult patients (age > 18y) who attended the outpatient HIV clinic between the period of July 2007 - December 2008. The clinic provides primary care services to approximately 750 HIV-infected patients. The clinic demographics include 47% women, 83% Blacks and 14% Latinos.

**Results:** 244 high risk HIV infected adult patients had at least one visit within the study period. All received anti-HAV screening, of which 87.3% (n=213) tested negative for anti-HAV and 12.7% (n=31) tested positive. Among those who tested negative, 12% (n=26) had only 1 visit and did not follow up. Of the remaining 187 patients who followed up, 60% (n=113) received at least 1 dose of hepatitis A vaccine and 40% (n=74) did not receive a single dose of vaccine. Among the total 244 patients, 18 had perinatally acquired HIV infection. 88% (n=16) of these patients with perinatal HIV had positive anti-HAV and are responsible for 52% of the total 31 patients with positive antibody. All the 31 patients with anti-HAV had more than 1 visit.

**Conclusion:** In our cohort of high risk HIV infected adults, patients with perinatal HIV are more likely to have anti-HAV. The seroprevalence of anti-HAV in our cohort is low (12.7%) and similar to the rate reported in other studies. The hepatitis A vaccination rate of 60% in our cohort is below the target of greater than 90% vaccination rate needed for herd immunity to lessen the impact of the disease in the community. The high rate of anti-HAV in patients with perinatal HIV highlights the effectiveness of the strategy for childhood hepatitis A vaccination schedule. Comparable success in adult patients will require a higher adherence to vaccination recommendations in order to prevent HAV infection in HIV patients.

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Central nervous system opportunistic infections (CNS-OI) in HIV-infected children from Buenos Aires, Argentina

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**Background:** CNS-OI is a frequent event in adult AIDS patients (pts) with decreasing rates in the HAART era. The spectrum of CNS disease is quite different in paediatric AIDS and there are very few reports about the incidence rates of CNS-OI in children.

The objective was to describe the clinical course and outcome risk of CNS-OI in a perinatally HIV-infected cohort from Argentina.

**Methods:** Retrospective study among 397 HIV infected children followed-up from 1998-2008. All pts with confirmed CNS-OI infection were included. Pts with congenital infections, other neurological disorders or without an etiologic agent identified were excluded.

**Results:** Out of 397 pts, 23 (6 %) developed 24 CNS OI during follow-up (0.8/100 person-years). Bacterial meningitis (BM) 6 (25%), which were *N meningitidis* 2, *St. pneumoniae* 2, *St. agalactiae* 1 and *L monocytogenes* 1. TB 5 (21%), *C neoformans* (Crypto) 4 (17%), toxoplasmosis 2 (8%), JCV 2 (8%), MAI 1 (4%), CMV 1 (4%), EBV-CNS lymphoma 1(4 %), measles SSPE 1 (4%).

Common initial symptoms were fever (61%), neurological deficits (26%) and headache (22%).

At diagnosis of OI, mean age: 7.6 ± 6 years, mean CD4: 15 ± 14%, mean viral load: 4.8 ± 1 log. CNS OI was the first manifestation of HIV in 6 pts (26%); 17 were on HAART, 11 of them (48%) had poor adherence. No IRIS was assumed.

BM and TB were more prevalent in the youngest pts (median age: 5 vs 12 years. p=0.03).

Median CD4 % of BM was higher than the rest (30 vs 5 p=0.007)

Neurological sequelae were present in 6. Six pts died (26%). Causes of death were 1 TB, 1 MAI, 1 JCV, 1 EBV-Lymphoma, 1 CMV and 1 Crypto. CNS-OI mortality was higher than in the rest of pts, 6/23 vs 31/374 (p=0.01) and KM survival log rank p=0.03.

**Conclusion:** In developing countries OI are still prevalent, especially in pts with late diagnosis of HIV, poor adherence or advanced AIDS disease. In this series, a significant high mortality was observed. BM and TB were more prevalent in younger children. In older children, the spectrum was more similar to adults and the relation with low CD4 was more accurate.

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**Type: Poster Presentation**

Parvovirus B 19 infection in immunodeficient patient

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**Background:** The parvovirus B 19 belongs to the Erythrovirus genus of the Parvoviridae Family. It was discovered in 1974 by Yvonne Cossart in an assay for hepatitis B. The Infection with Parvovirus B 19 is global. The normal mode of transmission is by droplet infection or from person to person through direct contact with respiratory secretions. A high prevalence of Ig G antibodies to B 19 parvovirus was found in the 75% of adult patients. Parvovirus B 19 has a specific tropism for erythroid progenitor cells. In HIV patients there may be persistent anemia with a pure red cell aplasia.

**Methods:** : A 36 year old patient with a medical history of smoking, drinking and addiction to inhalant drugs arrived at the hospital's emergency department with a severe anemic syndrome which had been evolving during three months. In addition, a week prior to the patient's arrival, he had suffered from a syncoptic episode. On examination, the patient was feverish, tachycardiac, normotensive, with no orthostatism, and dyspneic. The mucous and skin were pale. He presented a 3/6 heart murmurs without irradiation

**Results:** Serologic tests for the human immunodeficiency virus were positive CD 4 count of 186/mm<sup>3</sup>. The patient had a hematocrit of 6% and the hemoglobin level was 1,06 g per deciliter. The level of lactate dehydrogenase was 694. The mean corpuscular volume was 76,47  $\mu$ m<sup>3</sup> with a mean corpuscular hemoglobin concentration of 29g per deciliter. The levels of blood glucose, electrolytes, calcium, urea nitrogen, creatinine were normal. No reticulocytes were found on several examinations. Examination of the bone marrow biopsy showed pure red cell aplasia, Myeloid elements showed complete maturation. A direct Coombs test was negative. Anti parvovirus b 19 M antibodies were positive. The patient received specific treatment with intravenous human immunoglobulin 400 mg/kg/ day over 5 days. He showed improvement after immunoglobulin treatment. The patient was discharged from the hospital with a hematocrit of 22%. Finally, he followed up medical examination in an outpatient clinic.

**Conclusion:** Although parvovirus B 19 produces generally banal infections in immunocompetent patients, immunodepressed patients may develop acute or chronic anemia due to the lack of protective antibodies, along with hiccup or chronic bone marrow aplasia.

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Strategies to enhance Hepatitis B disease surveillance in Human Immunodeficiency Virus / Hepatitis B Virus co-infected patients

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**Background:** Human immunodeficiency virus (HIV) and Hepatitis B virus (HBV) infection share common routes of transmission and hence co-infection is common. Co-infection with HBV/HIV is associated with higher levels of HBV DNA, lower rates of spontaneous HBV e-antigen sero-conversion, more severe liver disease and increased rates of liver-related mortality.

Chronic viral hepatitis has become an important cause of morbidity and mortality in HIV co-infected patients. Starting treatment appropriately, monitoring both HIV and HBV response to treatment appropriately and surveillance for liver disease progression is imperative. Current guidelines recommend 6 monthly alpha fetoprotein (AFP) and liver ultrasound scans for hepatocellular carcinoma (HCC) surveillance.

In 2009 an audit of HBV/HIV co-infected patients at our hospital revealed that while HIV disease was being monitored as per guidelines, markers for HBV replication and sero-conversion were measured on a variable basis. We found suboptimal rates of monitoring for the development of HCC using either AFP or liver ultrasound. Finally, liver biopsies were infrequently requested in those who meet the criteria.

**Methods:** As a result of the above findings a number of interventions were made:

Generation of a frequent specific clinic for HIV/HBV co-infected patients with multidisciplinary team input.

Establishment of a regular teaching program with regards to current practice guidelines for managing and monitoring patients co-infected with HIV/HBV.

Collaboration with the Radiology Department to create a more efficient and effective method of surveillance radiology for these patients including automatic recall for surveillance scans.

**Results:** Since establishment of the co-infection clinic HBV DNA continues to be monitored appropriately. More patients had AFP levels checked on a 6 monthly basis (73% vs 20%). A greater proportion of patients (89% vs 45%) were booked for surveillance ultrasound.

**Conclusion:** Cohorting of HIV/HBV co-infected patients results in improved adherence to guidelines for HBV surveillance and will facilitate future HBV studies including HBV surface antigen quantification, delta virus surveillance and hepatic elastography.

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Risk of an outbreak of Kaposi's sarcoma associated with HIV/AIDS in Mozambique

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**Background:** Infection with HIV-1 has been considered an important co-factor in the development of Kaposi's sarcoma (KS), and antiretroviral therapy (ARV) has proven to be benefit to diminish KS-AIDS worldwide. In many African countries, KS has risen dramatically since the onset of the AIDS epidemic. Human herpesvirus 8 (HHV-8) is the etiological agent of KS. In Mozambique, no large study on HHV-8 infection has been conducted yet. The present study aimed to: (i) present and compare the frequencies and titers of HHV-8 antibodies in serum samples of KS and non-KS individuals from Mozambique, (ii) identify variables associated with HHV-8 infection, and (iii) search for predictive markers for KS development.

**Methods:** In-house immunofluorescence assays were employed for HHV-8 antibody detection in sera from individuals seen at public health centers in Northern ( $n=208$ ), Central ( $n=226$ ), and Southern ( $n=318$ ) Mozambique, and in 24 KS patients. HIV serology was used for comparative analysis. Socioeconomic, demographic and clinical data were assessed by interview. The study was approved by all participant institutions.

**Results:** The overall frequency of HHV-8 antibodies was 21.4% and, in spite of the diversity of epidemiological characteristics of the tested individuals, did not differ significantly among regions: 18.7%, 24.3% and 21.4% in Northern, Central and Southern Mozambique, respectively ( $X^2$ , 2.37;  $p=0.305$ ). The variables that were significantly associated with the presence of HHV-8 antibodies were gender, age, level of education, number of siblings and HIV serostatus, but these differed across the regions. The overall prevalence of HHV-8 seropositivity increased with age. High titers of HHV-8 antibodies also against latent antigens were detected in KS patients and in non-KS individuals co-infected with HIV-1, and this increased titer could have predictive value. Large number of participants (30% to 50%) unknown its real HIV serostatus, thus they were not under ARV therapy.

**Conclusion:** These results demonstrate that Mozambique is another country in Africa with endemic HHV-8 infection and that, because of the AIDS epidemic suggests universal access to antiretroviral treatment to avoid an outbreak of AIDS-KS.

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Severe febrile neutropenia (SFN) in HIV / AIDS

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**Background:** Neutropenia is one of the most common hematologic manifestations in HIV-infected individuals, present in 5-10% in early stages and more than 50% in advanced stages of the disease. Most of the data reported by SFN emerge from the analysis of cancer patients, did not know of specific data on HIV.

**Objective:** To determine the clinic-epidemiological and etiological SFN episodes in HIV / AIDS.

**Methods:** Prospective analytic study developed from June 2007 until October 2009, at the Hospital Rawson.

**Inclusion criteria:** confirmed HIV infection, more severe neutropenia (500 total neutrophils less), plus fever (T>38 °C).

**Results:** We recruited 18 patients with 22 episodes of SFN, the average age was 38.4%. 86.3% male. A single patient presented other comorbidities associated: chronic HCV infection. Probable causes of SFN (n: 22): Chemotherapy for NHL 5 (22.7%), disseminated mycosis 5 (22.7%). In 54.5% found only associated with drug toxicity as a likely cause, although it was present in all cases. In 59.1% of episodes the SFN was the cause of hospitalization. The 68.2% had pancytopenia. Probable origin of infection: respiratory 45.4%, 36.3% gastrointestinal, skin and soft tissue 27.3%, pharyngeal and odontogenic 18.1%, urinary tract 18.1%, retinitis 9%, and unfocused clinical 9%. The CD4 count was less than 200 in 19 episodes (86.3%). The average duration of neutropenia and fever was 7.6 and 9 days respectively. Of all episodes, 7 (31.8%) had microbiological isolation. Bacterial 5 (71.4%): GNB 4 and Gram-positive cocci 1. Fungi were isolated in 2 episodes (28.5%): 1 Cryptococcus and Histoplasma 1. Microbiological performance of the methods of diagnosis: 17.8% blood cultures, urine cultures 8.7%, bone marrow puncture 22.2%, CSF culture 20% lysis-centrifugation blood cultures by 6.6%. More empirical treatments used: piperacillin / tazobactam 31.8%, piperacillin and amikacin, 27.2%. Mortality was 18.2%. Bacteremia was associated with a 50% mortality.

**Conclusion:** Predominance in young males.

High frequency of severe neutropenia related only to drug toxicity.

Low yields of blood and urine.

GNB were isolated more frequently.

Low mortality

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**Session: HIV: Opportunistic Infections & Malignancies**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Abnormal vaginal cytology and colpocytology in a group of HIV infected women at Fundação de Medicina Tropical do Amazonas

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**Background:** The HIV infection in women is associated with a higher incidence of vaginal discharge, intraepithelial lesions and cervical cancer. Based on these information, it becomes important to know these alterations to develop strategies to provide screening programs. The aim of this work was to estimate the frequency of abnormal colpocytology in a group of HIV infected Women at Fundação de Medicina Tropical do Amazonas.

**Methods:** The study population consisted of HIV positive women, enrolled in a descriptive and transversal study to show cervical cytology and vaginal cytologic findings in HIV positive women. Inclusion criteria - Women HIV positive

Exclusion criteria: Women without Uterus, patients HIV negative or patients with cervical carcinoma diagnosed before these study.

**Results:** 101 patients aged between 16-65 years were examined. Great part of the examined patients is between 25 and 35 years old (44,6%), has low educational level (52%), is white (53,5%), married (58,4%), lives in Manaus (51,5%) and has a family income equal to or lower than the minimum salary established by the Brazilian government (approximately US\$ 120,00).

Vaginal wet smears with 10% potassium hydroxide and with normal saline showed that 41 patients had candidiasis, 28 bacterial *vaginosis*, 15 Trichomoniasis and no pathogen was noticed in 20 patients (normal cytology), 23 patients had more than one pathogen in wet smears. Pap smears showed 37,6 % of bacterial *vaginosis* infection, 31,7% of lactobacillus, 16,8% of unapparent Flora, 4,0% from *Candida*, 2,0 % from Trichomonas and 7,9 % from other bacillus. A total of 12 smears (12,1%) were normal, 71 smears showed inflammation (71,8%), 11 revealed low grade squamous intraepithelial lesions and or infection by Humana papillomavirus (NIC I/HPV) (11,1%), 2 smears diagnosed high grade squamous intraepithelial lesions (NIC II/NIC III e microinvasive carcinoma) (2%), 3 smears showed atypical squamous cells of indeterminate significance (ASCUS) and 2 cytology were not satisfactory.

**Conclusion:** HIV infected women showed an increased rate of bacterial *vaginosis* infection and 5,6 fold more chances to have abnormal Pap smears than uninfected women in Brazil, specially those with low levels of CD4 and high levels of viral load.

These results confirm the validity and importance of cervical screening by cytology.

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**Session: HIV: Opportunistic Infections & Malignancies**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Coinfection of tuberculosis and histoplasmosis in HIV patients from Medellin, Colombia

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**Background:** It is shown the retrospective analysis of 26 patients (21men/5women) infected with HIV and simultaneous diagnosis of tuberculosis and disseminated histoplasmosis (DH). The patients were seen in the Hospital La María and at the Corporación para Investigaciones Biológicas in Medellín, Colombia.

**Methods:** The diagnosis of TB was made by histopathology in 13 cases, direct examination of sputum smears for acid-fast-bacilli in 8 cases, culture in 8 cases and BAL direct examination in 3 cases.

The diagnosis of DH was made exclusively by histopathology in 8 patients, BAL direct examination in 4 cases and simultaneous direct examination and culture in 10 cases.

**Results:** The evolution time of the HIV infection was of 33 months (0-144), with T CD4 lymphocytes count of 55 (3-152) cl/ul and viral load of 256 copies/ul (400-1.000.000). At the moment of the consultation only 8 (30,8%) of the patients had received HAART.

The evolution time of the symptoms was of 74 days (7-270) and the most frequently compromised organs were the lungs in 17 patients (65%), followed by the lymph-nodes in 10 (38%), bone marrow in 3 (11,5%), liver in 3 (11,5%) and the intestine in 2 (7,6%).

Nineteen of the patients (73%) received Amphotericin-B as initial treatment, followed by Itraconazole. It was performed a monitoring of the Itraconazole serum levels, which were not detectable in 8 of 10 patients at the beginning and in 4 of 5 patients in positive controls.

It was used a conventional anti-TB treatment with rifampicin, isoniazide, pyrazinamide and ethambutol. The assessment of the therapeutic response was performed after 15 days, 1 and 3 months, finding improvements in 64%, 75%, and 77% respectively, and no improvement in 32%, 21% and 10% respectively. Mortality was present in 1 patient with the first two controls and 3 at the third month (16%).

**Conclusion:** This underlines the importance of considering TB in association with endemic mycoses such as DH, in severe-immunocompromised-HIV patients from third-world countries. This diagnosis is only possible after clinical suspicion and the performance of adequate laboratory tests. The association of these infections makes therapy difficult and increase mortality risk.

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**Session: HIV: Opportunistic Infections & Malignancies**  
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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Human Papillomavirus (HPV) detection in urine of HIV-infected women  
**I. Rosa-Cunha**, J. Prewitt, T. Guettouche, T. Hooton  
University of Miami, Miami, FL, USA

**Background:** HIV-infected women are at higher risk of cervical HPV infection persistence and related malignancy and the incidence of cervical cancer is likely to increase along with patient survival. The importance of screening in this population is thus evident, but costs of providing pap exams are considerable, the test is less than 70% sensitive, and nearly 1 in 4 HIV-infected women do not receive an annual Pap test. We assessed the feasibility of using urine samples from HIV-infected women to screen for HPV infection and cervical disease.

**Methods:** 18 HIV-infected women referred to HIV colposcopy clinic at Jackson Memorial Hospital, Miami, FL, for further evaluation of abnormal cervical Pap smear results agreed to collect urine sample for HPV testing. Colposcopy clinic practitioners performed colposcopic examination with biopsy of visible lesion(s) as per clinic routine. Participants self collected at least 15ml of urine. Presence of HPV in urine was evaluated using HPV Genotyping Microarray (Kurabo Industries Ltd, Osaka, Japan) with the capability to specifically identify 23 oncogenic HPV types.

**Results:** 100% of the women approached accepted to participate in the study. Baseline cervical Pap smear results that triggered colposcopy referral were: 12 women (67%) with low grade squamous intraepithelial lesion and 6 (33%) with atypical cells of unknown significance (ASCUS). Results for routine HPV testing using Hybrid Capture II assay (Digene Corp., Silver Spring, MD) were available for 12 participants with 9 samples (75%) being positive for HPV. Cervical biopsies were performed in 16 of the 18 patients referred to colposcopy; 13 patients had cervical intraepithelial neoplasia (CIN) I, 2 had CIN II and 1 had no abnormality. Urine HPV testing was positive in all but two patients; 46% of the samples were positive for multiple HPV oncogenic types; most isolated oncogenic HPV (71%) were non-16 and non-18 types.

**Conclusion:** Preliminary analysis suggests that self-collected urine sample for HPV testing was well accepted by HIV-infected women and could potentially have a role as a screening method for HPV infection and cervical dysplasia in this population. More studies are needed to further evaluate the potential of urine HPV screening in HIV-infected women.

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**Session: Obstetrical-Gynecological, Surgical and Sexually Transmitted Infections**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Prevalence of bacterial vaginosis amongst women of child bearing age

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**Background:** Bacterial vaginosis, previously known as non-specific vaginitis or Gardnerella vaginitis, is the most common cause of vaginal discharge. It may be the cause of up to one half of cases of vaginitis in all women and the cause of from 10 to 30 percent of cases in pregnant women. The clinical syndrome is now recognized as a polymicrobial superficial vaginal infection involving a loss of the normal lactobacilli and an overgrowth of anaerobes. Bv has mostly been associated to sexual transmission, though some controversy remains. It occurs commonly in women with more than one sexual partners, and can also occur in women who are not yet sexually active. Recent studies have confirmed its association with pelvic inflammatory disease and adverse pregnancy outcome. The aim of the research was to determine the prevalence of Bv infection among women of child bearing age with the view of evaluating epidemiological factors and risk factors responsible for the infection among individuals within this category and identifying possible ideal control measures.

**Methods:** In this cross-sectional study, 102 women of child bearing age ( comprising pregnant and non-pregnant) in selected hospitals and laboratory in Abakaliki and Afikpo, Ebonyi State, from December 2005 - March 2006, were screened to determine the prevalence of Bv among them, using three standard diagnostic techniques (wet mount, whiff test and gram stain). A structured questionnaire was also administered to 23 subjects in order to obtain vital epidemiological information necessary for the study.

**Results:** And to also link their laboratory result to the information and 16 (69.6%) tested positive. The data analysis was done using chi-square tests. Results show 49 (48.0%) were positive for wet mount, 58 (56.9%) for whiff test and 20 (23.5%) for gram stain. Age, douching, Use of IUD, and sexual activities of individual had no significant effect on prevalence of Bv, while symptomatology was a major indicator of infection.

**Conclusion:** Risk factors associated with significant infection were linked to ignorance of women, gynaecologist and laboratory technicians of the existence of Bv resulting to non-diagnosis of Bv infection, wearing of tight panties and poor hygiene conditions as well. Since Bv could be asymptomatic, routine screening of women, visiting gynecology, fertility clinics and antenatal care are advocated.

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**Session: Obstetrical-Gynecological, Surgical and Sexually Transmitted Infections**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Syphilis and HIV co-infection in Thai Medical School Hospital

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**Background:** HIV and syphilis affect similar patient groups and co-infection is common. In HIV infected patients, clinical manifestation of syphilis may be atypical and different from HIV uninfected cases. This study aimed to compare the characteristics of syphilis between HIV-seropositive and HIV-seronegative patients in Thai Medical School Hospital

**Methods:** Retrospectively chart review of new cases of syphilis who attended the STD clinic, Chulalongkorn Hospital, Bangkok, Thailand from 1st June 2001 to 31st May 2006 was conducted.

**Results:** There were totally 385 new cases of syphilis during the studied period and HIV screening was done in 325 patients. The prevalence of HIV infection among syphilis patients was 33.2%. We found that younger age, male gender, and men who have sex with men (MSM) were significantly associated with HIV infection ( $p < 0.05$ ). Secondary syphilis was more common manifestation in HIV infected individuals ( $p < 0.005$ ). Syphilis among HIV infected patients had significantly lower VDRL titer ( $p < 0.005$ ) and 15.9% had nonreactive VDRL.

**Conclusion:** HIV co-infection should be considered in syphilitic clients particularly with younger age, being male gender, MSM, and whose clinical manifestations are secondary syphilis. Non-reactive or low titer VDRL does not exclude syphilis in HIV infected patients and treponemal antibody test is needed for screening and diagnosis

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**Session: Obstetrical-Gynecological, Surgical and Sexually Transmitted Infections**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Genotyping of *Chlamydia trachomatis* and human papillomavirus in clinical specimens from North-Eastern Croatia

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<sup>1</sup>INSTITUTE of Public Health for Osijek-Baranya County, Osijek, Croatia, <sup>2</sup>School of Medicine, Osijek, Croatia, <sup>3</sup>School of Medicine, DNA Laboratory, Osijek, Croatia

**Background:** Human papillomavirus (HPV) and *Chlamydia trachomatis* (Ct) are major causes of sexually transmitted diseases. Clinical consequences of high-risk HPV (HR HPV) infection, especially with HPV types HPV 16 and HPV 18, include abnormal Pap test results, low and high-grade cervical intraepithelial neoplasia and cervical cancer, for which Ct may sometimes be a cofactor. The aim of the study was to determine the prevalence of Ct and age-related profiles of HR HPV infections among cases of other sexually transmitted infections in our county.

**Methods:** Due to the chronic and "silent" infection of Ct, fast molecular diagnostics and adequate therapy of the infected individuals are the crucial steps in the Ct spread control. During three-year period we tested 2327 genitourinary samples for Ct and 945 gynaecological samples for HR HPV with normal and abnormal cervical cytological diagnosis. Subsequently all Ct positive samples were analysed by sequencing of the amplified *omp1* fragments using Applied Biosystem 3130 Genetic Analyser. Genotyping and sequence mutation analysis were performed using ABI SEQSCAPE software and compared with the reference sequences of all known Ct serotypes. HR HPV positive samples with abnormal cervical cytological diagnosis were genotyped by Linear Array HPV Genotyping Test (Roche Diagnostics).

**Results:** The determined serotype of Ct and HPV genotypes distribution were compared with the Ct and HPV distribution pattern in other regions of the world. The association between certain HPV genotype and cervical intraepithelial neoplasia was determined as well as mixed infections with four or more HPVs. The most prevalent Ct genotype in Osijek-Baranya County was serotype E (in concordance with Sweden and Taiwan data), followed by F, K, G, D, B and J (differs from Sweden and Taiwan data). The sequence of *omp1* gene showed limited variation. HPV 16 and HPV 18 were the most common HR HPV genotypes in the cervical specimens with abnormal cervical cytological diagnosis. Obtained results also determined mixed infection of four HR HPV with one or more low risk HPV.

**Conclusion:** The obtained results for Ct and HPV and further analyses in this ongoing project could be useful tool for clinical and epidemiological characterization of circulating pathogens in our community.

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**Session: Obstetrical-Gynecological, Surgical and Sexually Transmitted Infections**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Frequent infections in Mexican women experiencing spontaneous pregnancy loss

**L. Conde-Ferráez<sup>1</sup>**, R. Suarez-Allen<sup>1</sup>, L. Manzano-Cabrera<sup>1</sup>, M. R. Gonzalez-Losa<sup>1</sup>, G. Koh-Tec<sup>2</sup>, M. Perez-Tuyub<sup>2</sup>, J. Camara-Mejia<sup>1</sup>, J. Carrillo-Martinez<sup>3</sup>, M. Puerto-Solis<sup>1</sup>

<sup>1</sup>Centro de Investigaciones Regionales, Universidad Autonoma de Yucatan, Merida, Yucatan, Mexico, <sup>2</sup>Universidad Autónoma de Yucatán, Merida, Yucatan, Mexico, <sup>3</sup>Instituto Mexicano del Seguro Social, Merida, Mexico

**Background:** Some maternal infections especially during the early gestation can result in fetal loss or malformations. Gestation represents an altered immunological state which could predispose to various infections, and in particular, latent viral infections might be reactivated during this period. In this study we aimed to determine the prevalences of viral and protozoan pathogens, within women at reproductive age experiencing spontaneous pregnancy loss in southeast Mexico, and the epidemiological variables present.

**Methods:** Women requiring medical attention for spontaneous abortion in a Social Security Hospital were randomly invited to participate. After consent, an interview was applied; serology tests for IgM antibodies against human cytomegalovirus (CMV), Herpes Simplex virus (HSV), and *Toxoplasma gondii* were performed. Cervical cells were PCR tested for Human Papillomavirus (HPV) infection.

**Results:** From 143 participants, 113 (79.6%) were younger than 35 years old, and only 11 (7.7%) were younger than 20 years old. 90% presented miscarriages on the first trimester. 19.6% had experienced a previous pregnancy loss; 33.6% had never given birth to a live child and 66.4% had delivered one or more live children.

From 141 serum samples tested, 60.3% (85/141) were positive to any of the infections: 17 (12 %) were *T. gondii* positive; 67 (47.5%) were CMV positive and 40 (28.4%) HSV positive. From 139 cervical samples tested, 34 (24.4%) were HPV+. Interestingly, 27.3% of HPV positive women have had repetitive abortions comparing to 17.4% of HPV negative women. Multiple infections were found in 42.4% of the seropositive samples, and importantly 19 (22.3%) were also positive to HPV cervical infection. One patient was positive to all infections.

**Conclusion:** Pregnancy loss is attributed to several factors including maternal age. It was worrisome finding a high frequency of spontaneous abortions at young age. Recognizing infections in the mother is an important part of prenatal care. HPV prevalences found were higher than previously reported for southeast Mexico. HPV studies in our region are particularly important because the incidence of cervical cancer is amongst the highest in Mexico. Also, the highest nationwide incidence of clinically identified genital herpes is reported for our State. Although our data are limited, the high prevalences of infectious agents often related to pregnancy loss and the high frequency of multiple infections found in women at reproductive age set important basis for further investigation.

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**Session: Obstetrical-Gynecological, Surgical and Sexually Transmitted Infections**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Maternal group B *Streptococcus* colonization: Prevalence, risk factors, phenotypical and genotypical characteristics in a Brazilian population

T. S. Pires<sup>1</sup>, **M. Turchi**<sup>2</sup>, M. C. D. P. B. André<sup>2</sup>, J. L. Cardoso<sup>2</sup>, L. E. P. R. Schmaltz<sup>1</sup>, O. M. Peres<sup>1</sup>, A. L. Andrade<sup>2</sup>

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**Background:** Maternal group B Streptococcus (GBS) colonization is considered to be the most important risk for invasive infections in the newborn. To reduce the incidence of neonatal infection two preventive strategies have been proposed: universal screening for all pregnant women or a risk-factor-based approach. However, prevention and treatment strategies have not yet been adopted in many developing countries. This study aims to estimate the prevalence, to investigate risk factors for GBS colonization and to describe phenotypical and genotypical characteristics of isolated strains from pregnant women in Goias-Central Brazil.

**Methods:** A cross sectional study was carried out among 198 pregnant women, > 32 weeks gestation, attending a reference public health unit, from March to June 2009. Socio-demographic and obstetric profiles were investigated using a standard questionnaire. Samples of vaginal and rectal secretion were collected and placed into selective enrichment broth Todd-Hewitt. Tests for GBS identification (gram, catalase and CAMP) followed by susceptibility test using antibiotic disk diffusion technique were performed. Genetic diversity was assessed by pulsed-field gel electrophoresis (PFGE). Descriptive and analytic statistical tests were applied (SPSS 13.0).

**Results:** Thirty pregnant women were colonized by GBS yielding a prevalence of 15.2% (IC95% 10.5-20.9). Pregnant women younger than 20 years and with low income had higher risk of GBS colonization, in univariate analysis ( $p < 0.05$ ). GBS was isolated from 28 vaginal and 14 rectal specimens. Twelve pregnant women were vaginal and rectal colonized. All 42 strains were susceptible to penicillin. Three strains (7.1%) were resistant to erythromycin and two (4.7%) to clindamycin. 19 pulsotypes and four clusters were identified. Nine out of 12 pairs of positive strains (vaginal and rectal) were genetically identical, two were strictly related and one pair was colonized by different strains. The same genetic profile was observed in more than one pregnant woman.

**Conclusion:** Socioeconomic and obstetric variables had low predictive value for GBS colonization among pregnant women, reinforcing the need for universal microbiology screening strategy in this population, in order to prevent neonatal sepsis. A high genetic diversity of GBS was found among pregnant women in our setting.

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**Session: Obstetrical-Gynecological, Surgical and Sexually Transmitted Infections**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Puerperal group A streptococci (GAS) infection: Re-emergence of a dreaded disease - A case series

**M. T. Busowski<sup>1</sup>**, M. Lee<sup>2</sup>, J. D. Busowski<sup>1</sup>, K. Akhter<sup>1</sup>, M. R. Wallace<sup>1</sup>

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**Background:** Puerperal sepsis due to GAS was responsible for two-thirds of deaths among childbearing women in the 18th and 19th centuries. This once feared complication of childbirth has declined throughout the twentieth century. Infection by GAS is now a rare cause of maternal morbidity and mortality. Recent sporadic reports in the literature indicate there may be a resurgence of serious infections caused by GAS.

**Methods:** We report on four late term pregnancies complicated by GAS infection resulting in either necrotizing infection or sepsis and profound multiorgan system failure (MOF).

**Results:** A 19 year old primagravida presented at 27 weeks with severe abdominal pain and vaginal discharge. Exam revealed extensive posterior cervical necrosis with culture confirmed GAS. She was treated successfully with penicillin.

A 27 year old woman developed severe sepsis one day following a vaginal delivery. Vaginal and blood cultures were positive for GAS. She required massive volume resuscitation and pressor support. She was treated with ampicillin-sulbactam and clindamycin and recovered without sequela.

A 31 year old woman presented seven days after an uneventful vaginal delivery complaining of burning abdominal pain. She was found to be hypotensive with profound sepsis. Emergent laparotomy revealed extensive myometrial infarction and necrosis with confirmed GAS infection. Her ICU course was complicated by MOF with acute renal and hepatic insufficiency and thrombocytopenia.

A fourth, and nearly fatal puerperal GAS infection involved a young woman who presented five days after a vaginal delivery with fever, chills, and abdominal pain. Her prolonged hospital course was complicated by MOF and peripheral necrosis, with loss of limbs.

**Conclusion:** This report highlights the return of serious GAS puerperal sepsis. The fulminant nature of GAS sepsis poses important diagnostic and therapeutic challenges. Recent reports suggest GAS remains a formidable pathogen late in pregnancy and raises the question of whether GAS should be included in antenatal screening programs. The presence of GAS in vaginal culture may have significant clinical implications. The pathogenicity and capacity of this organism to cause overwhelming, life-threatening infection warrants further study to determine the true incidence of vaginal contamination in late pregnancy and the potential efficacy of antibiotic prophylaxis.

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**Session: Obstetrical-Gynecological, Surgical and Sexually Transmitted Infections**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Potential impact of spouse's circumcision on herpes simplex virus type 2 prevalence among antenatal women in five northeastern states of India

**B. Borkakoty**<sup>1</sup>, D. Biswas<sup>1</sup>, K. Walia<sup>2</sup>, J. Mahanta<sup>1</sup>

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**Background:** Herpes simplex virus type 2 (HSV-2), the most common cause of genital ulcer disease worldwide has shown increasing evidence to have synergistic activity on human immunodeficiency virus (HIV) acquisition by two fold or more. So, there was a need to know the prevalence of HSV-2 to develop intervention strategy especially in the high HIV prevalent states of northeast India. A study was therefore conducted among antenatal women to assess the prevalence of HSV-2 infection as well the role of spouse's circumcision on HSV-2 prevalence.

**Methods:** A total of 1640 antenatal women from five different northeastern states of India, namely Assam, Arunachal Pradesh, Manipur, Meghalaya and Mizoram with diverse ethnic background were enrolled after informed consent. They were screened for IgG antibody status against HSV-2 using HerpeSelect 2 ELISA IgG kits form Focus Diagnostics, USA. A structured questionnaire was used to evaluate different risk variables.

**Results:** The median age of the subjects was 24 years (SD±4.8) with inter quartile age of 22-28 years. The overall prevalence of HSV-2 (IgG) positive was 8.6%, while prevalence was highest in Arunachal Pradesh (15%) and was lowest in Manipur (2.74%). There was a significant association of HSV-2 infection with history of vaginal discharge with pelvic pain (p=0.0001) and genital ulcer (p=0.02). Regular condom user's had a low HSV-2 prevalence of 1 % compared to 10.3 % in infrequent or non-condom users (OR=11.1, 95% C.I.= 3.5 – 35.2, p<0.0001). HSV-2 prevalence was 1.7% in women with circumcised spouses compared to 9.2% among uncircumcised spouse (OR= 5.7, 95% C.I.=1.4- 23.4, p=0.01). Prevalence was low among Muslims (3.8%) compared to 12.6% among Christians (p=0.0001)

**Conclusion:** The study documented a variable difference of prevalence of HSV-2 among the different states of northeast India. The important finding of independent association with spouse's circumcision with HSV-2 sero-status in pregnant women brings to light the importance of circumcision in decreasing the transmission of HSV-2 in the community. This may be of vital importance as it can be implemented as a modifiable preventive measure to decrease the prevalence of HSV-2 which will ultimately help in lowering the HIV prevalence in the community.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

The "PREVEN" urban community randomised trial of a combined intervention for sexually transmitted disease prevention in Nepal

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<sup>1</sup>Community Health and Environmental Society Nepal, KTM, Nepal, <sup>2</sup>District Public Health Office, Kail, Nepal

**Background:** A community randomized trial, (PREVEN trial), was conducted to explore the impact of combined sexually transmitted disease interventions in urban Nepal.

**Methods:** The intervention were: (1) improved syndromic management of vaginal and urethral discharge available through pharmacies, with social marketing of condoms and treatment packages and referral to previously trained physicians for cases of pelvic inflammatory disease and genital ulcers, and (2) screening and treatment of sex workers (SW) reached through a mobile team every two months, along with presumptive treatment for trichomoniasis (TV) and bacterial vaginosis with metronidazole, and provision of free condoms. A baseline survey of STIs prevalence was undertaken in the general population (GP) and amongst SW and their clients in 4 cities in 2005 to stratify cities for randomisation to intervention and control arms of the trial. 3 cities were selected for the trial. The impact of the interventions, was evaluated through surveys of STI prevalence. Testing for syphilis, GC, CT, TV and HIV were undertaken in the 20 randomised cities. Data analysis was blinded with respect to city identity until results were available for each infection where upon the prevalence of each infection in the matched intervention and control cities could be compared.

**Results:** The overall prevalences of STIs in the general population across the cities were as follows: males: gonorrhoea 0.11%, Chlamydia 4.7%, syphilis (titer > 1:8) 0.29%, HIV 0.51%; females: gonorrhoea 0.24%, Chlamydia 8.6%, syphilis (titer > 1:8) 0.20%, trichomonas 2.4%, HIV 0.17%. The overall prevalences of STIs in the female sex workers across the cities were as follows: gonorrhoea 1.2%, Chlamydia 17.6%, syphilis (titer > 1:8) 1.3%, trichomonas 3.7%, HIV 0.41%.

**Conclusion:** combined sexually transmitted disease interventions in urban Nepal is important for better prevention of STIs.

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**Session: Obstetrical-Gynecological, Surgical and Sexually Transmitted Infections**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

HIV and syphilis seroprevalence and associated factors in pregnant women and their couples in 6 Amazonian indigenous populations in Peru 2007-2008

M. Omaeche, **A. Whittembury**, M. Pun, L. Suarez-Ognio  
Ministry of Health from Peru, Lima, Peru

**Background:** HIV and Syphilis magnitude information in Amazonian indigenous population is scarce. In 2004-2006, HIV/AIDS cases have been reported from periurban indigenous population.

**Methods:** From June 2007 to december 2008 a seroprevalence study was conducted in 6 Amazonian indigenous populations: Shispibo-Konibo, Ashaninka, Kandozi, Shapra, Chayahuita, Matsigenka. Objective: to determine the magnitude of HIV and Syphilis and factors associated. Blood samples and written surveys were obtained. Informed consent was obtained from National, Regional and local Community leaders and participants. For HIV testing, Elisa and immunofluorescence tests were used; for Syphilis, RPR and MTPHA.

**Results:** 1274 pregnant women and 721 male couples were surveyed. HIV positive results: 2 women (0.16% [95%CI: 0.02 - 0.58]) and 2 males (0.28% [95%IC: 0.03 - 1.02]). Syphilis positive results: 20 women (1.60% [95%CI: 0.98% - 2.46%]) and 17 males (2.41% [95%CI: 1.41% - 3.83%]). Only 14.3% and 29.6% of women and males, referred having used condoms sometime. Sexual transmitted infections (HIV and Syphilis - STI) in pregnant women were associated to: 1) Male couple STI: OR=14.10 (95%CI: 2.73 - 65.21; p=0.003); 2) Vaginal secretion: OR=2.87 (95%CI: 1.15 - 7.14; p=0.021); 3) women Anti-HBc positivity OR=2.82 (IC95%: 1.13 - 7.23; p=0.013); 4) >1 sexual partner in last 12 months OR=2.96 (IC95%: 1.07 - 8.18; p=0.045); 5) >5 sexual partners in lifetime OR=3.50 (IC95%: 1.10 - 9.62; p=0.017).

Sexual transmitted infections in males were associated to: 1) history of sexual relations with another male in lifetime OR=5.9 (IC95%: 1.82 - 19.12; p=0.010); 2) sexual relations with another male in last 12 months OR=11.9 (IC95%: 3.01 - 47.26; p=0.005); 3) More than 10 sex partners in lifetime OR=3.29 (IC95%: 1.29 - 8.36; p=0.015).

**Conclusion:** HIV in Amazonian indigenous populations studied may be in a concentrated level (high risk groups information still needed). Syphilis triples national prevalence. Data suggests that transmission of these diseases to women is mediated by their couples. Lack of risk perception, poor STI knowledge, limited access to treatment and preventive measures, are other determinants identified. The development of a national plan of STI prevention and control is urgently needed in the Amazonian indigenous population in Peru.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Superoxide anion in lesions of cervix associated or not papillomavirus-infection

**Y. Carrero**, D. Callejas, R. Mindiola, L. Porto, F. Alana, M. E. castellanos, C. Silva, L. Costa  
Universidad del Zulia, Maracaibo, Zulia, Venezuela

**Objective:** To determine the expression of superoxide anions in premalignant and malignant lesions of the cervix associated or not with human papillomavirus (HPV).

**Methods:** A total of 74 patients (45 CIN I, 5 CIN II, 5 CIN III and 5 patients with Cancer) 7 women who had inflammation and 7 apparently healthy women as controls, were sampled for cytology, cervical exudate HPV and 2 biopsies determine: 1) fixed in 10% formalin for histological analysis, 2) component in OCT (Tissue Tek) to determine the expression of superoxide anions by reaction of Briggs, one carries out the identification and genotyping of HPV Hybrid Capture (HC2).

**Results:** Of the patients studied 23% were positive for HPV infection with a 58.5% high-risk genotypes were significant differences in the number of superoxide anion positive cells in both epithelium and stroma in different degrees of damage observed increased as the degree of lesion progressed

**Conclusion:** The results allow the inference that the superoxide anion plays an important role in the genesis and progression of lesions in the cervix, but can not be used as markers of tumor progression.

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**Final Abstract Number: 79.011**

**Session: Obstetrical-Gynecological, Surgical and Sexually Transmitted Infections**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Detection of human Papilloma virus DNA in cervical samples in Maracaibo-Venezuela

**D. Callejas**, M. E. castellanos, R. Mindiola, Y. Carrero, F. Monsalve, L. Porto, R. Atencio  
Universidad del Zulia, Maracaibo, Zulia, Venezuela

**Background:** Cervical cancer is the second leading cause of cancer death among women worldwide, mainly in countries in process of development. There are multiple risk factors involved in the genesis of pre-invasive lesions and cancer, such as sexual behavior, and some sexually transmitted infections especially Human Papilloma Virus. Objective: To perform molecular diagnosis of HPV

**Methods:** In a period from 2007 to 2009 have been treated 300 patients in Research Seminar in different health centers, each underwent a medical history, sampling for cytology and a citobrush (DNA collection device, Digene ®), for diagnosis of HPV by PCR using primers for L1 region (MY09/11, GP5 + / 6 +)

**Results:** Of the patients attended 40.5% were positive for HPV infection, of which one 22.55% were diagnosed by MY09/11 primers vs 31.98% GP5 + / 6 + sharing between 14,14% of cases

**Conclusion:** The PCR evaluated in this study showed significant differences, the use of primers GP5 + / 6 + possible to identify a larger number of cases, which is very important. The use of this technique applied in conjunction with colposcopy and / or cytology, are fundamental tools for definitive diagnosis and monitoring of cervical HPV infection.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Sexually transmitted disease recurrence in the department of defense

**A. McCabe**, G. Nowak

Navy and Marine Corps Public Health Center, Portsmouth, VA, USA

**Background:** Reinfection with chlamydia and gonorrhea is common; as much as 40% of the reported annual incidence of these infections occurred in people that had been previously infected. The objective of this project was to assess the differences between Department of Defense active duty personnel that had only one sexually transmitted disease (STD) and those that had multiple STDs.

**Methods:** All chlamydia and gonorrhea cases were extracted from Health Level 7 laboratory data between 01 Oct 2006 and 30 Sept 2008. Positive cases were classified as having either one infection or multiple infections during the study period. Demographic characteristics, treatment patterns and co-morbidities were compared between groups.

**Results:** There were 30,740 patients with chlamydia or gonorrhea, 13% (n=3,954) of whom had more than one infection. Patients treated for their first infection were significantly less likely to develop a second infection than those not treated (z-value=6.72, df=1, 99% CI). Significantly more patients with multiple infections were treated than patients with a single infection (z-value=7.12, df=1, 99% CI). Related outpatient encounters were identified for 94% of all cases in patients with multiple STDs.

**Conclusion:** The majority of patients with multiple infections had two infections. Reinfection could potentially be reduced if proper and timely treatment were provided to patients. Results also suggest additional clinical counseling may be necessary to prevent or reduce multiple infections. The study did not identify a specific demographic population with multiple infections that could be targeted for more specific intervention.

Disclaimer:

The views expressed in this abstract are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the U. S. Government.

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**Final Abstract Number: 80.001**

**Session: Pediatric and Perinatal Infections**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Effect of zinc supplementation on clinical course of acute diarrhea in patients admitted in Ali Asghar Children Hospital Medical Center Iran University of Medical Sciences Tehran Iran

**K. Mahlouji**

Iran University of Medical Sciences, Tehran, Iran, Islamic Republic of

**Background:** Pilot studies have been conducted in number of countries (Brazil, Egypt, Ethiopia, India, Mali, Pakistan and Philippines) prior to the implementation of large community-based studies to introduce zinc in the management of acute diarrhea, preliminary results of these studies show two interesting observations: ORS use rate increase; Antimicrobial use rate significantly decrease with zinc is prescribed with ORS solution.

**Methods:** We conducted an interventional study (clinical trial) in patients who admitted in Ali - Asghar Children Hospital, with one episode of acute diarrhea, the patients were divided into two groups(case=75 and control =75).

In the case group zinc sulfate was added as supplement.

In control group no supplement was added to usual treatments.

Zinc prescribed as zinc sulfate syrup for 14 days during and after diarrhea(10 mg /day for infants <6 mo of age and 20 mg / day for those >6 mo of age).

**Results:** Mean duration time of diarrhea in control group without Zinc Sulfate was 5.63 days and for that of case group using zinc sulfate was 3.21 days. Data was analyzed by SPSS-13 software using T-test and Chi-square Test, there was a significant difference between 2 groups in duration of diarrhea (P value=0.001).

**Conclusion:** In our study prescription of Zinc sulfate in acute diarrhea similar to other studies reduced the duration of acute diarrhea. Therefore Zinc Sulfate has an important role in treatment of acute diarrhea and should be considered as a supplement in all cases of diarrhea.

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**Final Abstract Number: 80.002**  
**Session: Pediatric and Perinatal Infections**  
**Date: Friday, March 12, 2010**  
**Time: 12:30-13:30**  
**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Neonatal tetanus cases in a region in Ghana: Contributory factors and consequential outcomes  
**E. KOOMSON**  
Ghana Health Service, CAPE COAST, Ghana

**Background:** Immunizing women in fertility aged group and pregnant women with tetanus toxoid vaccine is meant to protect them and their babies against tetanus infection. Irrespective of the prevalence of the vaccination activities at static and outreach clinics the incidence of neonatal tetanus is usually higher in the Central Region of Ghana for 2005. This paper throws more lights on factors which led to the infection of eight neonates who died within the year in a region in Ghana as a result of poor neonatal care, unvaccinated pregnant women and health seeking behavior exhibited by the families.

**Methods:** Review of hospital admission data of all (8) reported neonatal cases in 2005 at the Regional Health Directorate, using multidisciplinary approaches in 2006 by Ebenezer Koomson. The records of the cases were verified at the respective districts where they were first reported. After accepting the authenticity of the cases, then the researchers visited the health facility which attended to each case. The personal data and medical history of each neonatal tetanus case was assessed to the point of discharge or otherwise. The relatives of each case were also interviewed. Those who helped or supervised the delivery were also interviewed to gather the part each person played in the peri-and neonatal periods of each case.

**Results:** The level of adequate vaccination (receiving at least two tetanus vaccinations during pregnancy) for the mothers was very low (37.5%), four (50%) of the 50% deliveries were supervised by skilled health personnel; cord care was poorer for all the cases with improper observance of aseptic techniques, and subsequent death of seven (99%) of the patients. Fifty percent (50%) of cases were sent to a health facility on the day of onset of the condition. Timeliness of reporting was 0% (0/8) -none of the cases was reported to the next upper level timely. The range was 10-180 days. It should have been reported the same day. Completeness of reporting was inadequate- (50%) complete.

**Conclusion:** Adequate number of vaccinations, observance of aseptic techniques during and after delivery, proper health seeking behavior, health education and effective feedback of health data is crucial for neonatal care and survival.

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**Final Abstract Number: 80.003**

**Session: Pediatric and Perinatal Infections**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Role of adjuvant zinc in the treatment of severe pneumonia in young children: A meta-analysis of benefits and risks

**R. Das**<sup>1</sup>, M. Singh<sup>2</sup>, N. Gupta<sup>2</sup>, N. Shafiq<sup>2</sup>

<sup>1</sup>Post-Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India,

<sup>2</sup>Chandigarh, India

**Background:** Children with pneumonia have been found to have lower blood zinc levels as compared to uninfected children. Even in well nourished children suffering from acute lower respiratory infection, serum zinc levels have been found to be lower. A recent meta-analysis done on *preventive role* of zinc in childhood respiratory illness found significant reduction in the frequency and severity of respiratory illnesses. However no consensus has evolved for the *therapeutic role* of zinc when used as an additive in treatment of severe pneumonia in young children.

**Methods:** A comprehensive search was performed of the major electronic databases (e.g., CENTRAL, MEDLINE, EMBASE, CINAHL) till December 2008. Randomized controlled trials comparing treatment with zinc versus placebo in children < 5 years with severe pneumonia until discharge from hospital were included. Two reviewers independently applied eligibility criteria and assessed the studies for methodological quality. Data were independently extracted by two reviewers and analyzed using pooled weighted mean difference (WMD) or pooled odds ratio (OR) with 95% CI. RevMan version 4.2 was used for all the analyses.

**Results:** Three Randomized controlled trials (India = 2; Bangladesh = 1) (Jadad's quality score  $\geq$  4) including a total of 722 subjects were eligible for inclusion. There was no significant difference between the two groups in the time of resolution of severe pneumonia, WMD 1.12 (95% CI -8.17, 10.41;  $p = 0.81$ ). The duration of hospitalization was significantly shorter in the control group, WMD 56.28 (95% CI 48.86, 63.70;  $p < 0.00001$ ). No significant difference between the intervention and control group was noted for, in the duration of resolution of tachypnea, chest indrawing, fever and hypoxia. There was no report of any adverse events.

**Conclusion:** There is insufficient evidence to recommend zinc for the treatment of severe pneumonia in young children < 5 years age. Further studies are required to determine whether the findings are reproducible.

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**Session: Pediatric and Perinatal Infections**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

DNA amplification and sequence analysis of P1 adhesin gene fragments in *M pneumoniae* from pediatric respiratory infections

**S. Kumar**<sup>1</sup>, S. R. Saigal<sup>1</sup>, G. R. Sethi<sup>1</sup>, R. Chaudhry<sup>2</sup>

<sup>1</sup>Maulana Azad Medical College, New Delhi, New Delhi, India, <sup>2</sup>All India Institute of Medical Sciences, New Delhi, New Delhi, India

**Background:** *Mycoplasma pneumoniae* is genetically homogeneous and lacks variation. It possesses a surface-localized protein P1 that clusters at the terminus and is essential for cytoadherence. Direct amplification of P1 gene sequences from clinical specimens, bypassing culture is advocated for subtyping. This study amplified and sequenced fragment of P1 adhesin gene of *M. pneumoniae* employing nasopharyngeal aspirates (NPA) from children with lower respiratory tract infections.

**Methods:** A total of 276 children aged 2 months to 12 years, with community acquired lower respiratory tract infections (LRTIs) were investigated clinically and radiologically. An in-house PCR to amplify a 543 base pair fragment on P1 adhesin gene in *M. pneumoniae* using NPA was standardized. PCR products were purified and sequenced on automated DNA analyzer and prototype sequences aligned and compared with similar sequences of *M. pneumoniae* strain M 129 using Clustal W program.

**Results:** Pneumonia, was documented in 175(63.3%), bronchiolitis 90(32.6%), virus associated wheezing 9(3.3%), pneumonia with effusion 2(0.7%) and abnormal chest X-ray findings in 191(69.5%) children. Clinical and radiological features in *M. pneumoniae* positive and negative groups were comparable. Twenty one (7.6%) specimens gave positive PCR; 12(57.3%) specimens yielding weak amplification and in 18(89.5%) specimens PCR positive results were reproducible. Sequence comparison with Indian strains was not possible because of unavailability of published data whereas comparison with *M. pneumoniae* strain M 129(accession number 18639) using CLUSTAL W (1.83) multiple sequence alignment gave 98.07% homogeneity with one clinical isolate P-1-1, , while with in strain P-1-2, homogeneity was partial.

**Conclusion:** Our study underlined role of *M. pneumoniae* in community-acquired LRTIs, even in children < 12 months of age. Since isolation of *M. pneumoniae* is rarely attempted, multilocus sequencing typing system on P1 gene, directly on respiratory specimens, may yield more refined strain differentiation.

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**Session: Pediatric and Perinatal Infections**  
**Date: Friday, March 12, 2010**  
**Time: 12:30-13:30**  
**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Incidence and characterization of neonatal sepsis cases in a Colombian hospital during 2007-2008

**M. L. Raciny Aleman**, A. M. Castro Cordero, L. R. Ramos Hernandez, A. B. Muñoz Delgado  
Universidad de Cordoba, Monteria, Colombia

**Background:** The sepsis is an unacceptable but important cause of morbidity and mortality in newborns, for that is indispensable a precocious and specific diagnose, and an adequate antimicrobial treatment that permit to reduce these indices. In this study was estimated the incidence and characterized the cases of neonatal sepsis (NS) in a Colombian hospital.

**Methods:** A descriptive study was carried out. Were evaluated series blood cultures of neonates with suspicion of NS without previous antimicrobial therapy during a year. The isolates were identified by biochemistry techniques and the antimicrobial susceptibility was evaluated according to recommendations of CLSI. The isolated microorganisms were classified as etiological agents of late and early NS. The epidemiological and clinics variables were obtained from the clinic histories. The information was processes in the software Whonet 5.4 and Epi Info Version 3.4/2007.

**Results:** Were studied 50 patient suspicious, 14 (28%) were positive for NS. The more frequent etiological agent was *Staphylococcus aureus* (52%) which showed resistance to oxaciline in a 36,4%. The percentage of meticiline-resistant *S. aureus* (MRSA) was 31,8%. Epidemiologically most of the cases were classified as early NS (97,6%).

The majority of the mothers were greater of age, from rural zone, with low socioeconomic level. The main risk factors were premature membrane rupture > 18 hours of evolution (47,6%), chorioamnionitis and vaginosis (9,5%). In the newborn the principal risk factors were low weight (52,4%), premature gestational age (69%), masculine sex (66,7%) and regular general state (54,8%).

The subcostal retractions were the clinical signs prevailing; with diagnose of respiratory difficulty associate (28,6%).

There was association significant statistically between the presence of NS and extended delivery work and RPM > 18 hours. The reasons of prevalence for presenting NS showed greater probability in the masculine newborn than in the feminine, as well as the born of mothers with IVU respect to the born of mothers without IVU

**Conclusion:** Most of the cases of NS were caused by *S. aureus*, that additionally presented high percentages of antimicrobial resistance. For these reason the necessity of designing strategies to contain the dissemination of these bacteria in the institution

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Epidemiological data of infectious disease for 10 years in PICU

**F. Zavalani**<sup>1</sup>, I. bujari<sup>2</sup>, A. koja<sup>2</sup>, I. bakalli<sup>2</sup>, E. kola<sup>2</sup>, E. Kallfa<sup>2</sup>, S. sallabanda<sup>2</sup>, R. Petrela<sup>2</sup>, R. Lluka<sup>2</sup>, E. celaj<sup>2</sup>

<sup>1</sup>University hospital center "Mother Theresa", Tirana, AL, Albania, <sup>2</sup>University Hospital Centre "Mother Theresa", Tirana, AL, Albania

**Background:** This is a retrospective study to present the epidemiologic data of the infants hospitalized with infectious diseases in PICU during 1999-2008.

**Methods:** In this study are included 2128 infants from 1 month -14yrs. Admittance criters are based in anamnestic data, clinic situation (valuation of vital signs). The data interpretation is made in report with the kind of infection, with the age, sex, place and mortality.

**Results:** 2128 hospitalized infants with infectious disease in P I C U form 44.39% of all hospitalizations According to the kind of infection, the lower respiratory tract infections take the main place with 1086 cases (51.03 %), infectious diarrhea with 459 cases (21.56%), central nervous system infections with 239 cases (11.23%), septic shock with 89 cases (4.18%), bacterial meningitis with 74 cases (3.47%), upper respiratory tract infections with 66 cases (3.1%), hyperacute sepsis (meningocoxemia) with 47 cases(2.2%), leishmania with 19 cases (0.89%), heart infections (endocarditis, myocarditis, pericarditis) with 12 cases (0.56%), urosepsis with 10 cases (0.46%), skin infections (celulitis, dermatitis) with 10 cases (0.46%), pertussis with 6 cases(0.28 %), varicella with 5 cases (0.23%), tuberculous meningitis with 3 cases (0.14 %), tetanus with 2 cases (0.093%), salmonella typhi with 1 case (0.046 %).

According the age, hospitalized infants are fotmd by: 1 month-1 yrs with 1405 cases (66.02%), 1-5yrs with 425 cases (19.97%), 5-14 yrs with 298 cases (14.003%).

According the sex predominate males with 1198 cases(56.3%) and females with 930 cases(43.7%).

According the place, city's hospitalization (teamwise with suburb) are 1162 cases (54.6%) and from village 966 cases (45.4%).

Mortality of infectious diseases is 124 cases (5.827%) where the main place is taken by lower respiratory tract infectious deaths.

**Conclusion:** Infectious diseases take a main place in 1month-1yrs infant morbidity (66.02%).Mortality is with a high percentage of 5.827 %, where the first place is taken by lower respiratory tract infectious diseases with cardiopathia with 87 cases(4.088%).

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**Session: Pediatric and Perinatal Infections**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

The feasibility of rectal artesunate as pre-referral treatment for severe malaria in under fives at community level in rural Uganda

J. Rujumba<sup>1</sup>, **E. A. Mwozi**<sup>1</sup>, B. A. Mpeka<sup>2</sup>, T. Lutalo<sup>2</sup>, R. Azairwe<sup>3</sup>

<sup>1</sup>Makerere University College of Health Sciences, Kampala, Uganda, <sup>2</sup>Ministry of Health, kampala, Uganda, <sup>3</sup>WHO- Uganda Country Office, kampala, Uganda

**Background:** Malaria is the leading cause of child mortality especially in under five children. It is responsible for 1-2million deaths and 300-500million clinical cases worldwide annually (WHO 2003); 90% of the cases are in sub-Saharan Africa and majority are children under five. Most deaths result from delayed treatment due to various causes. Treatment of severe malaria requires parenteral drugs and skilled personnel yet peripheral health facilities are poorly equipped for this. Timely efficacious treatment at community can "buy" time for patients to reach health facilities. Artemisinin derivatives have been shown to cause rapid reduction of parasite load and resolution of clinical malaria symptoms. We conducted a study to design and evaluate systems for effective community based treatment of severe malaria in under-fives in 2 rural Ugandan districts using rectal Artesunate as pre-referral treatment.

**Methods:** Randomized community trial carried out from 2004-2007. Study phases were formative, development of IEC materials, selection and training of drug distributors, deployment and monitoring of study drug.

**Results:** Acceptability of Rectal Artesunate in treatment of severe malaria in under-five children at community level was high. A total of 1502 children with severe malaria were recruited and referred. A total of 1469/1502 (98%) of treated children recovered, 33(2 %) died mainly due to severe anaemia.

**Conclusion:** Majority (98%) of the treated children with severe malaria recovered. Use of rectal Artesunate at community level reduced mortality due to severe malaria in under-fives and community delivery systems for Rectal Artesunate are feasible and should be scaled-up in rural areas where accessibility to parental and anti malarial therapy is not feasible.

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**Type: Poster Presentation**

Clinical features predicting mortality (M) in high risk febrile neutropenic cancer children (HR-NTPCH) at Hospital de Niños Ricardo Gutiérrez (HNRG), Argentina

**N. Rivas**, A. Viña, G. Yerino, C. Ceccoli, F. Plat, C. Hascalovici, E. L. Lopez  
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**Background:** Mortality is the major concern in HR NTPCH. To detect associated factors on admission and during hospitalization is important to decrease it  
The objective was to identify clinical (c) and microbiological (m) factors predicting M in HR-NTPCH

**Methods:** Prospective cohort study from 1/02 to 12/07 at HNRG. HR-NTPCH were defined according to IDSA criteria. Exclusion: All NTPCH that did not fulfilled the HR criteria. Endpoint: Mortality . Predictive factors assessed for M: hospitalization in last 10 days (PH), underlying disease (UD), fever (F) and clinical focus in admission (CF), bacteremia (B), sepsis(S), positive culture (PC) and polymicrobial isolations (PI), intensity(IN) and duration of neutropenia (DN), duration of fever (DF), days of hospitalization (HD) and nosocomial infection. Demographic data were also analyzed. Chi 2 and Mann-Whitney test were used in the univariate analysis. All factors with  $p < 0.05$  were included in a regression model.

**Results:** Population 501 episodes/267 pts. M 13/501 (2,6%), survivals 488/501 (97,4%). Age(med) 87 (5-219)mo, male 231/501(46.5%) and UD: hematologic tumors 350/501(70%). No significant difference between groups in relation to age, gender or UD was observed. 58/501pts (12%) had not a focus, 216/501(43%) had m demonstrated infection and 227/501(45%) had only c findings. S was present in 85 pts , 12 of them in M group : 12/13 (92,3%) vs C 73/488(14,9%)  $p < 0,001$ . Nosocomial pneumonia(NP) 7/13(54%) vs 51/488(10.5)  $p < 0.001$ ; PI 5/13 (38.5%) vs 15/488 (3%)  $p < 0.001$ , B 7/13 (54%) vs 85/488 (17.4%),  $p = 0,0008$ ; PC 10/13 (77%) vs 204/488 (42%)  $p = 0,011$ ; and HD 22 (1- 100) vs 8(2- 110)d,  $p = 0,026$  also differed between M group and C. No statistical difference was found in F, CF, PH, IN, DN and DF. In multivariate analysis: S,  $p = 0.002$ -OR 33.6 ,NP,  $p = 0.009$  OR 7.2 and PI,  $p = 0.048$  OR 7.5 remained as independent risk factors for M. In M, PI were Gram - 7/10, Gram + 2/10 and fungi 1/10.

**Conclusion:** The presence of S, NP and PI should alert physicians to extreme health care attention in HR-NTPCH.

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Non-typeable *Haemophilus influenzae* and *Streptococcus pneumoniae*: Primary causes of acute otitis media in Colombian children

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**Background:** Acute otitis media (AOM) is one of the most common bacterial infections in childhood and the most frequent reason for antibiotic use. Bacterial conjugate vaccines have demonstrated some efficacy in preventing AOM, but impact may vary according to underlying pathogen and serotype distribution. Because data from Latin America are limited, this report provides novel data regarding bacterial etiology and serotypes of AOM cases within a routine clinical setting in two medical centers in Cali, Colombia to determine what proportion of disease may be vaccine-preventable.

**Methods:** Children aged  $\geq 3$  months to  $< 5$  years with new episodes of AOM (onset of symptoms  $< 3$  days) were included. Middle ear fluid (MEF) samples were collected by tympanocentesis or by sampling of spontaneous otorrhea ( $< 20\%$  of all cases). Recovered bacteria were identified and serotyped.

**Results:** 99 children with new episodes of AOM were enrolled between January 2008 and January 2009. 100 MEF samples from tympanocentesis (n=84) and otorrhea (n=16) were collected (1 subject had 1 sample collected in each ear). The median participant age was 29 months (range: 5 - 55 months), and 54.5% of subjects were male. Bacteria were cultured from 63% samples with at least one pathogen under study. *H. influenzae* was isolated in 31 (31%), 30 *S. pneumoniae* (30%), 2 *S. pyogenes* (2%) and 3 *S. aureus* (3%). 14 (46.7%) *S. pneumoniae* isolates were serotypes found in the two licensed pneumococcal conjugate vaccines (14, 19F & 23F), 7 (23%) were vaccine-related types 6A (n=5) and 19A (n=2) and 7 were non-vaccine types. 27/31 (87%) of *H. influenzae* isolates were non-typeable. No *M. catarrhalis* was isolated.

**Conclusion:** Non-typeable *H. influenzae* and *S. pneumoniae* were the leading bacterial causes of AOM in Cali, Colombia. A vaccine with efficacy against both pathogens would be most useful to prevent AOM.

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Risk factors (RF) for necrotizing enterocolitis (NE) in Pediatric oncologic patients (POP) with neutropenia (NTP)

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**Background:** NE is a life threatening complication in neutropenic patients

**Objective:** To identify risk factors for NE.

**Methods:** Retrospective analytical study Jan 03-Nov 07 at Hospital de Niños of Buenos Aires. NE: POP with NTP and abdominal symptoms (AS) with bowel wall thickness  $\geq 4$ mm by CT scan and/or ultrasonography. Controls (C): POP with NTP and AS with normal imaging. Exclusion: POP with NTP without AS. RF for NE: Underlying disease (UD), Cytosine arabinoside (ARA C) use, fever (F), diarrhea (D), vomiting (V), abdominal pain (AP), abdominal tenderness (AT), sepsis (S) and platelets count (PC). Demographic data, isolations (I), treatment (T), hospitalization days (HD) and mortality (M) were also analyzed. Chi<sup>2</sup> and Mann-Whitney tests were used in univariate analysis. All factors with  $p < 0.05$  were included in multivariate model. **Results:** Population 83 episodes/63 patients. NE 31/83 (37.3%) vs C 52/83 (62.7%). Age (median) 117(5-241)mo, male 43/83 (51.8%), UD: Hematologic malignancies 60/83 (72.5%). NE didn't differ from C in age, gender or UD. Groups were different in **F** 31/31 (100%) vs 44/52 (84%),  $p = 0.02$ ; **D** 31/31 (100%) vs 42/52 (81%),  $p = 0.009$ ; **AP** 29/31 (93.5%) vs 39/52 (75%),  $p = 0.03$ ; **AT** 22/31 (71%) vs 9/52 (17%),  $p < 0.001$  and **PC** (med) 17000/ul (3000-370000/ul) vs 49000/ul (6000-33000),  $p = 0.02$ , **S** 14/24 (58%) vs 18/58 (31%)  $p = 0.021$ . No differences were observed in the ARA C and V. In the multivariate analysis, only **AT**  $p < 0.001$ , OR 11.67 remained statistically associated. **I:** NE 13/31 (42%) vs C 13/52 (25%)  $p = 0.1$ . **I** in NE: *Klebsiella sp* 6/13 (46%), *E.coli* 5/13 (38.5%) and *Pseudomonas aeruginosa* 3/13 (23%). **T** in NE carbapenems-aminoglycosides (AMG) 10/31 (32%) or 3rd gen cephalosporins -AMG-metronidazole 20/31 (64.5%). NE vs C required surgical **T** 5/31 (16%) vs 5/52 (10%),  $p = 0.37$  and **HD** (med) 17(2-60)d vs 12(3-95)d,  $p = 0.85$ . **M** 6/83 (7.2%), NE 4/31 (12.9%) vs C 2/52 (3.9%),  $p = 0.12$ .

**Conclusion:** AT was the only independent risk factor for NE.

In POP with NTP and AS, images to confirm NE should be performed if AT is found

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Temporal variation of human rotavirus types circulating in Caracas during 2007-2008

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**Background:** Human rotavirus (RV) is considered the main viral cause of acute gastroenteritis in children in both developed and developing countries. The recent implementation of a vaccination program promises to effectively reduce the disease burden and health care costs of rotavirus-specific diarrhea. Surveillance is needed to assess the impact of immunization on the rotavirus diarrhea incidence and variability of the circulating strains. In the last decades, RV molecular genotyping has provided valuable information about the diversity of rotavirus outer capsid proteins (VP7/G and VP4/P) of strains circulating throughout the world. Previous studies have demonstrated a broad diversity in rotavirus strains circulating in Venezuela, with predominance of G1, G3 or G4 in combination with P[8] type. The purpose of the present study was to monitor the prevalence of the G/P-NSP4 genotypes of rotaviruses circulating in Caracas between February 2007 and April 2008 and detect any uncommon or novel types by means of molecular characterization.

**Methods:** A total of 164 rotavirus-positive stools from diarrheic pediatric patients aged between 2 months and 5 years collected in Caracas, were tested by multiplex seminested RT-PCR and/or sequencing of the VP4, VP7 and NSP4 rotavirus gene.

**Results:** The analysis revealed 5 common G/P-NSP4 combinations, being G2P[4]/NSP4A and G1P[8]/NSP4B the most prevalent (43% and 38%, respectively), while G3-, G4- or G9-P[8]/NSP4B were more sporadically found. Although present throughout the period studied, G2P[4]-NSP4A rotavirus was the most widely circulating type until November 2007, from then being prevalent G1P[8]-NSP4B strains. Four isolates showed an unusual genotype G8P[14], until now only described in Latino America among animal rotaviruses, 3 of the isolates being associated with NSP4C and one with NSP4A genotype.

**Conclusion:** This study highlights the wide genetic diversity among rotaviruses isolated in Caracas, the temporal variation of the predominant types and the occurrence of a unusual type, G8P[14], described for the first time among humans in our region, probably derived from interspecies transmission. Therefore, it is crucial to continuously monitor circulating rotavirus strains, to understand the effect of rotavirus variation on the efficacy of currently available vaccines.

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Kawasaki disease in a children hospital at Dominican Republic, period January 2006-October 2009

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**Background:** Kawasaki is a febrile illness characterizes by the inflammation of middle-size arteries that affect children, primarily younger than 5 years, of unknown etiology. Since its description by Tomisaku Kawasaki, in Japan, 1967, has been a target of epidemiologic, clinical and laboratorial interest. Early recognition is one of its principal challenges, because, it is related with a good prognosis. In Dominican Republic the first's cases were described in 1986.

The target of this research is to describe the clinical characteristics of the Kawasaki disease' cases in the principal pediatric hospital in Santo Domingo, Dominican Republic

**Methods:** This is a descriptive, observational and cross-sectional study to determine the clinical and epidemiological characteristics of patients admitted with the diagnosis of Kawasaki disease in the Hospital Dr. Robert Reid Cabral from January 2006 to October 2009

**Results:** During the observational period in the Hospital Robert Reid Cabral were admitted 17 patients with the diagnosis of Kawasaki disease. 10 (58.8%) were female, 7(41.2%) male; 6 (35.2%) younger than 1 year; 7 (41.2%) 1-5 years; 4 (23.5%) older than 5 years. Fever was present in all of the cases, nevertheless, duration was quite different, from 5 to 20 days in 6 patients (35.7%); the clinical findings more frequently reported were conjunctival injection in 15 (88.2%) patients, cervical lymphadenopathy 14 (82%); strawberry tongue and erythema of oropharyngeal mucosa 12 (74.5%); skin rash and desquamation 10 patients (58.8%). Only one patient presented cardiac abnormalities. Three (3) patients were treated with immunoglobulin. No mortality reported

**Conclusion:** The findings in the patients from the Dominican hospital were quite similar to those described in the medical literature, mismatching only in the female gender predominance, but it had little significance. Diagnosis was basically clinical, even though the apparent delay in the diagnosis and treatment, the outcome was satisfactory.

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Epidemiology, clinical and microbiology characteristic of multiresistant *Acinetobacter spp.* bacteremia in a pediatric hospital in Argentina

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**Background:** Multiresistant *Acinetobacter* species emerge as an important healthcare-associated pathogen. An understanding of the epidemiology is necessary in order to develop strategies to curtail their spread.

**Methods:** The aim was to analyze the epidemiology, clinical characteristics and antimicrobial profile of multiresistant *Acinetobacter spp.* bacteremia (MAB) in a pediatric hospital.

Methods: Demographic and clinical data from all MAB clinically relevant (2005 -2008) at were collected at Garrahan Hospital. Time-kill studies and a PCR assay with degenerate oligonucleotide primers were performed. Stata 8.0 was used for data analysis.

**Results:** A total of 50 MAB were analyzed, 66 % was confirmed as *A. baumannii* and five clones were detected (clone 1:34%). Time-kill curves tested in the different clones showed only bactericidal effects in 3/ 5 with ampicillin-sulbactam plus gentamycin and in 5/5 with polymixin .The median age of the patients was 13.5 months (IQR: 6 a 54 months). The median length of stay before bacteremia was 16 days (IQR: 8-32 days), 94% of the patients acquired bacteremia in PICU. Eighty eight percent of the patients had underlying conditions: congenital heart diseases (34%), burns (25%) and onco-hematological disease (13.63%) were the most frequent. Ninety four percent received broad spectrum antibiotics (carbapenems and third-generation cephalosporin), 20% immunosuppressive therapy and 46% had malnutrition. All patients had invasive procedures before bacteremia: 90% central venous line, 80 % had previous surgery, 76% mechanical ventilation and 52 % urinary catheter . The final diagnosis was: central venous catheter-associated bacteremia (40%), bacteremia related to skin and soft-tissue infections (26%) and bacteremia (24%). Antibiotics used for treatment were: colistin alone (52%), colistin plus carbapenem (26%), colistin plus piperacillin- tazobactam (10%), colistin plus ampicillin- sulbactam (4%) and ceftazidime plus colistin (2% respectively). The median length of therapy was 14 days (IQR: 14-21 days), 7/50 (14%) patients died but only 3 were related to bacteremia.

**Conclusion:** MAB was detected in patients with medical underlying conditions and prolonged hospitalization. In vitro bactericidal effect was similar with colistin alone as well with the different combination tested. The development of innovative control strategies is needed in order to limit the spread of these pathogens

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Spectrum of infections in children with nephrotic syndrome  
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**Background:** Infection remains an important complication in children with nephrotic syndrome. Besides being the commonest cause of mortality, infections result in significant morbidity and may also be responsible for a poor response to steroid therapy or induce relapse in a child who has already attained remission. The study was undertaken to observe the type of infection in children with nephrotic syndrome and to study the relation of infection to relapse .

**Methods:** A prospective study was done in children diagnosed to have nephrotic syndrome (satisfying ISKDC Criteria) and treated in the Department of Paediatrics, Kasturba Hospital, Manipal, India from August 2005- July 2007. Children with steroid resistant nephrotic syndrome were excluded from this study. Those who satisfied the inclusion criteria were monitored for infectious complications. Relevant investigations were carried out during the episode of infection. Data was analyzed using SPSS 11 data editor and the Z test for proportions .

Relapses (n=101)	Off steroid therapy (n=66)				On steroid therapy (n=35)					
	Only antibiotics		Start steroids + antibiotics		Same dose steroids antibiotics		Steroid dose increased + antibiotics		Immuno- modulators	
	n	%	n	%	n	%	n	%	n	%
With infection (n=78)	8	10.2	45	57.6	17	21.7	4	5.2	4	5.2
Without infection (n=23)	-	-	11	56.5	-	-	3	21.7	5	21.7

**Treatment of relapses of Nephrotic Syndrome**

**Results:** There were 101 episodes of relapse in 73 children with steroid sensitive nephrotic syndrome. Of these episodes, relapse was precipitated by an infection in 77.2% (n=78) of cases. Hence it was found that infections were associated with increased risk of relapses (p<0.005) which was statistically significant. Upper respiratory tract infection (50.4%) was the commonest infection followed by lower respiratory tract infection (22.3%), UTI (10.7%), acute gastroenteritis (9%), skin infections(4.1%), tuberculosis and peritonitis in 1.65% respectively. In children who were off steroid therapy, prompt control of infection with antibiotics induced remission in 8 episodes of relapse. In 45 episodes, children were started on steroids in addition to the use of antibiotics. Remission induction did not warrant modification of steroid dose as the infection was promptly controlled with appropriate antibiotic therapy during 17 episodes. Majority of the episodes of relapse occurred while children were off steroids (66/101) whereas 35 out of 101 episodes of relapses occurred while children were on steroid treatment.

**Conclusion:** Children with nephrotic syndrome are vulnerable for recurrent infections, which play a significant role in relapse. Prompt control of infection plays a crucial role in inducing remission in children with steroid sensitive nephrotic syndrome.

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Multicenter study of meningococcal disease in children in pediatric hospitals in Argentina

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**Background:** *Neisseria meningitidis* infections are an important cause of morbidity and mortality in children of all ages. Latinoamerican studies of this infection are scarce.

**Methods:** Retrospective reviews of medical and microbiologic records from Hospital de Niños Juan P Garrahan (HJPG) and Hospital de Niños Ricardo Gutierrez (HNRG), both in Buenos Aires, Argentina were done to identify patients with invasive *N. meningitidis* infections between 1998 and 2008. Demographic and clinical data were reviewed. **OBJECTIVES.** To analyse the epidemiology, clinical features and outcome of invasive infections caused by *N. meningitidis*

**Results:** One hundred and twenty-six cases (HJPG: 77; HNRG: 49) of invasive meningococcal infection were analyzed during the study period. The median age at presentation was 33 months (range, 1 to 180). Fifty-two percent had male and 15% had underlying disease. Eleven percent had previous antibiotic treatment. The median time of symptoms previously to admission was 1.5 days. The most common signs and symptoms at admission included fever (96%) (> 39°C: 40%; median duration: 3.5 days), rash (61%), nuchal rigidity (37%), vomiting (51%), irritability (59 %) and lethargy (64%). The final diagnosis were: meningitis (86 %), meningococemia without meningitis (10 %), septic arthritis (10%) and occult bacteremia (1.5 %). Twenty-four children (19%) required ICU hospitalization. The median time of ICU admission was 5 days (range: 1-45). Four patients died (3.1%) (serotype C: 2 and without typification (WT): 2). Long term sequelae were seen in 15 patients (12%) (serogroup B: 6, C:6 and WT: 3): brain damage (4), amputation (3), deafness (3), blindness (2), language disturbs (1), hydrocefalia (1) and skin scars (1) . The most frequent serogroup isolates were as follows: B (55); C (28); W135 (6) and Y (6). Almost all patients were treated with parenteral cephalosporin therapy alone. The median time of hospital admission was 8.5 days

**Conclusion:** Meningococcal infections remain an important cause of morbidity and mortality in children in Argentina. Although most patients recover without sequelae, there are a significant number who experience major morbidity and mortality as a result of this infection. Effective meningococcal vaccination recommendation could lessen considerably the burden of meningococcal meningitis.

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The association between vitamin D deficiency, folate deficiency and seropositivity to persistent pathogens among U.S. children

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**Background:** Studies have documented the association between vitamin deficiency and immune system dysfunction. Few studies have examined the relationship between vitamin deficiencies and infection with specific pathogens. The purpose of this study was to investigate whether vitamin D and/or folate deficiency (levels < 20 ng/ml and < 362.6 nmol/L, respectively) are associated with seropositivity to persistent pathogens including cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1) and *helicobacter pylori* (*H. pylori*) as well as with total pathogen burden (total number of the above pathogens for which a subject is seropositive, range 0-3).

**Methods:** We used data from the National Health and Nutrition Examination Survey III, including subjects 12-19 years of age who were tested for seropositivity to each pathogen and serum levels of vitamin D or folate (N=1275). Logistic regression was conducted to generate the odds ratio (OR) and 95 percent confidence intervals (CI) for seropositivity to each pathogen and ordered logit regression was used to generate the OR and 95% CI for seropositivity to an additional pathogen (ie., increasing total pathogen burden).

**Results:** In crude models, vitamin D deficiency was associated with seropositivity to CMV (OR 2.09, 95% CI (1.60, 2.74), HSV-1 (OR 1.53, 95% CI (1.14, 2.05) and seropositivity to an additional pathogen (OR 1.64, 95% CI (1.10, 2.44). Only the association between vitamin D deficiency and CMV remained after controlling for gender, race/ethnicity and poverty income ratio (OR 1.38, 95% CI (1.01, 1.89). Folate deficiency was associated with seropositivity to HSV-1 (OR 1.32, 95% CI (1.04, 1.68) in the crude model, however this association was no longer significant after controlling for confounders.

**Conclusion:** Vitamin D deficiency was associated with CMV seropositivity in this U.S. representative sample of children age 12-19. Vitamin supplementation could serve to lower the prevalence of CMV infection in children in the U.S.

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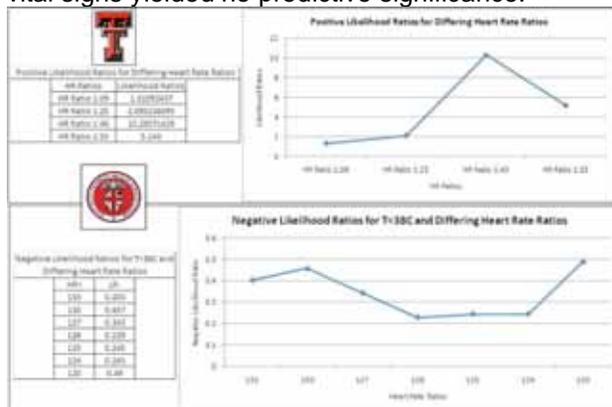
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Pilot study to determine feasibility of development of an influenza clinical diagnostic tool  
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**Background:** Influenza is a costly healthcare burden worldwide. Diagnosing influenza in a timely and accurate manner allows the proper appropriation of scarce healthcare resources and antiviral medications. Unfortunately, current testing technologies are either insensitive (rapid antigen tests), nonspecific (clinical diagnosis) or untimely and expensive (rt-PCR). Ultimately, treatment and management decisions for influenza are suboptimal due to lack of diagnostic accuracy. The need for differentiating influenza from other viral infections led us to the hypothesis that influenza may have a large affect on certain vital signs allowing development of a clinical diagnostic tool.

**Methods:** We prospectively collected data, including demographics, vital signs and rapid influenza antigen testing results from patients who presented to primary care clinics with fever and a cough or fever and a sore throat (influenza like illness) of 2 days or less duration. A total of 38 children were included in this analysis. Subjects' heart rates were converted to a uniform unit (heart rate ratio) by dividing their heart rate by the median heart rate for age. Likelihood ratios (LR) were determined for apparently significant vital sign thresholds.

**Results:** 14 children were influenza A positive by rapid antigen testing. Compared to rapid antigen negative controls, clinically significant likelihood ratios were determined for heart rate ratio greater than 1.4 (Positive LR = 10 (CI 1.4-76.9)) and a combination of current temperature less than 38C with a heart rate ratio less than 1.25 (Negative LR = 0.24 (CI 0.06-0.99)). Other vital signs yielded no predictive significance.



#### Graphs of Likelihood Ratios

**Conclusion:** Although limited by its small size and the use of the rapid antigen test as a proxy to a gold standard, we feel that this pilot study highlights the feasibility of developing a clinical influenza diagnostic tool. Children presenting for medical care with HR ratios >1.4 may be prioritized to treatment based on the higher post-test probability of influenza. Perhaps of more utility, patients with Temp<38C upon presentation with a heart rate ratio of less than 1.25 may have low enough post-test probability to avoid antiviral therapy or allow earlier return to school. We feel this issue deserves additional study.



**Nomogram Showing Pre-test to Post-test change in Probability**

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**Room: Poster & Exhibition Area/Ground Level**  
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Data management, design and analysis challenges in the prevention of perinatal sepsis (PoPS) randomized control trial, Soweto, South Africa

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Wits Health Consortium, Johannesburg, South Africa

**Background:** The World Health Organization highlights neonatal sepsis as the fourth leading cause of death among children <5 years, and the second leading cause of neonatal death. An inexpensive and feasible intervention, to cleanse the birth canal during labor and to wash the newborn at birth with the wide-spectrum microbicide chlorhexidine, holds great promise as a neonatal sepsis prevention tool. Randomized trials done in Malawi and Egypt had design flaws that led to limited uptake, and the efficacy of the intervention remains under debate.

**Methods:** Our study (PoPS) was a single center, prospective, randomized controlled trial evaluating the efficacy of 0.5% chlorhexidine wipes of the birth canal during labour and neonatal skin after delivery at preventing serious neonatal peripartum infections. The purpose of this paper is to describe the design characteristics and challenges of the trial and of our method of dealing with the lack of double-blind outcome assessment. To deal with the complexity of having 13 data collection forms and about 30 data tables we developed a relational database and used a double data entry system for cleaning and editing the database. Because the trial was not double-blinded, we elected to monitor some baseline characteristics by treatment group to detect any potential problems with implementation of the randomization procedures. Propensity scores analysis was planned to address imbalances. Serious Adverse Events (SAEs) were monitored and analyzed separately for mothers and infants. The primary analysis included all births and twin births were assumed to be independent events. An interim analysis was undertaken to evaluate the balance between the treatment arms and to decide on whether to stop the trial for compelling efficacy.

**Results:** At the conclusion of the trial, balance was found acceptable. The rate of neonatal sepsis within three days of birth did not differ between the chlorhexidine (3.5%; 141 of 4 058) and placebo arms (3.7%; 148 of 4 036; p=0.65).

**Conclusion:** Data management in epidemiological studies should receive high priority. Alternate strategies are required to reduce the high burden of neonatal sepsis in developing countries.

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**Type: Poster Presentation**

RCT Methodology, population profile and pneumonia rates in socio-economically deprived infants of Kabul: Data from a RCT investigating the effects of vitamin D supplementation on the incidence of infant pneumonia

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**Background:**

Approximately 150 million children under-5 suffer from pneumonia each year worldwide and South-east Asia has the highest incidence of pneumonia (0.36 episodes per child-year). Two hospital-based case-control studies from Ethiopia and India suggest that vitamin D deficiency may substantially increase the risk of severe pneumonia among children under-5. In Afghanistan Dietary intake of vitamin D is low and exposure to sunlight is also limited due to widespread use of Burqua. Thus it was hypothesized that supplementation of vitamin D would reduce the incidence of pneumonia in young children particularly in areas where vitamin D deficiency is common.

**Methods:** Study Design: Double blind placebo controlled trial

Setting: 4 urban districts in Kabul, Afghanistan with a socio-economically deprived population living in overcrowded housing.

Subjects: 3048 children (half taking vitamin D and half placebo) aged 1-11 months at recruitment (Nov-Dec 2007) followed up for 18 months through active and passive surveillances.

Intervention: 3-monthly oral 100,000IU vitamin D versus Placebo (olive oil).

Analysis: Rate ratio for episodes of pneumonia was calculated for the whole population using survival analysis. Kaplan Meier plots display the survival curves. Covariates in the models control for potential confounders and/or interactions

**Results:** Socio-demographic Profile Sex ratio of infants was balanced (males 52.2% and females 47.8%). The mean age of children was 6.5 months (SD 3.1) at recruitment.

Incidence rate of Clinical Pneumonia (IMCI definition): As the trial is not complete yet, the data for the first 12 months is used for this presentation The incidence rate (95% CI) for first episode of simple pneumonia was 1.5/child year (1.5, 1.6), while for repeat episodes was 1.7 (1.7, 1.8). The incidence rate of the first episode of severe pneumonia was 0.1/child year (0.09, 0.12) and for repeat episodes was 0.1 (0.11, 0.13). The incidence rate of all episodes of pneumonia was 1.8 (1.7, 1.8).

**Conclusion:** RCT with 18 months follow up amongst socio-economically deprived population in Kabul can be successfully implemented. Lessons learned will be shared in the presentation. The incidence rate for pneumonia seems high compared to South Asia rates

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Morbidity and mortality for neonatal sepsis in a Hospital in Havana City, 1998-2008

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**Background:** To compare the epidemiological, clinical and microbiological profiles between patients with neonatal sepsis who lived or died.

**Methods:** The medical records of patients with neonatal sepsis were retrospectively reviewed at Hospital Universitario Docente América Arias, between 1998 and 2008. Neonatal sepsis cases were classified as surviving or not after 30 days of postnatal follow-up. The survivor and deceased groups were compared using Mann-Whitney

's U test for continuous variables, and the chi-squared test or the Fisher's exact test for categorical variables. Significantly associated variables were included in a Cox proportional hazards model. A p-value <0.05 was considered statistically significant for all analyses.

**Results:** A total of 116 patients with neonatal sepsis were included (65 live and 51 dead). Multivariate analysis showed that fetal distress, respiratory distress, a delayed capillary fill up, a low platelet count, and a positive hemoculture for Staphylococcus Coagulasa Positive and Klebsiella Pneumoniae were significant risk factors for death.

**Conclusion:** Conclusions. Epidemiological, clinical, laboratory, and microbiological variables are significant predictors of death in newborns with neonatal sepsis.

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Age and sex specific pattern of urban malaria and diarrhea mortalities in Accra

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**Background:** Health outcomes are not evenly distributed across different populations and different individuals in a given population differ in susceptibility to different risk factors. Study objectives: To investigate age and sex specific malaria and diarrhea mortality patterns in a rapidly urbanizing area in Ghana. Design: A time-point pooled analysis of observed cluster-level malaria and diarrhea mortality in an urban area in Ghana.

**Methods:** All-cause mortality data for the period 1998-2002 were obtained from the Ghana Vital Registration System (VRS) and cluster-level Proportional Mortality Ratios (PMRs) computed separately for age and sex.

**Results:** While there was no sex-specific mortality difference for both malaria and diarrhea, there was some evidence of differences in mortality levels across age-groups. In particular, children under-1 year old were partially protected against malaria specific mortality which was highest among those between 1 and 5 years. In contrast, diarrhea specific mortality was highest in infants and decreased progressively with age.

**Conclusion:** Child survival programs will be more effective if the programs more appropriately reflect these risk patterns for the two childhood killers.

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Paediatric rotavirus disease in The Gambia: A hospital based sentinel study

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**Background:** Diarrhea is a major cause of pediatric morbidity and mortality with an estimated 3 million deaths per year of which about 20% are caused by rotavirus.

After more than a decade of the last published work on diarrheal disease in the Gambia, we aimed to determine the disease burden and epidemiology of rotavirus diarrheal disease at the Royal Victoria Teaching Hospital (RVTH), Banjul during the documented peak period of rotavirus transmission.

**Methods:** From 1st January to 31st March 2006, eligible children aged 5 years and below admitted with gastroenteritis had their stools collected within 48 hours of admission and tested for rotavirus, using ELISA (Dako ID EIATM Rotavirus test kits). Socio demographic data was obtained using a study questionnaire.

**Results:** Out of 536 admissions, 187 (34.9%) had acute diarrheal disease and 148 stools tested for rotavirus. Of the 148 specimens tested, 111 (75.0%) were positive for rotavirus antigen and 37 (25.0%) were negative. Ninety (81.1%) of the positive cases, were aged 12 months and below. There was no significant difference in the age specific prevalence rates ( $\chi^2 = 0.50$ ,  $p = 0.48$ ). The mode of feeding, and other identifiable possible risk factors like socio economic class, maternal education, level of hygiene practiced by the mother, method of excreta disposal, water source, did not appear to have a significant effect on the risk of rotavirus infection. There was a well-defined, period of peak transmission occurring between the third week of January and second week of February 2006.

Mortality rate for acute diarrhea over the study period was 7.5%, with 50.0% of the stools of those who died being positive for rotavirus.

One hundred and twenty (81.1%) of the patients had complete routine vaccination for age, 16(10.8%) had incomplete vaccination, while 12(8.1%) did not have available records.

**Conclusion:** Rotavirus remains a leading cause of diarrhea disease in children in the Gambia, with significantly high mortality in the under 5 year old children during periods of high transmission. Because of the high vaccination coverage noted among the children studied, introduction of vaccines against rotavirus may be an effective control measure.

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The role of viruses in the aetiology of IRA in Peruvian children

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**Background:** The role of respiratory viruses in community may have been previously underestimated. We aimed to study the incidence and clinical characteristics of acute respiratory infections (IRA) in children adding PCR to routine conventional laboratory tests.

**Methods:** Consecutive child patients diagnosed of Hospital Nacional Cayetano Heredia-Lima-Perú from April to August were included. Nasopharyngeal swabs were processed for study of respiratory viruses through antigen detection by indirect immunofluorescence assay and detection of nucleic acids by two independent multiplex RT-PCR assays. According to the aetiology, patients were categorized in 4 groups: group 1, only virus detected; group 2, only bacteria detected and group 3, viral and bacterial

**Results:** Of 200 patients diagnosed with IRA, 200 had nasopharyngeal swabs available and were included in this study. Aetiology was established in 200 patients: group 1, n=57 (28.5 %); group 2, n= 23 (11.5 %); group 3, n= 25(12.5 %). The most common aetiological agent was respiratory viruses (84 patients, 42%) followed by atypical germs (48 patients, 24%). Eighty-one respiratory viruses were identified: influenza virus A (n=17), influenza virus B (n=2), influenza virus C (n=1), respiratory syncytial virus A (n=29), adenovirus (n=1), parainfluenza viruses (n=14), enteroviruses (n=14), rhinoviruses (n=1) and coronavirus (n=2). There were eleven patients coinfecting with respiratory virus. Forty and five atypical germs were identified: 21 *Chlamydia pneumoniae* (n= 21) and *Mycoplasma pneumoniae* (n=24). There were sixteen patients coinfecting by both atypical germs. Immunofluorescence 41 and PCR 81. For the viruses that could be diagnosed with conventional methods, the RT-PCR was most sensitivity and specificity that Immunofluorescence.

**Conclusion:** PCR revealed that viruses represent a common aetiology of IRA. There is an urgent need to reconsider routine laboratory tests for an adequate diagnosis of respiratory viruses, as clinical characteristics are unable to reliably distinguish viral from bacterial aetiology.

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Clinical and epidemiology characterization of children hospitalized with influenza A H1N1 (FLU AH1N1) during the first wave of 2009 outbreak, Santiago, Chile

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**Background:** In Chile flu AH1N1 affected mainly children between 5-14 years old (4500/100,000 pop) with highest hospitalization in children ≤5 years (90,8/100,000 pop). Objectives were to describe epidemiological, clinical, virological and laboratory findings and to determine risk factors for severe disease in pediatric patients.

**Methods:** Descriptive study of hospitalized children with confirmed flu AH1N1. We studied the presence of the virus in biological samples (respiratory secretions, blood and urine) using real-time RT-PCR and viral culture, at admission and at 3rd and 5th days of treatment with oseltamivir. Viral load from respiratory samples was standardized by copies/100,000 cells.

**Results:** 20 children were hospitalized with flu AH1N1. Twelve girls (60%), mean age 2.9 years (1m-16y). Most were infected at home 30% (6), hospital 25% (5) and nursery/kindergarten 15% (3). 65% had underlying diseases: recurrent wheezing (7), congenital heart disease (2) and immunosuppression (2). The leading cause of hospitalization was oxygen requirements (50%), respiratory failure (20%), deterioration of the underlying disease (10%), fever of unknown origin and hyperemesis (5%). Fever (95%), cough (90%) and dyspnea (70%) were the most frequent symptoms. Ten patients required ICU for an average of 16 days, median 8 days (1-60), 3 of them had underlying diseases (recurrent wheezing and liver transplantation). Six patients required mechanical ventilation and three of them needed high frequency oscillatory ventilation. Complications: wheezing 55% (11), secondary bacterial infection 45% (10), atelectasis 30% (6) and global respiratory failure 15% (3), no mortality. The average of the lymphocyte count in ventilated patients was 715 ± 517 cells/ml and 3251 ± 2225 cells/ml in non ventilated patients (p< 0.0001). Presence of underlying disease and viral load were not associated with severe respiratory failure. 14/15 patients had detectable virus in nasopharynx after 24-48 hours of oseltamivir (127,000 to <200 copies/ml), at 48-72 hrs 2/17 remained positive (66,000 to less 200 copies/ml). 2/5 remained positive after treatment. Virus was not found in blood and urine samples.

**Conclusion:** Hospitalization due to flu AH1N1 in children was associated with presence of underlying diseases. High proportion of children needed ICU but no mortality was observed. Lymphopenia might be used as a risk factor for severe disease.

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**Type: Poster Presentation**

Rapid diagnostic tests for neonatal malaria: How reliable?

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**Background:** Congenital malaria has a prevalence of 11.5% in Northwestern Nigeria with low parasite densities which range from 40-120 $\mu$ L. Neonatal malaria in this region has a prevalence of 8.25% with most of these babies presenting with fever in the first week of life. Diagnosis has been dependent on blood film examination for asexual forms of *Plasmodium* parasites. Due to the inherent difficulties in identifying very low levels of parasitaemia by light microscopy, this study was carried out among febrile and afebrile neonates to compare microscopy with a malaria rapid diagnostic test (RDT) to determine sensitivity and specificity of the two in diagnosis of neonatal malaria.

**Methods:** Presence of malaria parasitaemia were assessed in 50 febrile and 50 afebrile neonates using thick and thin blood film smears, air dried, stained with freshly prepared Giemsa stain and examined under a light microscope at X1000 magnification as well as by use of optiMAL rapid diagnostic malaria kits.

**Results:** 42/50 of the febrile neonates were positive for *falciparum* malaria parasites by microscopy while 19/50 were positive by optiMAL. Thus sensitivity of the light microscopy was 84% and that of RDT was 38%. The true negatives were 48/50 and 50/50 by microscopy and RDT respectively giving a specificity of 96% for microscopy and 100% RDT. OptiMAL had positive and negative predictive values of 38% and 100% respectively.

**Conclusion:** The rapid diagnostic test was easy to perform and results were obtained more quickly than the microscopy. However its performance in accurately diagnosing patients with neonatal malaria is unacceptably low. About 2 out of every 10 children with neonatal malaria will also be missed by light microscopy. Peripartum malaria prevalence which is highly predictive of congenital/neonatal in this area is about 22% with higher parasite densities up to 400/ $\mu$ L. Since optiMAL is known to have sensitivity of >95% when parasite densities are up to 200/ $\mu$ L, it is recommended that optiMAL be routinely performed when women present in labor to identify and followup newborn at risk of malaria.

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**Type: Poster Presentation**

Detection of rotavirus antigen in stools samples collected from children in parts of Nigeria

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**Background:** Rotavirus is the most common cause of severe diarrhoeal disease in infants and young children all over the world. About 40% of more than 125 million cases of diarrhoea each year in the world are attributed to rotavirus. According to reports of the Center for Disease Control and Prevention (CDC) in the United States, each year, rotavirus causes approximately 111 million infections, 25 million hospitalizations, and 440,000 deaths in children less than five years of age, worldwide, and the total associated medical costs due to rotavirus infection are estimated to be enormous.

In Nigeria, about three hundred children under the age of five are thought to be lost annually to diarrhoea.

**Methods:** Data including stool samples and background information were collected from 157 diarrhoeic children aged less than five years; from eight different hospitals in the southern part of Nigeria between June, 2006 and July 2009. Viral RNA was extracted from the stool samples with the RNA mini-kit (Qiagen) and a fragment of the VP7 gene was amplified with the Reverse transcriptase polymerase chain reaction (RT-PCR), from the extracted RNA.

**Results:**

Of the 157 specimens collected from these hospitals, Rotavirus was amplified from 29 (18.5%) of them. There was no significant sex preponderance observed ( $p < 0.05$ ). However the peak incidence of Rotavirus diarrhoea was seen among children between 12 and 24 months.

**Conclusion:** Rotavirus gastroenteritis remains a serious public health problem in the developing world and given the extraordinary diversity of the virus in some countries, adequate surveillance is needed to establish rotavirus disease burden. Moreover, as various multinational health organizations take on projects for producing and circulating the rotavirus vaccine, to reduce rotavirus infection in developing countries and to curtail medical costs in developed countries, the precise situation in developing countries like Nigeria needs to be adequately projected so as to effectively enforce the use of the Rotavirus vaccine.

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Bed net coverage, usage and condition in fishing villages of Suba district, Western Kenya

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**Background:** Past studies showed that bed nets can reduce child mortality. Currently, the Kenyan government is subsidizing the price of bed nets, and several non-governmental organizations (NGOs) are distributing nets with little charge. However, information on coverage, usage and condition of nets is lacking, particularly in the remote areas.

**Methods:** We investigated bed net coverage and condition in seven fishing villages on the shore of Lake Victoria, Western Kenya. Usage of bed nets was examined through direct observation in early morning. Residents were asked whether they were trained on proper usage and maintenance of nets. As locals have started to replace traditional papyrus mats with bed nets for spreading and drying small fish, we visited seven beaches to investigate how widely bed nets have been used for capturing and drying fish.

**Results:** On overall, coverage was 71.9% and 41.1% for mainland and island villages respectively. Out of 262 nets only 100 (38.2%) were used. Seventy seven (29.4%) of them were hanged but not used, 51 (19.5 %) were not hanged properly, while 34 (13%) were kept in boxes. We observed three types of nets; long-lasting treated bed nets (LLTNs) 47.8%, ordinary 38.5% and Insecticide treated nets (INTs) 13.7%. Eighty seven percent of the observed nets had holes more than 1cm. On the beaches, 234 bed nets were used for drying fish and 194 (82.9%) of them LLTNs, while 40 (17.1%) were non-LLTNs. Forty one bed nets were used for capturing fish from the lake. Locals preferred LLTNs for drying or capturing fish because they were stronger and fish dry faster and straighter. An NGO distributed 150 LLTNs in one village eight months before this survey, and we counted 52 (36.7%) of them were used for either drying or capturing fish. Out of 244 residents interviewed 236 (96.7%) said they did not get any training on proper usage and maintenance of nets.

**Conclusion:** In addition to pursuing high coverage of bed net more efforts should be made to ensure that nets are kept in good condition. Education component should be included in the ITN distribution to ensure proper usage and care.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Quantitatively estimated the global burden of disease of chronic and infectious diseases interface in 2002

**Y. Yan**<sup>1</sup>, B. Choi<sup>2</sup>, H. Morrison<sup>2</sup>, T. Wong<sup>2</sup>, J. Wu<sup>2</sup>

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**Background:** Many chronic diseases are original from infectious agents, and some infectious diseases involve to chronic conditions. If according to current classification for disease control and research, it is easy to produce a neglected area, the interface of chronic diseases and infectious diseases. We try to estimate the global burden of the kind of diseases.

**Methods:** The number of deaths, mortality rate and disability adjusted life years (DALY) of chronic and infectious diseases in the world in 2002 come from the Databases in Data and statistics in WHO website. There are 3 chronic and infectious disease interface areas: (1) chronic disease with infectious origin; (2) chronic disease with short-term morbidity; (3) Infectious disease with long-term morbidity. We reviewed the literature to formulate our own evaluation, and calculated the subtotals and percentages of the 3 categories by sex, age, cause and level of development.

**Results:** The percentage of the estimated global number of deaths of chronic disease with infectious origin in all number of deaths and DALY of all chronic diseases were 10.46 % and 7.96%. This kind of chronic disease mainly affected on aged of 45+ people. The number of deaths in developing countries accounts for over 2/3, but the mortality rate is higher 1/3 in developed countries. The percentages of the estimated number of deaths and DALY of chronic diseases with short-term morbidity in all chronic diseases were 19.14% and 8.70%. The mortality rate (503.84 per 100, 000) of aged 45+ in male was higher than ones in female (335.28 per 100, 000). The percentages of estimated number of deaths and DALY in developed countries were higher than ones in high mortality developing countries. The percentage of number of deaths of long-term morbidity infectious disease was near to one third in all of infectious diseases. And it's affected aged of 15-44 years adolescents and adults, especially to the DALY.

**Conclusion:** The infectious origin chronic diseases should be paid more attention not only in developing countries, but also in developed countries. To take effective health care action in male and in developed countries will play a key role for preventing chronic diseases with short-term morbidity. Through integrate the methods of chronic and infectious disease epidemiology, the kinds of diseases will be controlled effectively more.

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**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Application of international health regulations in the Singapore pandemic influenza experience

**P. L. Ooi**

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**Background:** The global spread of novel influenza A (H1N1-2009) constituted a public health emergency of international concern (PHEIC). Singapore's defense against this pandemic strain comprised public health measures in compliance with WHO's International Health Regulations (IHR), 2005. We report herein on the epidemiology and control of the first 350 cases notified in May-Jun 2009.

**Methods:** We investigated into the first 350 laboratory-confirmed cases of novel influenza A (H1N1-2009) identified from the healthcare institutions between 27 May and 25 Jun 2009. Epidemiological details of these cases were retrieved and analysed. Contact tracing and active case finding were also instituted for each reported case, and relevant particulars including flight information provided to WHO and overseas counterparts.

**Results:** The first 350 novel influenza A (H1N1-2009) cases comprised 221(63%) imported cases, 124 (35%) locally acquired cases and 4(2%) cases with unknown source. The imported cases consisted of three waves involving the US, Australia and Southeast Asia. In the first wave, 11 (69%) of the 16 imported cases had visited the USA within seven days prior to their onset of illness between 25 May and 4 Jun 2009. In the second wave, 20 (74%) of the 27 imported cases between 5 Jun and 12 Jun had traveled to Melbourne, Australia. In the third wave, 90 (50.6%) of the 178 imported cases between 13 Jun and 25 Jun were acquired from intra-regional travel in Southeast Asia. Specifically, 49 cases were from the Philippines and 40 (82%) of them had traveled to Manila. A total of 667 communications were effected through the IHR mechanism, majority within 24 hours of disease notification.

**Conclusion:** Singapore experienced an unprecedented level of international cooperation for surveillance and response to this novel Influenza A (H1N1-2009) pandemic. Through the timely sharing and open reporting of major public health events, we established a network of collaboration which facilitated not only notification of confirmed cases, but also bilateral communications in a manner that was rational, predictable and productive. The IHR mechanism has served as an effective channel to engage in regional cooperation concerning disease surveillance and data sharing.

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**Type: Poster Presentation**

Models and data for determining transmission routes

**J. Koopman**

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**Background:** Multiple modes of transmission are possible for respiratory infections. For example influenza might be spread via small droplet aerosols that get down into the lungs, airborne medium size droplets that settle on upper airway mucosa, or large droplets that might travel directly between individuals or contaminate hands or fomites that carry infectious agent to a new host. Methodology to study the frequency of different transmission modes and the role that they play in amplifying or disseminating transmission throughout populations has been lacking.

**Methods:** Environmental Infection Transmission System Models were formulated that range from highly abstract to highly detailed. These models follow the infectious agent out of one person, through the environment, to another individual, and then through the immune elements that could eliminate them until they start an infection. These models are analyzed mathematically and numerically. Then they are run with different hypothetical types of data in order to determine what types of data would be most helpful in determining what modes of transmission are acting in the real world.

**Results:** The abstract models show that different modes of transmission will be expected to have different relationships that constrain the number of individuals that can be infected via that mode. The more detailed models show that for influenza, different routes of transmission will dominate under different conditions. The framework for determining infection mode from real world data involves relating a hierarchy of data types to a hierarchy of model types in a manner that assesses the robustness of inferences about modes of transmission to realistically relaxing extreme simplifying assumptions in the models and then finding a reasonable data set that can estimate model parameters.

**Conclusion:** Data from a single venue on who gets infected given different levels of environmental contamination and their exposure to that contamination will be very difficult to gather in a manner that allows for definitive conclusions about transmission mode. Data on genetic variation patterns of pathogens in different environments where different modes of transmission might dominate could be more productive.

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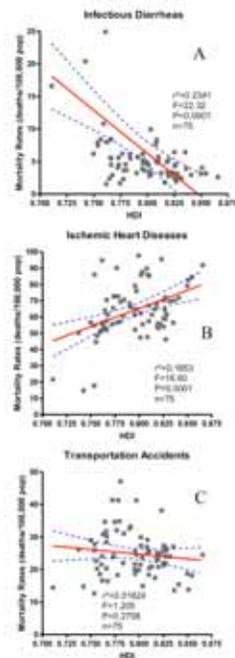
Epidemiological transition in Venezuela: Relationships between infectious diarrheas, ischemic heart diseases and transportation accidents mortalities and the human development index (HDI) in Venezuela, 2005-2007

**A. Rísquez Parra**, L. Echezuria, A. Rodriguez-Morales  
Universidad Central de Venezuela, Caracas, Venezuela

**Background:** The objective of this study is to describe potential relationships between the Human Development Index (HDI) and its components and the mortality due to infectious diarrheas (ID), ischemic heart diseases (IHD) and transportation accidents (TA) in the 23 States and DC of Venezuela between 2005-2007 as a reflect of epidemiological transition in the country.

**Methods:** Socioeconomical data (classified according the WB) was obtained from the National Institute of Statistics, including the HDI and its components life expectancy, literacy, education, standard of living, and GDP per capita; and the epidemiological data (mean rates) from the Ministry of Health, both from Venezuela. For the analysis regression models were done.

**Results:** The HDI varied in the states from 0.710689-0.865577. ID mortality disparity ranges from rate of 1.55-49.62 deaths/100,000pop; IHD mortality from 14.77-97.78 deaths/100,000pop; and TA mortality from 12.63-47.05 deaths/100,000pop. Linear regression models evidenced that the relationship between epidemiological and social variables and HDI was negative for ID, positive for IHD and neutral for TA. Those states with higher HDI and its components had lower ID mortality rates ( $r^2=0.2341$ ;  $p<0.0001$ ) (Figure 1A) and higher IHD mortality rates ( $r^2=0.1853$ ;  $p=0.0001$ ) (Figure 1B). In the case of TA there was no significant variation regard the HDI and its components ( $r^2=0.01624$ ;  $p=0.2758$ ) (Figure 1C).



**Figure 1. A. Linear regression between Infectious Diarrhea Mortality Rates and HDI. B. Linear regression between Ischemic Heart Diseases Mortality Rates and HDI. C. Linear regression between Transportation Accidents Mortality Rates and HDI. 2005-2007.**

**Conclusion:** These data reflect the significant influence of socioeconomical indicators of development, such as the HDI, on the ID and IHD mortality rates in Venezuela, with different patterns, compatible with the epidemiological transition in the country. With better socioeconomical conditions, reflected in the HDI and its components, mortality rates from ID are lower but from IHD are higher.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Medical needs assessment and infectious diseases concerns in rural Honduras - Implications for medical relief planning

M. P. Stevens<sup>1</sup>, K. Elam<sup>1</sup>, L. F. Stevens<sup>1</sup>, S. Shodhan<sup>1</sup>, D. Markley<sup>1</sup>, R. Hemrajani<sup>1</sup>, K. Sanogo<sup>1</sup>, **G. Bearman**<sup>2</sup>

<sup>1</sup>Virginia Commonwealth University, Richmond, Va, USA, <sup>2</sup>Richmond, VA, USA

**Background:** Socio-environmental conditions vary across rural Honduras. As medical relief missions target rural communities, planning should focus on highly prevalent concerns. We assessed the needs and disease perceptions of people in the Department of Yoro area of rural Honduras

**Methods:** A needs assessment survey was administered in June 2008 in the Department of Yoro area of Honduras. The survey consisted of 29 multiple-choice questions that collected data on demographic information, environmental health pressures such as home environment, access to clean water and sanitation. The survey also assessed access to healthcare and the top 3 perceived critical issues affecting health. Surveys were randomly administered at multiple clinic sites. Surveys were voluntary, anonymous and in Spanish. A descriptive analysis of the responses was performed. The association between concern for infectious diseases and socio-environmental factors was explored. The Chi-square test was employed for statistical significance.

**Results:** 70 surveys were collected. The mean age was 38. Twenty-one men and 49 women completed the survey. Only 8% of the respondents had completed 12 years of education. Thirty-eight (54%) were employed. The average number of adults living in a given home was 4.4. Forty-eight (69%) of respondents obtained their water from plumbing, with 14 (20%) reporting no purification treatment of water. Forty-seven percent of respondents used outhouses or latrines. Thirty-nine (56%) reported the presence of farm animals within their home. Sixteen percent of homes were constructed with adobe and 39% of all homes had dirt floors. The most common healthcare concerns were water sanitation (19, 32%), nutrition (19, 32%), infectious diseases (18, 31%) and access to doctors and medications (18, 31%). There was no statistically significant association between concern for infectious diseases and crowded household (>4 people/house) ( $p=0.90$ ), dirt flooring ( $p=0.48$ ) or the presence of farm animals in the home ( $p=0.48$ ).

**Conclusion:** Despite differences in socio-environmental factors in rural Honduras, the principal health concerns were water sanitation, nutrition, infectious diseases and access to doctors and medications. There were no associations between infectious diseases concerns and socio-environmental factors. Medical relief missions to rural Honduras should anticipate widespread infectious diseases related concerns and should prepare accordingly.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Redevelopment and evaluation of EpiData: A practical software tool for use in the public health field

**A. Arthur**<sup>1</sup>, J. Garay<sup>2</sup>, B. Guarda<sup>3</sup>, L. Sieswerda<sup>4</sup>, A. Stevens<sup>5</sup>

<sup>1</sup>Toronto Public Health, Toronto, ON, Canada, <sup>2</sup>York Region Community and Health Services Department, Newmarket, ON, Canada, <sup>3</sup>Public Health Agency of Canada, Ottawa, ON, Canada, <sup>4</sup>Thunder Bay District Health Unit, Thunder Bay, ON, Canada, <sup>5</sup>Brant County Health Unit, Brantford, ON, Canada

**Background:** EpiData Entry and EpiData Analysis are key tools for case and outbreak data management, both in Canada and internationally. Based on field input and pilot testing, the full scope of EpiData capability has yet to be realized. This project focuses on the development and evaluation of standardized tools used for epidemiological training and field management of outbreaks on a national and international scale.

**Methods:** With funding received by the Public Health Agency of Canada (PHAC), the EpiData Association in Denmark and the Association of Public Health Epidemiologists in Ontario (APHEO) have been collaborating since 2007 on the redevelopment and evaluation of EpiData software applications. EpiData is a free software suite designed to assist epidemiologists, public health investigators and other public health professionals in entering, managing, and analyzing data in the field. The software was created in 1999 and is maintained by the EpiData Association in Denmark. It is also used as a training tool internationally, including the Public Health Agency of Canada's (PHAC) Canadian Field Epidemiology Program.

Through in-person meetings, targeted surveys, and solicited feedback from key public health partners, gaps in knowledge and awareness regarding the functionality and use of EpiData have been assessed, and used to define the knowledge products for development.

**Results:** To date, software development has focused on functionality applicable to a range of public health tasks, including the investigation and management of infectious disease outbreaks. Knowledge translation of these advances has been conducted through development of field guides and hands-on workshops. The next phase of the project will focus on developing tools for the use of EpiData in areas outside of communicable disease investigations, including surveys, program evaluation, and management of chronic disease data.

**Conclusion:** This poster provides an overview of the Evaluation and Redevelopment EpiData project, and outlines the initiatives currently underway.

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**Final Abstract Number: 81.008**

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**Type: Poster Presentation**

Similarities and differences between persons of higher and lower income in Argentina, concerning their knowledge on vertically transmitted diseases

M. Mayer Wolf<sup>1</sup>, A. V. Sanchez<sup>2</sup>, C. Ezcurra<sup>1</sup>, V. Rodríguez<sup>1</sup>, **C. Freuler<sup>1</sup>**

<sup>1</sup>Hospital Alemán, Buenos Aires, Argentina, <sup>2</sup>Sala Angel Bo, William Morris, Buenos Aires, Argentina

**Background:** The fact that individuals in higher income brackets have easier access to general information raises the question whether the individuals in lower brackets are receiving insufficient medical knowledge. The countrywide vaccination campaign against rubella allowed us to observe a young and healthy adult population that was a fair representation of two social strata; this was ideal for the purpose of our investigation.

So, we tried to assess the degree of knowledge regarding vertically transmitted diseases by these two groups of citizens of different social and cultural levels.

**Methods:** Our population performed two groups, the first one attending a community hospital of a very high social and cultural level in the city (GROUP #1, with 508 males and 966 females), and a second one belonging to a low income health center in a lower income area outside the city (GROUP #2, with 84 males and 94 females). Both groups responded anonymously to the survey on vertically transmitted diseases.

<b>Results: Disease</b>	<b>Hospital Alemán</b>	<b>William Morris</b>
<b>Toxoplasmosis</b>	46%	46%
<b>HIV</b>	81%	76%
<b>HBV</b>	53%	40%
<b>Rubella</b>	24%	48%
<b>Syphilis</b>	24%	30%
<b>HPV</b>	21%	35%

GROUP #1 showed to have a better knowledge than GROUP #2 regarding HIV and Hepatitis B (81% to 76%). However GROUP #2 identified Chagas disease and Syphilis more readily than GROUP #1 (30% to 24% and 35% to 21% respectively) as vertically transmitted diseases; for German Measles the proportion was even higher (GROUP#1: 24% and GROUP #2: 48%).

**Conclusion:** Possibly, the higher incidence of Chagas disease and the compulsory testing for Syphilis in the health center attended by GROUP #2 are the variables that led to a slight increase of awareness of these illnesses by the latter. It is surprising though that, despite the fact that this study was conducted at the time of the German Measles vaccination process, both groups showed a low recognition level of this disease being vertically transmitted.

The degree of awareness of vertically transmitted infections is generally low, and better access to information does not seem to play a determining role in the level of awareness amongst a healthy, young population. Other strategies have to be tried.

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**Session: Public Health Interventions, Modeling & Training**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Socioeconomical aspects of malaria morbidity and mortality in Venezuela: An analysis of their correlations with the Human Development Index and its components

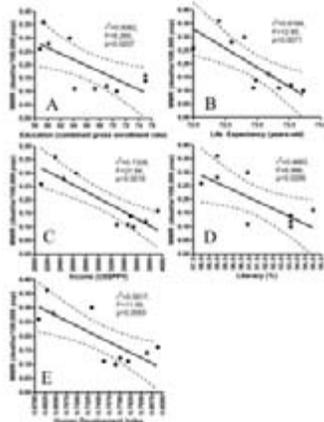
**A. Rodriguez-Morales**<sup>1</sup>, J. Benitez<sup>2</sup>, R. Harter-Griep<sup>3</sup>, M. A. Lopez-Zambrano<sup>4</sup>

<sup>1</sup>Instituto Jose Witremundo Torrealba (former Center for Parasitological Research Jose Witremundo Torrealba), Universidad de Los Andes, Trujillo, Venezuela, <sup>2</sup>Environmental Health and Sanitary Control (former Malariology), Ministry of Health, Maracay, Venezuela, <sup>3</sup>Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil, <sup>4</sup>Public Policy Research Unit, Universidad Simon Bolivar, Caracas, Venezuela

**Background:** Describe potential relationships between the Human Development Index (HDI) and its components and the morbidity and mortality of malaria in Venezuela in the period 1995-2004.

**Methods:** Socioeconomical data (classified according the World Bank) was obtained from the National Institute of Statistics, and the epidemiological data from the Ministry of Health, both from Venezuela. For the analysis the annual variation of the variables was assessed and also regression models were done.

**Results:** The HDI varied in the period from 0.6746 (1995) to 0.8144 (2004). An increase in the morbidity rate was observed from 1.01 to 1.79 cases/1,000 pop (22,056 cases to 46,244 cases), but mortality rate decreased from 0.36 to 0.10. Analyzing the linear regression models, it was observed that the relationships between epidemiological and social variables were significant. There was an inverse relation between both types of variables; when education increased the malaria mortality decreased ( $r^2=0.5082$ ,  $F=8.265$ ,  $p=0.0207$ ) (Figure 1A), similarly for life expectancy, when increased, mortality decreased ( $r^2=0.6164$ ,  $F=12.85$ ,  $p=0.0071$ ) (Figure 1B); when income increased malaria mortality decreased ( $r^2=0.7328$ ,  $F=21.94$ ,  $p=0.0016$ ) (Figure 1C); and for the literacy also its increase was associated with a malaria mortality decrease ( $r^2=0.4662$ ,  $F=6.986$ ,  $p=0.0296$ ) (Figure 1D). As expected, the influence of HDI on malaria mortality was also significant, with the improvement of HDI a reduction of mortality was seen ( $r^2=0.5917$ ,  $F=11.59$ ,  $p=0.0093$ ) (Figure 1E). In Bolivar and Amazonas states (higher endemic states), these relations were similar, being significant between socioeconomical variables and malaria mortality ( $p<0.05$ ) but not for morbidity ( $p>0.05$ ).



**Figure 1. Linear regressions between malaria mortality (MMR) and socioeconomical variables (A, education, B, life expectancy, C, income, D, literacy and E, HDI), Venezuela, 1995-2004.**

**Conclusion:** These data reflect the significant influence of socioeconomical indicators on malaria epidemiology in Venezuela, being an inverse relationship between both types of variables; with an increase or improvement in the socioeconomical indicators, the morbidity and, significantly, mortality decreased.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

FluZone: A national decision support system for the H1N1 flu pandemic in England

**C. Kara-Zaïtri**<sup>1</sup>, M. Schweiger<sup>2</sup>, R. Hamilton<sup>1</sup>

<sup>1</sup>inFact UK, Bradford, United Kingdom, <sup>2</sup>Health Protection Agency, Leeds, United Kingdom

**Background:** This work is the continuation of a seven-year development programme of a web based decision support system for health protection in England called HPZone. The recent H1N1 outbreak has provided a real test bed for a new national system facilitating a response in the most effective way by coordinating all the arrangements for the clinical assessment and management of the first few and subsequent cases of Swine Flu. FluZone has been developed using a bottom-up and top down approach in a rapidly changing environment.

**Methods:** FluZone is a web based system linking ten Flu Response Centres throughout England. It includes a triage page commensurate with a continuously changing case definition and provides a variety of prompts for appropriate action in different circumstances, such as arranging nasal and throat swabs, and chemoprophylaxis. The application has enabled 1500 multi-disciplinary users enter appropriate data about H1N1 Enquiries, Cases, Contacts and Outbreaks across the entire country.

**Results:** FluZone functions include:

1. Screen calls by non-clinical staff against an agreed protocol, triage (Clinical, Laboratory and Epidemiology) calls by clinical staff commensurate with the latest algorithm re testing and antiviral treatment, and then handover Probable and Confirmed Cases to dedicated Clinical Staff.
2. Facilitate the multidisciplinary management of Enquiries, Cases, Contacts and Situations based on a continuously updated protocol of conditioned actions and enabling direct data entry by the laboratories.
3. Provide a real-time tabular and geographical dashboard of all Possible, Probable, Confirmed and Discarded Cases locally, regionally and nationally.

The combination of an advanced query facility on the rich data set captured and time and spatial visualisation methods in FluZone, has provided a powerful tool for improved case management, quick contact tracing and advanced epidemiological analyses.

**Conclusion:** The system has performed well at peak times with over 350 concurrent users with minimal training. FluZone has demonstrably contributed to the containment phase of the pandemic supporting the delivery of the nationally agreed approach to management of cases and contacts and the capture of high quality local, regional and national epidemiological data in real time to inform future planning of the national response.

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**Session: Public Health Interventions, Modeling & Training**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Economic burden of health care at household level: Examination of out-of-pocket expenditure on sexual and reproductive health care

**T. Akalu**

Tulane University Technical Assistance Program Ethiopia, Addis Ababa, Ethiopia

**Background:** In Ethiopia although public health care is provided at normally low prices and for free to those that do not afford to pay, the health care consumers are still required to make considerable amount of out-of-pocket expenditures (OOPE) for various reasons. NHA 2004 has showed that 33.9% accounts for households as financing agents on reproductive health care. However, out-of-pocket expenditure remains poorly visible in resource tracking efforts.

**Methods:** Study area: Butajira Demographic Surveillance site

Study time: From October 2007 to March 2008

Study was done by collecting household level expenditures made for financing sexual and reproductive health care.

Study design: cross-sectional study

Sampling technique: simple random sampling using single population mean formula

Sample size: 370 Households

Data entry and analysis: by statistical package software, using central tendency measurement tools, regression analysis

**Results:** Total annual gross out-of-pocket expenditure showed ETB 85,900.90 (\$9357.40) with ETB 282.57 (\$ 30.78) per each household.

72.4% of the expenditure spent on pregnancy related services; where as 27.6% spent for FP and other sexual and reproductive health care services.

6.5% of the total OOPE as percentage of the annual household expenditure is spent for sexual and reproductive health care.

The net health service expenditure has a 64.08% gap from the gross OOPE.

The study assessed the difference on the direct and indirect costs incurred on different intervention components.

Out-of-pocket expenditure has a regressive distributional impact and poor and very poor people are expected to pay more.

These expenses were covered from regular income which can lead to catastrophic household economic crises.

**Conclusion:** Out-of-pocket expenditure has a regressive distributional impact and poor and very poor people are expected to pay more.

These expenses were covered from regular income which can lead to catastrophic household economic crises.

Recommendation: Other alternative health care financing mechanisms like community based health insurance, social insurance; and other community based organizations should be part of the process.

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**Date: Friday, March 12, 2010**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Testing the effectiveness of dengue vector control interventions

**L. Sanchez**<sup>1</sup>, J. Maringwa<sup>2</sup>, Z. Shkedy<sup>3</sup>, M. Castro<sup>4</sup>, N. Carbonell<sup>5</sup>, P. Van der Stuyft<sup>6</sup>

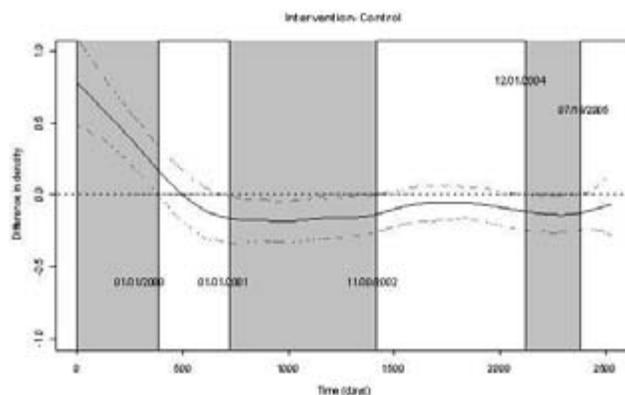
<sup>1</sup>Tropical Medicine Institute "Pedro Kouri", Havana City, Cuba, <sup>2</sup>European Organization for Research and Treatment of Cancer, Brussels, Belgium, <sup>3</sup>Center for Statistics, Hasselt University, Belgium, Hasselt, Belgium, <sup>4</sup>Tropical Medicine Institute, Havana City, Cuba, <sup>5</sup>University Center of Sancti Spiritus, Sancti Spiritus, Cuba, <sup>6</sup>Institute of Tropical Medicine, Antwerp, Belgium, Antwerp, Belgium

**Background:** Community-based strategies to dengue vector control have been implemented in different countries worldwide, but there is a need for more evidence on their effectiveness. We present an application of a semiparametric mixed model to evaluate the result obtained through intersectoral coordination and community empowerment in one study carried out during six years in Playa Municipality, Cuba.

**Methods:** A longitudinal assessment comparing one intervention and one control area was conducted. The intervention encompasses two main stages separated by two dengue outbreaks. The first stage, focused on strengthening intersectoral coordination, was initiated in January 2000. In August 2003 a complementary strategy, focused on community empowerment was initiated in half of the intervention area. In the control area, routine dengue control activities continued without additional input. We used entomologic surveillance data from January 1999 to December 2005 to assess the effectiveness. We computed the Breteau indices (BI, number of positive container per 100 premises) per area and the 95% confidence interval for the difference between the BIs at each time point. A semiparametric mixed model to capture the evolution in time of *Ae. Aegypti* larval densities was fitted.

**Results:** The Figure shows the fitted models for the differences in *Ae. aegypti* larvae densities between areas. The BIs in the control area had the lowest values before starting the intervention. This was reversed one year after the launch of the intersectoral activities for dengue control in the intervention area. In spite of spraying actions carried out during the intensive campaign that started in January 2002 in all areas, the differences in BIs between intervention and control areas remain significant until December 2002. Although for the next two years no differences were observed, they become significant again in December 2004, which corresponds with the implementation of the complementary community-based vector control strategy in the intervention area.

**Conclusion:** The model fitted allowed us to identified monotonous trends over time and reversal trends at particular moments. The confidence bands indicate sections with significant differences between areas. Our data augment the evidence that intersectoral coordination and community empowerment strategy to *Ae. Aegypti* control is effective.



**Figure. Fitted models and confidence bands for the differences in *Aedes aegypti* larvae densities between areas. Playa municipality, Havana, 1999-2005.**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Age at sexual initiation and its determinants among youths in NE Ethiopia

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**Background:** For behavioral as well as physiological reasons, early sexual debut increases young peoples' risk for infection with HIV and other STIs. It is crucial to understand the determinants of early sexual initiation in a broader context for designing and implementing effective interventions targeting youth.

**Objective:** The objective of the study was to determine the median age at first sexual intercourse and the determinants of sexual initiation among rural and urban youths (age 15- 24 years).

**Methods:** A comparative cross sectional study was conducted between, March 1 -15, 2008, in Dessie town and Dessie Zuria Woreda. To draw a total sample of size 1294 (647 urban and 647 rural), a multistage cluster sampling was used. Bivariate and multivariate analyses were employed. Moreover, Kaplan Meier survival analysis was used.

**Results:** About half, 51.3% of the youths have ever had sex. Rural youths initiate sexual intercourse at lower age than their urban counterparts with mean ( $\pm$ SD) (16.49 $\pm$ 2.11) for rural and (17.18 $\pm$ 2.32) for urban youths. The hazard ratio for sexual imitation was significant (AHR [95% CI] =1.45 [1.19, 2.55]. Multivariate analysis showed that factors like female by gender, chewing Khat, drinking alcohol, viewing pornographic materials and less connectedness with parents were independent predictors of early sexual initiation.

**Conclusion:** Early sexual initiation prevails more in rural than urban youths. Delaying sexual debut can be achieved through well designed sexual education programs at earlier life. Strengthening the norm of virginity should be advocated. Equally, ways to access condoms and other contraceptives especially to rural youths should be sought for those who already initiate sexual intercourse.

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**Type: Poster Presentation**

World Rabies Day Campaign: Evaluating a global initiative

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**Background:** World Rabies Day (WRD) is the only worldwide event of its kind. To evaluate the 2007 and 2008 initiatives, the authors utilized the CDC evaluation framework.

**Methods:** Process measures from the campaign implementation were captured, and outcome measures were tracked using data from three sources: web traffic, feedback survey, and media monitoring. Evaluation of the inaugural WRD resulted in recommendations for the second year evaluation. A modified survey was constructed and translated into four languages. During both years, the survey was distributed via the WRD listserv, advertised by partners, and remained open for several months.

**Results:** After redesigning the evaluation strategy and survey design, the WRD team received more completed surveys in year two (259 vs. 98). Respondents indicated that the WRD website is a useful resource, and larger events were significantly more likely to utilize these resources ( $p < 0.001$ ). In 2008, WRD reached 15% more countries (from 74 to 85) and again educated millions. Seven types of events were analyzed: commemorations, conferences/symposiums, educational outreach, fun runs, concerts, parades, animal vaccination clinics. Vaccination clinics were successful both years, increasing the number of vaccines administered from approximately 604,000 to 1,053,388. The WRD website has currently received over 180,000 visitors from more than 200 countries/territories; the majority (81%) of which are new visitors and remain on the site for an average of 2 minutes.

**Conclusion:** The success of WRD is promising, and web traffic demonstrates interest from a broader scope of potential participants. The evaluation metrics serve as a continuous feedback loop into the yearly campaign design and global outreach plan.

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**Type: Poster Presentation**

From strategy to action: The vital roles of trained field epidemiologists and laboratory management professionals in epidemic control and prevention in Tanzania

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**Background:** As Africa and other resource limited continents of the world battle with the double burden of diseases, integrated disease surveillance and response (IDSR) strategy was adopted by African World Health Organization region (AFRO) to curtail the occurrence of, and morbidity and mortality related to, outbreaks of epidemic prone diseases. The strategy also supports proper management of diseases of public health importance and those targeted for eradication/elimination. Since its adoption in 1998, there have been challenges in implementation due to lack of qualified personnel until the paradigm shift to training of field epidemiologist.

**Methods:** The US Centers for Disease Control and Prevention (CDC) working in a smart collaboration with several African Ministries of Health, WHO, AFENET, Universities and USAID initiated and inaugurated the Field Epidemiology and Laboratory Training Program (FELTP) to train health workers to predict, identify, timely respond to and control outbreaks to minimize morbidity and mortality. Trainees are either trained in long courses or short courses. The program aims to empower trainees to mitigate common infectious causes of morbidity and mortality. Recent Cholera outbreaks in Tanga and Kigoma, Measles in Dar Es Salaam, H1N1 in Zanzibar, Dar es Salaam and Mbulu and Chemical poisoning in Mara had seen these trained health-workers promptly responding and thus shift the epidemic curve to the left

**Results:** There are now functional rapid response teams at the national levels for all African countries with established FELTP programs, prompt investigation and response of outbreaks, minimal morbidity and mortality rates, and effective community involvement in the management of these outbreaks. In addition, common drivers of epidemics are identified and monitored through intensified surveillance systems. The availability of standby teams ready to investigate and mitigate any outbreak irrespective of location and nature is an added advantage.

**Conclusion:** Although the IDSR strategy is a veritable tool in outbreak investigation and response, its implementation needs train health workers in field epidemiology and laboratory management. Such programs should have mechanisms that train, empower and retain the graduates within the national health care system for a period of time. This is the task that FELTPs through AFENET is spearheading within the African continent.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Continuous medical education: Teaching infectology using the internet

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**Background:** The use of new technologies in information and communication offer alternatives through e-learning, which integrates those with pedagogical and didactic resources useful for graduate and post-graduate students.

Evaluate the experience of the "Actualization of infections in adults courses" delivered through the Internet during 2007 and 2008, using INTRAMED, a web medical portal in Argentina. Analyze its advantages and difficulties, the number of participants and the results obtained with this methodology, describe the demographic characteristics of the professionals and propose strategies for the training actualization and update using the distance learning.

**Methods:** We evaluated two courses delivered through IntraMed with free registration. We assessed age, sex, level of graduate training and participation in one or both courses. The teachers developed reading material and also selected bibliography and complimentary material. Each module had a final evaluation and clinical cases resolution. The students also had a final evaluation. A forum was provided and an organized survey and a qualitative evaluation of the 2008 course was carried out.

**Results:** 4791 professionals carried out both courses, 3173 (66.2%) were physicians, 1149 (23.9%) medical students and 469 (9.7%) other healthcare professionals. The pass rate was of 80% per year and the desertion rate was among 52.8% and 56.9%, respectively for the years 2007 and 2008. The 34.3% of the students that completed the 2008 course answered the survey. Between 44 and 55% considered appropriate or very appropriate the contents and among the 36 and 52% valued the tutor and the technical support as adequate. 86% of the students did not consider necessary tutoring and nearly 90% expressed they have changed the management and treatment of diseases after the course.

**Conclusion:** In both years we have provide updated and free information to 4791 professionals. This learning methodology does not intend to replace the in-person training, as it is impossible to evaluate skills and competence. However, we believe that this is an additional resource, useful to acquire or reinforce the existing ones, especially in those cases in which for different reasons professionals cannot access in-person training courses.

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**Type: Poster Presentation**

Measles epidemic with complications in Bosnian children during 2008- Prevention strategy

**A. Bajraktarevic**

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**Background:** Morbilli is a childhood viral disease manifested as acute febrile illness associated with cough, coryza, conjunctivitis, spots on the buccal mucosa, and rash starting on the head and neck and spreading to the rest of the body. Treatment for mild cases of measles is supportive.

**Aim:** The objective of this review was to assess the effects of antibiotics given to children with measles in reducing pneumonia, other morbidities and mortality during epidemiological infections unvaccinated children during last epidemic 2008 in Bosnia and Herzegovina.

**Methods:** Randomized controlled trials (RCTs) and quasi-RCTs comparing antibiotics with placebo or no treatment to prevent complications in children with measles. Diagnosis was usually clinical, by identifying Koplik's spots or the rash. Alternatively, laboratory diagnosis of measles had done with confirmation of positive measles IgM antibodies or isolation of measles virus RNA from respiratory specimens.

**Results:** Bronchopneumonia occurred in up to 10% cases producing serious respiratory difficulties in Bosnian epidemic 2008. Severe cases of measles required hospitalisation in 16% cases of kids measles infection. Low rate of complications had a group of kids patients that received antibiotics prophylaxis (6%) compared with group of sick kids without antibiotics (21%). Gypsies children had significantly lower immunization coverage (37%), compared with other Bosnian national groups (89%) that time period.

**Discussion:** Antibiotics may be given to treat secondary bacterial infections from complications such as otitis media, infectious diarrhoea, pneumonia and sepsis. Despite this, measles remains a leading cause of vaccine preventable death worldwide.

**Conclusion:** There is no specific treatment for measles which is why immunisation is so important. These results show that a significant number of kids patients with measles develop complications and require admissions. The majority of patients are nine months to five years old. Combined measles, mumps and rubella (MMR) vaccine is currently part of routine immunisation programmes in most countries, including Bosnia and Herzegovina.

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**Type: Poster Presentation**

GLaDMap: Global Laboratory Directory Map

S. Mukhi<sup>1</sup>, L.-K. Ng<sup>1</sup>, M. Chu<sup>2</sup>, B. Swaminathan<sup>3</sup>

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**Background:** The International Health Regulations (IHR) 2005 calls for strengthening core capacities of the 194 World Health Organization (WHO) member countries. No single country has the resources to control spread of infectious diseases, and cross border collaborations and information sharing are important for early mitigation of a public health event of international concern (PHEIC). Given the disparity among countries, especially in the laboratory capabilities in many less developed countries (LDC) for the detection, risk assessment of, and response to public health events, one way to improve capacity is to tap into the vibrant communities of laboratory networks. To connect the expertise and to map global laboratory resources, WHO has launched the Global Laboratory Directory (GLaD). GLaD is conceived as a *support system* to encourage laboratory networks to be part of a global community of peers. It is to connect laboratory networks to leverage capabilities and capacities in support of effective preparedness in compliance with the IHR. GLaD comprises of three components: GLaDMap, GLaDNet and GLaDResource

**Methods:** This abstract focuses on the GLaDMap component which is based on a combination of the "yellow pages" directory concept with the links of a social "facebook" community. It is a list of networks and their member laboratories using a web based interactive mapping technology. Laboratory networks upload information through a detailed questionnaire and their specifics are uploaded and displayed dynamically, highlighting their specialties, geographic location, partnerships and activities.

**Results:** A dynamic display of laboratories and their connections will be demonstrated: A series of mapping exercise scenarios will highlight how peer communities are utilizing the mapping tool, focusing on networks for cholera, food safety, influenza and biosafety.

**Conclusion:** GLaD is positioned to strengthen the WHO's capability to perform fully and effectively the functions entrusted to it under the IHR, in particular to link more isolated laboratories in LDCs with others for better global connectivity and readiness to mitigate PHEICs.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Comprehensive community hygiene promotion in peri-urban Cape Town: gastrointestinal and respiratory disease reduction in families

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**Background:** The previous report of our community-based participatory research demonstrated the effectiveness of a comprehensive household hygiene program in the reduction of a spectrum of gastrointestinal and respiratory illnesses in children <5 years. As the burden of infectious diseases in poverty communities affects all household members, evidence-based programs that demonstrate positive impacts of participatory hygiene promotion on the family unit have a greater likelihood for sustaining long-term reductions in morbidity and mortality.

**Methods:** Effects of intensive hygiene education alone and in combination with the use of hygiene products (soap, surface cleaner/disinfectant, and antiseptic) were assessed. Four communities and 922 households participated: two of government (RDP) housing (indoor tap/flush toilet) and two of informal (INF) housing (communal tap/latrines). Trained community facilitators monitored illness symptoms weekly and reinforced disease-prevention behaviors established through participatory learning and action focusing on handwashing/bathing with soap, and cleaning toilet and food surfaces. RDP and INF communities were co-located in two geographic areas, with one area receiving education and products (intervention), and the other receiving education only (control). Illness data were gathered from Jun-Nov 2006 (baseline), and for the same 2007 period following education and product introduction (intervention).

**Results:** Household family members in all communities experienced significant reductions in gastrointestinal and respiratory illnesses over time. Communities that received hygiene products in addition to reinforced hygiene education had lower illness incidence rates. Higher reduction differences in respiratory illnesses (INF 63.7%, RDP 58.2%) as well as gastrointestinal illness reduction (INF 84.0% , RDP 75.9% ) were observed for hygiene education plus product use (intervention) versus hygiene education alone (control) Hazard analysis was also completed to assess disease risk potential. RDP controls were more likely to experience gastrointestinal (HR = 1.22, CI: 1.03–1.45) and respiratory (HR = 1.21, CI: 1.02–1.43) illnesses at follow-up than intervention counterparts. INF controls were more likely to experience gastrointestinal (HR = 1.26, CI: 1.05–1.51) and respiratory (HR = 1.28, CI: 1.06–1.55) illnesses at follow-up than intervention counterparts.

**Conclusion:** Hygiene promotion encompassing both comprehensive participatory hygiene education and the use of personal and home hygiene products (soap, surface cleaner/disinfectant, and antiseptic) was shown to be an effective approach to reducing the burden of gastrointestinal and respiratory illnesses among all household family members in at-risk communities.

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

An alternative standard for radiological pneumonia in children

**G. Mackenzie**, U. Uchendu, D. Ameh, O. Adeyemi, D. Saha  
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**Background:** Although the WHO standard for paediatric radiological pneumonia has been useful in vaccine trials, it lacks sensitivity for some significant pneumonic lesions and inter-observer agreement may be limited. We assessed inter-observer variation in interpretation of WHO standardised radiographs and evaluated an alternative standard.

**Methods:** Four clinicians were trained using the first 122/222 WHO classified radiographs. The remaining radiographs were used to assess agreement between clinicians and WHO. The alternative standard used WHO definitions for quality, other infiltrate and pleural effusion, while the alveolar infiltrate category (end-point consolidation, EPC) was separated into two categories: EPC-A (alveolar infiltrate spanning  $\geq 2$  intercostal spaces), and EPC-B (alveolar infiltrate spanning  $\geq 1$  &  $< 2$  intercostal spaces). The alternative standard was applied to the first 100 WHO films. Clinicians then undertook a consensus review of the alternative standard using the first 100 films. The alternative standard was then applied to the final 100 WHO films. Prevalence and kappa values were calculated.

**Results:** Kappa for agreement between readers and WHO was 0.55-0.88 (EPC) and 0.16-0.52 (other infiltrate). Inter-observer kappa for EPC was 0.56-0.86 and 0.03-0.53 for other infiltrates. Prevalence of EPC in the first 100 WHO classified films was 23% (right) and 6% (left). Prevalence of EPC-A was 21-32% (right) and 3-8% (left) and prevalence of EPC-B was 2-18% (right) and 1-5% (left). Prevalence of combined EPC-A and B was 24-50% (right) and 4-12% (left). Alternative standard inter-observer kappa was 0.33-0.73 (EPC-A), -0.02-0.38 (EPC-B), and 0.27-0.77 for combined EPC-A and B.

Prevalence of EPC among the final 100 WHO classified films was 37% (right) and 10% (left). Prevalence of EPC-A was 27-43% (right) and 6-7% (left) and prevalence of EPC-B was 5-9% (right) and 0-4% (left). Prevalence of combined EPC-A and B was 36-50% (right) and 6-21% (left). Alternative standard inter-observer kappa was 0.47-0.85 (EPC-A), -0.02-0.66 (EPC-B), and 0.43-0.92 for combined EPC-A and B.

**Conclusion:** Compared to WHO, the alternative standard showed greater sensitivity for alveolar infiltrates and similar levels of agreement. Improved agreement with training suggests the alternative standard may be used in a training package. This alternative standard should be evaluated for further use.

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**Type: Poster Presentation**

Field evaluation of ZeroVector™ Durable Lining as an alternative to indoor residual spraying (IRS) for the control *Anopheles* vectors of malaria in rural villages of Obuasi, Ghana

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**Background:** ZeroVector™ Durable Lining (DL) is a novel vector control tool that releases a suitable insecticide impregnated in a textile which is used to cover surfaces where malaria vectors rest after taking a blood meal. The product has the ability to remain effective for three to four years. The technology utilizes the best features of both long-lasting insecticide-treated bed nets (LLINs) and indoor residual spraying (IRS) by being long-lasting and requiring no behavioural change after installation. This study was conducted in 2008-09 to evaluate the acceptability, durability and residual activity of ZeroVector™ DL in two selected rural villages of Obuasi.

**Methods:** Anwona and Mmemiriwa #1, located at the periphery of the Obuasi Municipality, were selected for the field trial. Data on the installation process of ZeroVector™ DL was captured. At three weeks and nine months post-installation, acceptance and durability surveys were conducted at the household level to obtain data on user impressions of the product appeal and appearance over time and also to compare resource demand in contrast to IRS. The residual activity of deltamethrin-impregnated DL against field and susceptible *An. gambiae* s.s was assessed monthly and compared with deltamethrin spray (K-Othrine) on both cement and mud surfaces using WHO cone bioassay kits. Twelve houses, six representing each surface, were selected for each intervention at each village.

**Results:** ZeroVector™ DL was preferred over IRS by 95.1% of household heads (58/61), primarily for aesthetic reasons. They claimed the textile added beauty to their rooms. At 12 months post-installation, the Durable Lining was generally in good physical condition. Mortality of *An. gambiae* s.s was 100% for ZeroVector™ DL installed on both cement and mud surfaces at the end of the 1 year study. However, the residual life for the IRS was app.6.5 months on cement surfaces and 4 months on mud surfaces.

**Conclusion:** The high user acceptability and residual efficacy of ZeroVector™ Durable Lining makes it an ideal substitute for IRS in the control of *Anopheles* vectors of malaria, especially in rural communities where houses are constructed with mud or with mud rendering.

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Mass screening and treatment for malaria among gold miners in Suriname

**L. Villegas**, H. Cairo, A. Huur, H. Vinisi, H. Pereira, N. Jozuazon, F. Refos, S. Summerville, L. Hardjopawiro, M. Jordaan, S. Mitro, H. Hiwat, C. Alensi, P. Malaria Technical Committee  
Ministry of Health, Paramaribo, Suriname

**Background:** The activities and habits of small to medium scale gold miners -and their interaction with both the environment and the local population- are recognized as one of the main determinants of the current occurrence and dispersion of malaria in the Amazon Region. Gold miners are the group with highest risk for malaria in the region. Their exploration techniques are based on damming creeks and rivers, to provide water that is used in high pressure hoses, to dig for gold. This practice, multiplies the potential breeding sites of the main vectors in the region. We assessed the malaria prevalence in gold mining areas in Suriname.

**Methods:** Mass screening and treatment for malaria was conducted in 4 main gold mining areas in Suriname. A standardized national malaria form was used to collect the information. Malaria blood films were stained with 4% Giemsa and read using microscopy (1000X). 100 microscopic thick film fields were inspected before a slide was being declared malaria negative. For positive-malaria smears, parasite species were identified and treatment was given following the national malaria protocol.

**Results:** Between June and October, 2009, there were 2543 people screened for malaria in 4 main gold mining areas in Suriname. These 4 areas had 261 gold mining camps (Victoria:26; Benzdorp:88; Sellacreek:79; Maku:68). Sixty-six percent of the people seen were Brazilian. Malaria infections were reported among 85 people with an overall prevalence of 3%. There were 42, 40 and 3 cases of *Plasmodium falciparum*, *P. vivax* and *P. malariae*, respectively. Parasite specific prevalence ranged from 1-4%(Pf), 1-3%(Pv) and <1%(Pm). All malaria cases were uncomplicated infections and treated. No cases were detected among pregnant women.

**Conclusion:** Despite a steep decline of malaria morbidity in Suriname (~95% reduction), "hot spots" still exist. From the 4 gold mining areas evaluated, Sellacreek was the only malaria-free region. Gold mining activities implies population movements and transmission of malaria which make diagnosis and treatment, a critical gap for the sustainability of the impact obtained in other areas. A strategy to increase access to adequate diagnosis and treatment for malaria -training locals and serving their populations- in gold mining areas is ongoing.

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Almost 10.000 second opinion consultations in infectious diseases. A telemedicine program in Argentina

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**Background:** To describe a on line-second opinion consultation system (SOC) in a telemedicine program (TP) for public hospitals in Argentina (PHA).

**Methods:** A TP for physicians and nurses (HCW) of PHA was started in April 2002. At each PHA, the TP designates a local medical coordinator, an administrative assistant and provides a computer and Internet connection. A specially designed secure website gives HCW free access to medical education (ME) and SOC. SOC have been designed as a complement to ME but their main purpose is to provide rapid access to experts' opinion on complex medical situations. SOC presented by HCW are received by the local TP coordinator, who replies or transfers SOC to the TP headquarters, to be evaluated by the TP Area Directors, who can reply, ask for additional information, or direct the SOC to an external consultant.

**Results:** Up to 10/31/09, 13.989 HCW (6.150 physicians, 5.846 nurses and 1.993 others) from 50 PHA have received ME and access to SOC in our TP.

A total of 9.028 SOC have been processed in the period: 3.204 (35.4 %) on pediatricians (P), 1.651 (18 %) on perinatologist (Pe) and 3.192 (35.3 %) on adults (A). Average reply time is 37 hs. Most frequent topics in SOC about P deal with vaccines, use of antibiotics, HIV, infection control, pneumonia; skin and soft tissue infections, immunocompromised host, TB, chickenpox; in A: HIV, bone and joint infections, non-HIV immunocompromised host infections, severe infectious diseases from the community; skin and soft tissue infections, epidemiologic questions, CNS infections, antibiotics and in Pe: sepsis, epidemiology, nosocomial infections, fever in the newborn, antibiotics, syphilis, toxoplasmosis, puerperal infections, exposure to infections, antibiotics, early membrane rupture and preterm pregnancy.

**Conclusion:** Our TP has organized a network of HCW at PHA actively engaged in scientific knowledge exchange, and SOC prove to be an effective tool as a complement to ME and to help HCW solve complex medical situations locally.

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7 Years' experience in a telemedicine program in infectious diseases in Argentina

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**Background:** To describe a telemedicine program (TP) combining medical education (ME) and second opinion consultations (SOC) for public hospitals in Argentina (PHA)

**Methods:** At distance ME is a useful teaching methodology for healthcare workers (HCW); SOC as a complement to ME would also contribute to HCW daily practice. We describe a TP for PHA, which provides ME in Infectious Diseases (ID) combining e-learning and on site tutorial meetings with a SOC system exclusively over the Internet. The TP is provided free of charge, since it is financed through unrestricted grants from philanthropic organizations. The TP was started in April 2002. The TP designates a local medical coordinator and an administrative assistant at each PHA and provides a computer and Internet connection. The initial phase lasts 2 yrs when ME and SOC are administered directly from TP headquarters (PHQ). After this period, TP is opened to replication by local coordinators, with permanent support and update of ME contents from PHQ.

**Results:** Up to 10/31/09, 13,989 HCW (6,150 physicians; 5,846 nurses; 1,993 others) from 50 PHA have joined the TP. A total of 9,028 SOC have been processed in the period, with an average reply time of 36 hs. In the period 2007-09, 3,047 HCW (1,635 physicians and 1,412 nurses) joined the TP in the 25 PHA entering the second stage. As a result of permanent surveying and feedback, the TP strategy has been redesigned to include other resources:

Epidemiological Research Division; Microbiology Branch; Immunizations Branch; intervention actions in HIC including WHO's 5 moments initiative. The TP has given quick response to emerging situations (A-H1N1 influenza and Dengue) and has incorporated external experts to comment on key SOC.

**Conclusion:** The TP has successfully organized a network of HCW at PHA actively engaged in scientific knowledge exchange; SOC prove to be an effective tool as a complement to ME. The TP can be successfully replicated locally. Its main impact is its potential to unify diagnostic, therapeutic and prevention approaches in ID and its capacity to provide rapid response to emerging situations. Continued surveillance and feedback has opened the ground for new TP strategies besides ME and SOC.

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Sustained malaria control in Suriname after 3 years of effective interventions

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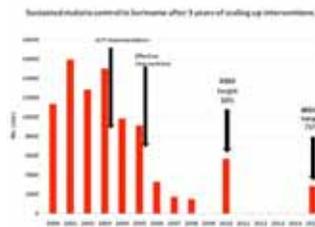
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**Background:** Suriname along with Guyana and French Guyana have contributed with a high concentration of malaria cases in the region. In Suriname, 10% of the population lives in malaria endemic areas. Falciparum malaria is the predominant parasite followed by *Plasmodium vivax* and *Plasmodium malariae*, representing an important public health problem. Effective control measures have impacted malaria morbidity and mortality and changed the epidemiological profile in the country in the last 5 years. We assessed the achievements in malaria interventions in Suriname after 3 years of scaling up control measures.

**Methods:** National malaria data per year was obtained from the national malaria surveillance system. Artemisinin-derivatives combination therapy (ACT) was implemented in 2004 and in 2006, a combination of interventions were implemented in different strata. Insecticide-treated long lasting nets (LLNs), aggressive active case detection (ACD), media campaign (IEC/BCC), Indoor residual spraying (IRS), Mass screening and treatment, Primaquine, passive surveillance and impregnation/re-impregnation were carried out in the interior of the country. Malaria diagnosis was performed with light microscopy and rapid diagnostic tests.

**Results:** After the national implementation of ACT in the country (2004-2005), a 33% reduction of malaria cases was recorded. By 2006, evidence-based interventions were implemented: LLNs (coverage 95%), IRS (60% coverage high risk areas), IEC/BCC (>15 people reached), ACD (20.000+ people screened). Malaria morbidity dropped from 15006 cases in 2003 to 1498 cases in 2008. Malaria-related hospitalizations declined dramatically (>70%) between 2006-2008 and no deaths due to malaria have been reported since 2006. A reduction of 92% of the number of *P.falciparum* infections was recorded between 2003-2008.

**Conclusion:** Malaria morbidity and mortality have decline significantly in Suriname and this reduction, was higher when interventions were combined (2006-2008). Coverage of most malaria control interventions were above 95%. The burden of malaria in Suriname has significantly changed, reaching the Roll Back Malaria objectives and Millennium Development Goals targets in a very short time. The national malaria epidemiological profile was changed focusing now on "hot spots" among gold miners in remote areas. This is the first country in the Amazon basin that have decided to go for elimination of malaria at the national level.



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**Session: Trypanosomiasis, Leishmaniasis & Schistosomiasis**

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**Type: Poster Presentation**

Inhibition of entry and survival of *Leishmania donovani* by down regulation of TACO gene on human macrophages using Vitamin D3 & Retinoic Acid

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**Background:** A gene coding for tryptophan-aspartate containing coat protein (TACO) has been recognized to play a crucial role in the survival of *Mycobacterium tuberculosis* within human macrophages. Host molecule TACO has been shown to play a crucial role in the arrest of such a maturation process. At the onset of infection promastigotes of *Leishmania* are internalized by macrophages into phagosomes. Phagosomal maturation arrest is known to play a central role in the survival of pathogenic *Leishmania* within macrophages. We are postulating that the stable association of TACO with phagosomes would prevent the vacuole from maturation. It would be of interest to know whether this protein is associated with *Leishmania*-containing phagosomes.

**Methods:** In this study, We evaluated the dose dependent effect of treatment with vitamin D3/RA what impact TACO gene down-regulation has on the uptake/survival of *Leishmania donovani* within macrophages. The reverse-transcriptase polymerase chain reaction employed in this study, revealed that the major component of D3/RA had the inherent capacity to down-regulate TACO gene transcription within human macrophages and accompanied by inhibition of promastigote survival within macrophages as assessed through flow cytometry and microscopy.

**Results:** Coat protein TACO gene down-regulation observed with vitamin D3/RA dose dependent treatment occurred through modulation of this gene via the VDR/RXR response sequence present in the promoter region of TACO gene. Treatment of macrophages with vitamin D3/RA allows maturation of promastigote containing phagosome, leading to degradation of the parasite inside the phagosome.

**Conclusion:** Our results shows that vitamin D3/RA treatment inhibits promastigote entry as well as survival within macrophages, possibly through rescue of phagosome maturation arrest. The developing knowledge in this study suggests that vitamin D3/RA may be of importance in the treatment of intracellular infection, particularly Leishmaniasis without any side effects. The major practical application anticipated would be in the treatment of visceral leishmaniasis especially resistant cases wherein it may help in elimination of the parasite. The major attraction here is the use of molecules which are already in routine use and in case these are effective, they could be put to immediate use.

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**Session: Trypanosomiasis, Leishmaniasis & Schistosomiasis**

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A diagnostic approach to detect murine *Shistosoma mansoni* infection using a polymerase chain reaction

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**Background:** Traditional diagnosis of *Schistosoma mansoni* infection involves direct microscopic detection of eggs in feces. The use of some stool concentration techniques may increase the diagnostic yield. However, it seems that the sensitivity of parasitological methods diminishes when prevalence and intensity of infection are low, making these methods less appropriate for low-endemic areas and in post treatment situations.

In 2001, a new antischistosomal drug, Mirazid® was introduced into the Egyptian market by Pharco Pharmaceutical Company (Alexandria, Egypt). Extensive advertising efforts have encouraged physicians in private clinics to use Mirazid. The chemistry of myrrh is not fully studied. Reports on its anti-schistosomal effect in human or experimental animals seem to be controversial.

**Methods:** Our present study is a laboratory trial aimed at using conventional PCR technique for studying the schistosomicidal effect of Mirazid® in the murine model, and comparing the results with the parasitological results (ova and worm count).

**Results:** Microscopic results showed that there was no eggs passing till the 41st day of infection by examining fecal samples of all infected mice by Kato-Katz technique. Only on the 42nd day, the first batch of eggs appeared in stool samples. Comparable results were found in the number of *S. mansoni* worms recovered from sacrificed mice of infected groups. By using PCR for detection of *S. mansoni* specific DNA sequences in murine fecal samples of infected control group, all fecal samples showed positive results by using feces from the 45th day after infection. On the other hand, all fecal samples of uninfected control group showed negative results.

Regarding results of the Mirazid® treated group, six fecal samples (60%) showed positive results for feces from the 77th day after infection. Interestingly, the four mice diagnosed negative by PCR were among the eight mice that diagnosed negative by the microscopic technique.

**Conclusion:** The used PCR technique was more sensitive than the Kato-Katz thick smears. Mirazid® showed some schistosomicidal effects against murine *S. mansoni*. However, it was not efficient enough to cure any of the mice. Thus, re-evaluation of myrrh as a schistosomicidal drug must be considered because of its recommendation by some Egyptian physicians motivated by its natural origin.

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**Session: Trypanosomiasis, Leishmaniasis & Schistosomiasis**

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**Type: Poster Presentation**

*Leishmania aethiopica*: The unusual etiologic agent of cutaneous leishmaniasis in Ho District of the Volta region of Ghana

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**Background:** Leishmaniasis is a parasitic disease of significant public health importance. An outbreak of suspected cutaneous leishmaniasis (CL) was first seen in the Volta Region of Eastern Ghana in 1999, and has remained an endemic area ever since. To improve the level of understanding regarding leishmaniasis in West Africa, particularly in Ghana, there is a need to provide information for the management of the disease. The study focused on the identification of species of *Leishmania* parasites responsible for leishmaniasis infections reported in the Volta Region of Ghana.

**Methods:** The Ho district, located in the middle zone of the Volta region in the south-eastern part of Ghana, was the study site. It borders on the east with Togo in the West African sub-region. Forty four samples were taken for the study. Skin scrapings were collected from the sites of active lesion(s). Primers P5 and P6, were used to amplify a fragment of ~1500 bp of the intergenic region between the ribosomal protein genes RPS7A and RPS7B on chromosome 1 and second primers P1 and P2, were used to amplify an internal fragment of ~1350bp in the nested PCR. Products obtained from the nested PCR were digested using *MspI* enzyme.

**Results:** The bands produced from some samples showed a match to the control sample *L. aethiopica*.

**Conclusion:** PCR was shown to be a useful diagnostic tool for Ghanaian CL.

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**Session: Trypanosomiasis, Leishmaniasis & Schistosomiasis**

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The role of colonoscopy and squash preparations in diagnosis and follow-up of stool negative enteric schistosomiasis: A case report of double infection with *S. mansoni* and *S. intercalatum*  
**M. Astriti**<sup>1</sup>, K. Tzanetou<sup>2</sup>, K. Sarri<sup>2</sup>, K. Ntinou<sup>2</sup>, G. Adamis<sup>2</sup>, V. Delis<sup>2</sup>, G. moustakas<sup>2</sup>, M. Choreftaki<sup>2</sup>, P. Gargalianos<sup>2</sup>

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**Background:** To present a case of simultaneous infection with two types of schistosoma. To underline the role of colonoscopy in diagnosis.

**Methods:** : A 30 year old Mauritian male was admitted to the Nephrology department as he had been recently diagnosed with hypertension due to chronic renal failure. Basic laboratory tests revealed leucocytosis (13,100 WBC/ $\mu$ L), eosinophilia (5,100/ $\mu$ L), elevated IgE 1050 UI/ml. The patient mentioned having constipation and periodic diffuse abdominal pain. The upper abdominal ultrasound showed increased echogenicity of both kidneys. C/T scan demonstrated bilateral nodular lesions <1 cm. Bone marrow aspiration was non diagnostic. The patient finally underwent urinary bladder cystoscopy that was normal and 24 hour urine collection showing no ova. Renal biopsy was not performed due to increased risk of complications. In spite of these results, it was decided that a total colonoscopy with squash preparations and biopsies was needed.

**Results:** Colonoscopy showed terminal ileum having normal appearance, transverse colon having 2-3 mucosal folds with whitish nodules interlapping with normal mucosa and finally rectosigmoid being erythematous, with small ulcers which were friable and again whitish nodules surrounded by normal mucosa. Biopsy was performed the result of which showed schistosomal ova in different phases of necrosis (rectosigmoid), chronic eosinophilia and inflammation (right and transverse colon). Squash preparations taken during colonoscopy were examined under the microscope: the samples from rectum and sigmoid showed multiple granulomas around *S. intercalatum* ova and the samples from transverse colon showed a few granulomas around *S. intercalatum* ova. The patient received 2 courses of treatment with praziquantel. Because his symptoms as well eosinophilia were still present after the treatment, it was decided to give a 3rd course. Follow-up colonoscopy 10 days later showed normal colonic mucosa. Eosinophilia and symptoms regressed.

**Conclusion:** Diagnosing enteric Schistosomiasis is difficult because stool samples are often negative. At times where parasitic infection is strongly suspected, total colonoscopy and squash preparations may be needed to establish the diagnosis.

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**Session: Trypanosomiasis, Leishmaniasis & Schistosomiasis**

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Trypanocidal activity of novel alkanediamide-linked bisbenzamidines and bisbenzamidoximes

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**Background:** Human African trypanosomiasis (HAT) is caused by the protozoan parasites *Trypanosoma brucei gambiense* (*T.b.g*) and *Trypanosoma brucei rhodesiense* (*T.b.r*) and is usually fatal when left untreated. It is one of the most neglected tropical diseases in the world causing an estimated 50,000 deaths annually. Current drug therapy suffers from high toxicity, undesirable intravenous route of administration and emergence of parasite resistance. The present study is to evaluate the trypanocidal activity of a novel series of alkanediamide-linked bisbenzamidines and bisbenzamidoximes against several clinical isolates of *Trypanosoma brucei*.

**Methods:** A series of 20 bisbenzamidines and bisbenzamidoximes were synthesized and tested in vitro against a drug-sensitive strain of *T. b. brucei* Lab 110 EATRO and a drug-resistant strain of *T. b. r.* KETRI 243. The bisamidoximes were designed to improve oral bioavailability by functioning as orally-active prodrugs of the most active bisamidines. The in vivo efficacy of 8 bisbenzamidines and bisbenzamidoximes were evaluated using mice infected with the drug-sensitive (*T. b. brucei* Lab 110 EATRO) or drug-resistant strains of *T. b. r.* KETRI 2002 and KETRI 2538.

**Results:** The tested compounds generally showed similar in vitro potencies against both strains of *T.b.* The most potent compounds were bisbenzamidines linked with a hexanediamide, heptanediamide or octanediamide group (inhibitory concentration for 50% (IC<sub>50</sub>) = 1-3 nM). Several of the most potent bisbenzamidine compounds were effective in curing mice infected with the drug-sensitive or drug-resistant strains of *T. b. rhodesiense*. Curative doses were < 15 mg/kg/day for 3 days given by the intraperitoneal injection in the mouse model of infections. However, replacing the terminal basic bisamidines with less basic bisamidoxime groups resulted in prodrugs that were not orally effective against *T. b. brucei* infected mice.

**Conclusion:** The results suggest that alkanediamide-linked bisbenzamidines are highly effective against *T. brucei*, but further optimization of the prodrug strategy is needed to improve their oral bioavailability.

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**Session: Trypanosomiasis, Leishmaniasis & Schistosomiasis**

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**Type: Poster Presentation**

A critical role of CD2 as an immunoprophylactic agent to combat visceral leishmaniasis

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**Background:** A major Concern for VL prevention appears to be the inability of their CD4+ T cells to mount an adequate TH1 response which ensures the possible cure of the disease. Similarly the effectiveness of SAG in intact animal is determined by the host cell-mediated immune response. The present study aims at evaluating the use of CD2 antibody as an immunotherapeutic agent along with SAG in ensuring treatment of BALB/c mice induced with experimental Visceral leishmaniasis.

**Methods:** Mice were infected with *Leishmania donovani* promastigotes. Another set served as control. After seven week of infection, a set of mice from infected group was subjected to SAG treatment and another group was subjected to SAG treatment along with stimulation with antiCD2 antibody. CD4 cells expressing CD-25 were immunophenotyped and cytokines like IL-2, IFN- $\gamma$  and TNF- $\alpha$  were assessed using FACS. We also looked into cell cycle pattern, expression of CD25+ cells on T cells, percentage of lymphocytes converted into lymphoblasts, percentage of activated T lymphocytes and IL-2 production. These parameters were evaluated in T cells both before and after stimulation of their CD2 antigen.

**Results:** We recorded a substantial enhancement of protective cytokines which are essential for combating visceral leishmaniasis infection. We also observed a significant reduction in parasitic load when drugs are used in combination with this immunoprophylactic agent

**Conclusion:** CD2 proved to be an important immunoprophylactic agent which if used in combination with drugs can provide a suitable and substantial cure against visceral leishmaniasis

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**Type: Poster Presentation**

Patients with suspected visceral leishmaniasis in Istanbul

**H. Cakan**<sup>1</sup>, S. Saribas<sup>2</sup>, V. Oz<sup>1</sup>, E. Polat<sup>3</sup>, M. Aslan<sup>2</sup>, B. Kocazeybek<sup>3</sup>

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**Background:** Visceral Leishmaniasis (VL) is a parasitic disease caused by *Leishmania infantum*. It is transmitted through bites of infected sand flies (female Phlebotomus). We aimed to investigate bone marrow and blood samples obtained from the patients with suspected VL.

**Methods:** Fifty-nine patients with suspected VL from Istanbul were included in this work. Bone marrow and blood samples of these patients were tested for possible VL infection using several methods including serological tests, microscopy, PCR.

**Results:** Nineteen (32.2%) patients had positive results for VL after one or more of the tests performed, while only 7 patients (11.8%) had positive results with all the tests including Giemsa stain. Four (6.8%) patients had negative results based on all the serological tests performed except for positive results with Giemsa stain, culture and PCR. The other 4 (6.8%) patients had positive results with Formol-gel, ELISA IgG (>1.1 ISR) and IFAT IgG, (>1/256) but negative results were obtained with direct microscopic examination, culture and PCR. Using PCR *Leishmania infantum* DNA was detected in 11(18.6%) of the (*Leishmania*) cultures originated from the bone marrow samples. *Plasmodium vivax* was found in 2 (3.4%) patients and leptospira was detected in 1 (1.7%) patient. One (1.7%) patient was diagnosed with *Pneumonia* (*Streptococcus pneumoniae*). Forty (67.8%) patients had negative results after direct microscopic examination, culture, serological tests and PCR. The kappa coefficients  $\kappa = 0.80$ ,  $\kappa = 1.00$ ,  $\kappa = 0.51$ ,  $\kappa = 0.55$  and  $\kappa = 0.45$  were evaluated for PCR and direct microscopic examination, PCR and culture, PCR and ELISA, PCR and IFAT and PCR and Formol-Gel, as perfect agreement, perfect agreement, moderate agreement and moderate agreement fair moderate, respectively. The probability values (p) for comparisons of all the above tests with PCR showed a significant correlation (p<0.000)

**Conclusion:** In conclusion, we found that no single method alone was sufficient enough to diagnose VL accurately; however, combined with PCR, all these methods can reveal better and sensitive results ultimately leading to a correct diagnosis.

We also suggest that PCR has to be applied with other laboratory diagnostic tests in order to increase the sensitivity in diagnosis and decrease the possible defects in diagnosis.

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Immunological profile of CD18-deficient mice during *Schistosoma mansoni* infection

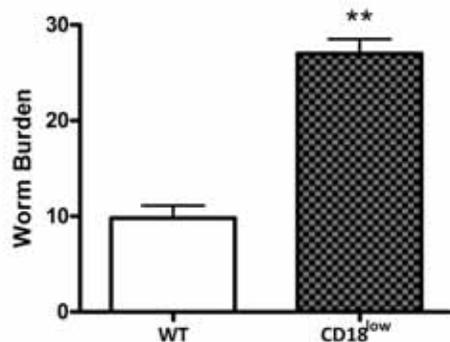
**M. S. Espíndola, F. G. Frantz, L. H. Faccioli**

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**Background:** Schistosomiasis is recognized as the most important human helminth infection in terms of morbidity and mortality harboring around 200 million people world-wide, being considered a risk for travelers. A study focusing the role of integrins, which are involved on cellular migration, antigen presentation and T cell activation, is necessary on the knowledge of immunopathology during schistosomiasis. The aim of this work is to evaluate the role of CD18 molecule, a  $\beta 2$  integrin, in modulate the immune response and pathology during the development of experimental schistosomiasis.

**Methods:** C57BL/6 (WT) mice and CD18<sup>low</sup> mice were percutaneously infected with 50 cercariae and the parasitological evaluation was done 48 days after infection. The adult worms were recovered from the hepatic portal system and the liver by perfusion with citrate saline. Ten and 48 days after infection, the cellular recruitment to the bronchoalveolar lavage fluid (BALF), as well the number of inflammatory cells present on the peripheral blood and the cytokines production in the lung homogenates were evaluated. To determine the proliferation of T CD3+CD4+ cells and the cytokines production *in vitro*, splenocytes were stimulated with concanavalin-A.

**Results:** CD18<sup>low</sup> mice showed an increased susceptibility to infection with *S. mansoni* since the worm burden was 135% higher than in the WT group. Nevertheless, the cellular recruitment to the BALF was similar between WT and CD18<sup>low</sup> mice, while CD18<sup>low</sup> mice showed a markedly enhancement on the accumulation of mononuclear cells in the peripheral blood, suggesting that less effector cells could migrate through blood to the inflammatory focus. Moreover, T cells from CD18<sup>low</sup> mice presented reduced potential to proliferate in the presence of Con-A than cells from infected WT mice. Ten days after infection the measurement of TNF- $\alpha$ , IL-12, IL-5, IL-10 and IL-4 in the lung homogenates was always lower in CD18<sup>low</sup> mice. Although, 48 days after infection, only IL-5 and IL-12 in CD18<sup>low</sup> mice showed slightly inferior levels. After *in vitro* stimulation of splenocytes with Con-A, just IL-5 production from CD18<sup>low</sup> mice was lower than WT.



**CD18<sup>low</sup> mice are more susceptible to infection with *S. mansoni* than WT mice. Worm burden was obtained by perfusion of the hepatic portal system with citrate saline. \*\* p<0.01**

**Conclusion:** The deficiency of CD18 molecule causes an uncontrolled parasite burden and changes of immune patterns, magnifying the severity of disease.

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**Session: Trypanosomiasis, Leishmaniasis & Schistosomiasis**

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Control of Chagas disease patients with chronic form of its treatment after Benznidazole treatment

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**Background:** Chagas disease is caused by the parasite *Trypanosoma cruzi* (TC). It's estimated that around sixteen million people are infected in Latin America and represents a serious blood safety problem due to increasing immigration from these countries.

Following the acute phase of the infection, untreated Chagas' disease enters a chronic phase that is initially asymptomatic or unrecognized. Between 20-30% of patients develop cardiac abnormalities, 10% digestive complaints, and less than 5% of patients develop a neurologic form of disease.

The aim of this work is to determine the effect of Benznidazole treatment (5 mg/kg/day) 60 days, in patients with chronic Chagas disease.

**Methods:** In 53 samples of patients we were tested by enzyme linked immunoassay (ELISA Dade Behring CHAGO560DB) for IgG antibodies against TC, and indirect immunofluorescence (IFI Biocientifica SA Immunofluor Chagas NF09-60) as confirmatory test, for IgG (Bio-Merieux 75 692) antibodies, with serial serum dilutions to determine the level of these antibodies.

In addition, all PCR were performed before treatment and at the end of it.

As a criterion of cure was established a significant (more than 2 degrees) in the rate of antibody after treatment

**Results:** About the 53 patients studied, 37 were performed two or more determinations of antibodies before and after treatment and in 80% of them, a diminution of the levels were found. The PCR was negative after treatment in all cases.

In 13 patients was carried out only antibody titer before treatment, administering it even with low rates from them, because they had organ involvement suggestive of Chagas.

**Conclusion:** More studies are needed to clearly establish the criteria for cure of Chagas disease. Furthermore, because in these patients with chronic Chagas disease parasitaemia sometimes is intermittent and low have to question the result of a negative PCR.

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**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Population structure of *Leishmania infantum* from Morocco

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**Background:** Visceral leishmaniasis (VL) is endemic in northern Morocco where it is caused by *Leishmania infantum*. It predominantly affects children under 4 years with incidence of 100 cases/year. Genetic variability and population structure has been investigated for 55 strains isolated from infected dogs and humans in Morocco.

**Methods:** A multilocus microsatellite typing (MLMT) approach was used in which a MLM type based on size variation in 14 independent microsatellite markers was compiled for each strain. MLMT profiles of 21 European strains which belonged to zymodeme MON-1 and non-MON1 according to multilocus enzyme electrophoresis (MLEE) were included for comparison.

**Results:** A Bayesian model-based approach and phylogenetic analysis based on genetic distances inferred two populations of the Moroccan *L. Infantum*; population A consists of 25 strains and population B consists of 30 strains. These populations were significantly different from the European MON 1 and Non MON 1 strains which constructed two different populations respectively.

Gene flow was noticed between populations A and B. Five strains have shown mixed A B genotype indicating possible recombination between the two populations

**Conclusion:** No genetic differences were detected between parasites isolated from dogs and humans emphasizing the role of dogs as reservoir. MLMT has proven to be a powerful tool for epidemiological and population genetic investigations in *Leishmania*.

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**Session: Trypanosomiasis, Leishmaniasis & Schistosomiasis**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Propolis and derivatives of megazol: *In vitro* and *in vivo* activity on *Trypanosoma cruzi*, mechanism of action and selectivity

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**Background:** One hundred years after its discovery, Chagas disease, caused by *Trypanosoma cruzi*, still represents an important health problem and in need of alternative drugs for the treatment of chagasic.

**Methods:** Ethanolic extract of Brazilian green propolis (Et-Bra) was assayed on amastigotes proliferation, trypomastigote by transmission and scanning electron microscopy and flow cytometry. Thirty two 1,3,4-thiadiazole-2-arylhydrazones of megazol were synthesized and assayed on trypomastigotes.

**Results:** Et-Bra was active against amastigotes proliferation inside mammalian macrophages and induced plasma membrane damage in the infective trypomastigote forms as determined by transmission and scanning electron microscopy and flow cytometry. In non-infected mice, propolis induced no toxicity as determined by the GPT, GOT and CK plasma levels. Treatment of *T. cruzi*-infected mice (up to 300 mg Et-Bra/kg/day for 10 days) led to a significant reduction of the mortality but not of the parasitemia, did not reversed the hepatic, renal or muscular damage induced by the parasite.

The most active analogues *in vitro* -S1 to S8- were assayed *in vivo* by a single oral dose at 5 dpi, being selected S1, S2 and S3, together with megazol for subsequent *in vitro* and *in vivo* studies. In trypomastigotes, ultrastructural analysis revealed that the compounds led to alterations at kDNA, mitochondrion and flagellar membrane and rounding and torsion of the parasite's body. S1 and S2 inhibited the amastigotes proliferation inside macrophages, while in cardiac muscular cells only S1 was active. The administration of 10 consecutive doses (50 and 100 mg/kg) of S1 caused no effect on the course of infection, while S2 led to a significant decrease of the parasitemia and S3, only of the mortality. The three analogues were not toxic for the animals based on the levels of GPT, GOT and urea.

**Conclusion:** Our results demonstrate the promising activity on *T. cruzi*, especially S2 and S3, justifying *in vivo* assays with longer period of treatment and the continuity of the investigation of new analogues.

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**Session: Trypanosomiasis, Leishmaniasis & Schistosomiasis**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Alanine 163 in loop C of *Leishmania major* aquaglyceroporin LmAQP1 resides near the pore mouth of the channel

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**Background:** The *Leishmania major* aquaglyceroporin, LmAQP1, is responsible for the transport of trivalent metalloids, arsenite and antimonite. We have earlier shown that down regulation of LmAQP1 provides resistance to trivalent antimony compounds whereas upregulation of LmAQP1 in drug resistant parasites can reverse the resistance. We have implicated this transporter to volume regulation and osmotaxis, two important characteristics for successful infection. Recently we have shown that a single change in the loop C of LmAQP1 changes its substrate specificity. Beitz et al (2004) have shown that E125 in loop C of PfAQP is close to the mouth of the pore and is critical for high water permeability of PfAQP (Beitz, 2004). Loop C of PfAQP is twelve residues shorter than LmAQP1. Therefore, E125 of PfAQP lines up with A163 in the primary sequence. To confirm that A163 is near the pore mouth we have created different mutants of LmAQP1 and their transport properties were compared.

**Methods:** Different mutants of A163 were created using site directed mutagenesis method. Wild type and mutant LmAQP1 were transfected into wild type *L. donovani*. Metalloid sensitivity, transport and osmoregulation ability of the transfected parasites were measured. cRNA of wild type and mutated LmAQP1 were injected into *Xenopus levis* oocytes followed by the determination of metalloid, water and solute transport.

**Results:** Change of A163 to glutamate, glutamine or aspartate made it impermeable to water, metalloids and other solutes. However, changes to serine and threonine did not change the transport properties of the channel. Water transport through LmAQP1 is mercurial insensitive. Introducing cysteine (s) in loop C in the vicinity of A163 made the water transport through the channel mercurial sensitive.

**Conclusion:** We report that A163 in loop C is localized near the pore mouth of LmAQP1.

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**Session: Trypanosomiasis, Leishmaniasis & Schistosomiasis**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Chagas disease: Mother to child transmission (MTCT). A single experience in a public hospital from Buenos Aires

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**Background:** Mother to child transmission of Chagas Diseases (ChD) rates was reported from 1 to > 10%. Factors reported to increase risk include younger maternal age, human immunodeficiency virus infection, and parasite strain.

**Methods:** Retrospective analysis of children assisted in infectious diseases department from 2001 to 2008. Inclusion criteria was: every child born from a mother suffering of ChD with accessing a diagnostic procedures before 24 months of life and never traveled to endemics areas until finish diagnostic process. Diagnosis of ChD among mothers and children over 6 months old were performed by serology (ELISA and HAI). Diagnosis of ChD among children ≤ 6 months was performed with parasitemia investigation.

**Results:** We studied 307 children born from mother with ChD, 165 were ≤ 24 months of life. Ratio man/woman 87/78. MTCT was 26/152 (17%). Diagnosis was performed by parasitemia in 20/26: the 1st parasitemia performed was positive in 13 cases, 2nd parasitemia was positive in 5 cases and 3rd parasitemia was positive in only a case. In six patients, ChD diagnosis was performed by serology.

Mothers were from: Argentina=28, Bolivia=96, Paraguay=19 and without records=22.

Only 11/26 chagasic children were symptomatic. Most frequent symptoms were: anemia 9/11, hepatosplenomegaly 3/11, hydrops fetalis 1/11, sepsis 1/11, abnormalities in serum transaminases 1/11 and cardiac abnormalities 1/11.

Prematurity was observed in 5/26 with ChD vs 2/111 no ChD. Low birth weight was present in 5/26 (19.2%) with ChD vs 6/111 (5.4%) no ChD.

Three patients born from mother with HIV infection. MTCT were 3/3 (100%) vs 23/145 (15.8%) no HIV. No children were infected with HIV.

The study of newborn allowed us performed study of ChD among 97 siblings never studied; in 11 of them ChD was diagnosed.

Benzindazol was indicated in all cases of vertically acquired ChD; 9 of the were lost of follow up and 5 had adverse reactions that.

**Conclusion:** MTCT was higher than previously reported. Even if sample was small; it can be possible that mothers with HIV co-infection have more MTCT than no HIV. We have the opportunity to study all the members of family and bring treatment if it is necessary.

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**Session: Trypanosomiasis, Leishmaniasis & Schistosomiasis**

**Date: Friday, March 12, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Trypanosoma cruzi expressing luciferase for drug screening using a bolivian strain

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**Background:** The commonly used drugs against *T. cruzi* are nifurtimox and benznidazole but they both have adverse effects for humans. Clinical symptoms of Chagas disease also depends on the strain present in each region. Drug screening methods are tedious and expensive. High-throughput systems are required for screening anti-*T. cruzi* agents. The use of transfected parasites expressing reporter genes like  $\beta$ -galactosidase has facilitated drug screening. However, *luciferase* (*luc*) has a higher sensibility. In our studies we found clinical and serological differences between the Peruvian and Bolivian strain. The Bolivian strain seems more virulent as it has been documented with a high number of acute and chronic cases. Here we developed transgenic parasites expressing luciferase gene for having a new drug screening method.

**Methods:** Bolivian strain (DH29) was isolated from a child who acquired Chagas Disease by congenital infection based on microscopic examination and parasites were recovered by *in vitro* cultivation. The open reading frame of *luc* was inserted in two vectors: pBS:CL-Neo-01/BC-LacZ-10 (pLAC) and pTREX. Stable transfectans were isolated with G418 following integration of the circular transforming plasmids into the nucleus of epimastigotes by electroporation. Luciferase was detected by PCR, western blot and its activity with a luminometer.

**Results:** Two plasmids were developed pBS-Neo-Luc and pR-Luc-Neo. Drug (G418)-resistant parasites were isolated after three weeks post electroporation. PCR (1.7Kb) and western blot (61 KD) detected luciferase gene. Transfected parasites incubated with luciferin showed luciferase activity. The clones that failed to express luciferase were found to have integrated the neomycin gene. The clones that expressed luciferase were stable for more than six months without selection. Two clones (L95 y L97) expressing luciferase were isolated from DH29 strain. There is a correlation between the enzyme activity and number of parasites. Luciferase assay was able to detect 102 parasites ml<sup>-1</sup>.

**Conclusion:** The constructed plasmids pBS-Neo-Luc and el pR-Luc-Neo are useful for stable expression in *T. cruzi*. Two clones L95 and L97 from DH29 strain of *T. cruzi* expressing luciferase gene were developed. There is a linear correlation between luciferase activity and the number of parasites ml<sup>-1</sup>. Luciferase assay is able to detect at least 102 parásitos ml<sup>-1</sup>.

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**Final Abstract Number: 83.001**  
**Session: Vaccines and Vaccine Development**  
**Date: Thursday, March 11, 2010**  
**Time: 12:30-13:30**  
**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

The effectiveness of one dose of varicella vaccine  
**Z. Kurugol**  
Ege University, Izmir, Turkey

**Background:** Breakthrough varicella rates range from 10 to 33%, it may be related to the regional epidemiology of varicella. In this study, we investigated that the effectiveness of varicella vaccine in vaccinated children who had received a single dose.

**Methods:** This study was conducted in 4 elementary schools and 9 nursery schools in Izmir in Turkey between October 1 and December 31, 2008. We distributed a questionnaire to parents and above to collect vaccination history and varicella disease. Varicella disease was defined as an acute, pruritic, generalized, maculopapulovesicular rash without other apparent cause and it was defined as varicella like rash that developed >6 weeks after vaccination in vaccinated children.

**Results:** In the study, 3630 questionnaire were distributed and 2523 of them were resumed. One thousand three hundred and eighty-four children who had received a single dose of vaccine were included the study. Exclusion criteria were: existence of incomplete questionnaire and the children who were vaccinated with two doses. The varicella history was determined in 424 patients (30.7%) among patients who received a single dose of vaccine. The mild form of varicella was found to be 65.8 %, and the moderate-severe type of disease was determined in 34.2% of in this patients. The disease was diagnosed in 90 percent by a doctor. The median age of children was 95 months and the median age at vaccination was 13 months. According to the duration of vaccination, the varicella disease rate was found 15% (< 5 years), and 38.4% (≥ 5 years). Children who had been vaccinated 10 years or more were at greater risk for varicella disease (71%) than those who had been vaccinated the more recently.

**Conclusion:** A single dose of varicella vaccination provided poor protection against varicella, however the vaccine provided sufficient protection against moderate and severe disease. Probability of breakthrough varicella was increased when a interval since vaccination was longer.

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**Final Abstract Number: 83.002**

**Session: Vaccines and Vaccine Development**

**Date: Thursday, March 11, 2010**

**Time: 12:30-13:30**

**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Production and characterization of a chimeric recombinant protein from synthetic genes consisting antigenic domain of EspA, Intimin and Tir of *Escherichia coli* O157 and evaluation as a candidate vaccine antigen in animal model

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**Background:** Enterohaemorrhagic *Escherichia coli* (EHEC) comprise an important group of zoonotic enteric pathogens. In humans, some EHEC infections result in bloody or non bloody diarrhea, which may be complicated by hemorrhagic colitis and severe renal and neurological sequelae, including hemolytic uraemic syndrome (HUS).

Intimin, Tir, and EspA proteins are expressed by attaching-effacing Enterohemorrhagic *E. coli*. EspA proteins are part of the type three secretion system (TTSS) needle complexes that delivers Tir to the host epithelial cell, while surface arrayed intimin docks the bacterium to the translocated Tir. This intimate attachment leads to attaching and effacing (A/E) lesions.

Vaccination strategies have been proposed for the prevention of this bacterium including the choice of potential antigens. EspA, intimin, and Tir are three predominant virulence factors of EHEC, and each of them has proved to be capable of inducing partial protective immunity.

**Methods:** In this study, we constructed a trivalent recombinant protein designated EIT that is composed of C-terminal 120 amino acids EspA (E), C-terminal 282 amino acids of intimin (I) and Tir fragment containing 103 amino acid residues 258 to 361 (T) which attached together using four repeat of five hydrophobic amino acids as a linkers. For high level expression of this chimeric gene in *E. coli* system the modified genes (1680bp) encoding EspA, Intimin and Tir (EIT) was synthesized in accordance with the G+C content and increasing in the codon bias of *E. coli* genes without altering the encoded amino acid sequence. The multimeric effector was produced by using chemically induced T7 promoter in *E. coli* expression system. The immunization was assayed in mouse model with purified recombinant protein.

**Results:** The structure of synthetic gene, its mRNA and deduced protein and their stability was analyzed by bioinformatic softwares. Furthermore the immunogenicity of this multimeric recombinant protein consisting of three different domains was predicted.

Experimental results showed that immunization with EIT induced strong humoral response to EspA, intimin and Tir and protected mice against the challenges with live EHEC.

**Conclusion:** This work suggests that for EHEC control a combination of antigenic EspA, Intimin and Tir appears to be more effective than using any of these immunogens alone.

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**Session: Vaccines and Vaccine Development**

**Date: Thursday, March 11, 2010**

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**Room: Poster & Exhibition Area/Ground Level**

**Type: Poster Presentation**

Variability of PspC (Pneumococcal surface protein C) in strains isolated in the University Hospital of the University of São Paulo (Brazil)

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**Background:** *Streptococcus pneumoniae* is one of the most common causes of respiratory tract infections. The vaccine composed of different capsular polysaccharides (PS) purified from pneumococci has low efficacy in children and the elderly, besides not being able to induce immunological memory. Although the 7-valent PS vaccine conjugated to CRM197 was an advance, the production cost is still a major barrier for its widespread use. A proposal to increase vaccine coverage at a low cost consists in the identification of protein antigens present in all pneumococcal isolates. PspC (Pneumococcal surface protein C) has been described for its role in both colonization of the nasopharynx and in invasive infection. PspC is highly polymorphic, being divided into 11 groups. Thus, the evaluation of the variability of this antigen in clinical samples is of great importance to determine the ideal vaccine formulation.

**Methods:** Pneumococcal strains were obtained from the University Hospital of the University of São Paulo (Brazil). Strains were serotyped by PCR and 13 isolates were chosen based on the serotypes present in the new 13-valent conjugate vaccine (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F). The complete *pspC* locus was cloned and the gene was sequenced for each isolate. BALB/c mice were immunized with two different recombinant PspC variants for the production of antibodies that were used for Western blot analysis.

**Results:** From the 13 pneumococcal isolates analyzed, six were found to be from group 3, three isolates from group 6, one isolate from group 5, one isolate from group 8 and one isolate from group 9. A duplication containing PspC from group 4 and from group 10 was also found. An antiserum raised against PspC3 was able to recognize the majority of pneumococcal extracts in Western Blot analysis, showing a broad cross-reactivity. On the other hand, an antiserum raised against PspC8 was able to recognize only the isolate expressing PspC from group 8.

**Conclusion:** These preliminary results suggest that PspC3 would be a suitable vaccine antigen, being able to induce antibodies with broad cross-reactivity.

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**Type: Poster Presentation**

Combination of whole cell pertussis vaccine and the pneumococcal surface protein A (PspA) antigen: proposal of a combined vaccine against pertussis and pneumococcal diseases  
M. L. Sarno de Oliveira, D. M. Ferreira, A. T. Moreno, P. C. D. Ferreira, E. Miyaji, **P. Ho**  
Instituto Butantan, Sao Paulo, Brazil

**Background:** *Streptococcus pneumoniae* (pneumococcus) is one of the major agents of respiratory acute diseases, accounting for 1 million deaths per year around the world. In developing countries, pneumococcal diseases kill 800,000 children annually. PspA is a surface pneumococcal antigen, shown to elicit protection against animal models of pneumococcal infections, in different vaccine formulations. The whole cell pertussis vaccine (WCP) is widely used in developing countries and its first dose is indicated to be given at 2 months of age. As an inactivated bacteria-based vaccine, it has the potential to exert adjuvant activity upon co-administration with other antigens.

**Methods:** Here, we analyzed the adjuvant activity of WCP in combination with PspA and tested the efficacy of WCP-PspA nasal vaccination against a pneumococcal challenge in mice.

**Results:** Nasal immunization of Balb/C mice with WCP-PspA elicited higher amounts of anti-PspA IgG and sIgA than the immunization with PspA alone. Mice immunized with the combination were 100% protected after a respiratory lethal challenge, whereas immunization with PspA alone produced around 60% protection. In time course experiments of challenged mice, IL-6, TNF and IFN-g cytokine signaling was observed in vaccinated animals, with a peak 12h after challenge followed by a quick reduction in secretion, indicating controlled inflammatory responses. In contrast, cytokine secretion in control mice remained high long after challenge, showing that these animals are unable to control the inflammatory response elicited by pneumococcal infection. Pneumococci loads increase in the lungs of control animals and reach the bloodstream 30h after infection, leading to death at 72h after infection. On the other hand, vaccinated mice displayed steadily pneumococcal numbers in the lungs in the first hours and a marked decrease 72h after challenge. In this group, pneumococci did not reach the bloodstream. Sera collected from WCP-PspA immunized mice displayed increased capacity of induction of complement deposition on bacteria surface, indicating a potential role of antibodies in the protection observed. Moreover, sera from WCP-PspA immunized mice protected naïve mice in passive immunization experiments.

**Conclusion:** These results open the possibility for the combination of WCP vaccines with PspA, taking advantage of the adjuvant activity of WCP.

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**Type: Poster Presentation**

Protection against pneumococcal nasopharyngeal colonization in mice immunized with PspC ("Pneumococcal Surface Protein C")-based vaccines formulated with whole bacteria as carriers or adjuvants

M. De Lúcia Hernani, D. M. Ferreira, P. C. D. Ferreira, A. T. Moreno, E. Miyaji, P. Ho, **M. L.**

**Sarno de Oliveira**

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**Background:** *Streptococcus pneumoniae* (pneumococcus) is responsible for the majority of pneumonia cases around the world. Despite the effectiveness of conjugate vaccines, the costs of the available formulations are prohibitive for mass vaccination in developing countries. Protein antigens are alternatives for the constitution of low costs-formulations that can elicit immunity to different pneumococcal serotypes. PspC is a virulence factor that plays a role in the adhesion of the pathogen to epithelial cells and evasion from the immune system. Intact bacteria display adjuvant activities when administered in association with antigens, through the binding of their components to receptors present on immune cells surface.

**Methods:** In this work, mucosal vaccines composed of PspC and two different bacteria as carriers and/or adjuvants were tested in mice. The first strategy consisted in *Lactobacillus casei* expressing PspC (*L. casei*-PspC), as a live vaccine. The second consisted in the combination of recombinant PspC with the whole cell pertussis vaccine (WCPV).

**Results:** Nasal immunization of mice with *L. casei*-PspC did not stimulate the production of systemic and mucosal anti-PspC antibodies. Despite the absence of specific antibodies, this vaccine inhibited pneumococcal nasopharyngeal colonization of immunized mice, when compared with mice inoculated with saline or *L. casei*. In this same model, sublingual immunization with *L. casei*-PspC did not produce a significant effect on pneumococcal colonization. On the other hand, nasal immunization with PspC-WCPV induced high levels of anti-PspC antibodies. Mice immunized with PspC-WCPV presented a significant inhibition of pneumococcal colonization, when compared with mice inoculated with saline or WCPV. The reduction in pneumococcal colonization was much more accentuated for the PspC-WCPV formulation than for *L. casei*-PspC. Analysis of cytokine secretion by spleen cells from mice 5 days after the colonization challenge showed two different patterns. Mice immunized with *L. casei*-PspC displayed a dramatic reduction in IFN-g and TNF-a secretion whereas mice immunized with PspC-WCPV displayed increased IFN-g and IL-17 secretion.

**Conclusion:** Our results indicate that two different mechanisms are likely to be involved in the protection elicited by both vaccines. The mechanisms elicited by PspC-WCPV seem to be more effective in the control of pneumococcal nasopharyngeal carriage.

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**Type: Poster Presentation**

Immunogenicity of interleukin 12 and DNA vaccine prime-BCG boost against *Mycobacterium tuberculosis*

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**Background:** BCG as an only effective vaccine to TB played variable efficacy. New vaccination strategies are required. We used interleukin 12 with the combined DNA prime-BCG boost strategies to observe whether the immunogenicity of vaccines against M.Tuberculosis would be improved.

**Methods:** Plasmid pcDNA- Ag85A and pc-Esat-6 were constructed for vaccination .

The mice were divided into 4 immunity groups: BCG group(1), DNA/BCG group(2), DNA+IL-12/BCG group(3) and DNA/BCG+IL-12 group(4). All mice received three immunizations at 2-week interval For prime-boost experiments, animals were twice vaccinated intramuscular injection with combined DNA (Ag85A and ESAT-6) or mixed DNA-IL-12 and combined DNA, then boosted with BCG or mixed with DNA-IL-12 and BCG. ELISA was used to determine IgG antibody specificity and the proliferative responses of lymphocytes and the phenotype was detected by flow cytometry. Production of INF- $\gamma$  was detected at 4-week, 6-week, and 8-week after boost.

**Results:** The antibody titer of group 1, group 2, group 3 and group4 showed a positive reaction. Group3 and 4 compared with group2 and 1, induced high antibody titer. The antibody titer of all four groups increased gradually, and achieved a higher level at 8 week after booster. Group 1, group 2, group 3and group4 all showed significant difference of proliferative responses ( $P<0.05$ ). Group 3and group 4 were much higher that group 2 and group1 ( $P<0.05$ ).

The group 3 and the group 4 induced stronger antigen-specific IFN- $\gamma$ . The mean IFN- $\gamma$  responses of group 3and the group 4 were not only significantly higher than group PBS ( $P<0.01$ ) but also higher than group 2and group 1 ( $P<0.05$ ). The mean percentage of CD4+ and CD8+ T cells vaccinated with DNA/BCG+IL-12, DNA+IL-12/BCG, DNA/BCG, or BCG were significantly higher compared to group PBS( $P<0.05$ ). A higher mean percentage of CD4+ and CD8+ T cells were observed in mice vaccinated with DNA+IL-12/BCG or DNA/BCG+IL-12 when compared to the DNA/BCG or BCG groups.

**Conclusion:** Our results showed that the strategy of using human interleukin 12(IL-12) associate DNA priming followed by BCG boosting is an effective way to increase the immunogenicity of tuberculosis, not only increasing cell immunity but also maintaining a stable humoral immunity.

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The role of inactivated polio vaccine (IPV) in achieving polio eradication in India

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**Background:** Since ancient times polio has lamed millions of young children before they had learned to walk. The World Health Organization since 1988 is spearheading the global efforts to eradicate poliomyelitis. The global initiative to eradicate the disease has been successful in reducing the incidence by 99% and it has prevented more than 5 million cases of polio in past 21 years. The disease has been eradicated throughout the western hemisphere and in most developing nations by use of the Sabin Oral Polio Vaccine. At the present crucial end stage of polio eradication, the wild virus is circulating in Afghanistan, India, Nigeria and Pakistan. The studies in developing countries have demonstrated that even after more than 3 doses of OPV, the immune response generated in children is not satisfactory and not all the children seroconvert. Studies have concluded that that OPV/IPV sequential schedule generate far superior humoral and intestinal immune response. It is therefore essential for the policy makers to consider the role that could be played by the sequential IPV/OPV schedule in achieving the goal of polio eradication for this important international public health challenge.

**Methods:** To achieve the objectives, we used peer reviewed journal articles published since 01-01-1985 till 31-07-2009. The articles were searched via Pub Med and EMBASE electronic data base system using key words/phrases. The criteria used for limiting the search while using PubMed and Embase have been described in Table: 1. The references of the selected articles were also searched for additional literature. The seroconversion rates among the study population after OPV only, IPV only and sequential OPV/IPV schedule were compared with each other.

Table:1 Limiting criteria used for literature search on PubMed and Embase		
Limiting Criteria	PubMed	EMBASE
Full Text	√	√
Date	01/01/1985 to 31/07/2009	01/01/1985 to 31/07/2009
Language	English	English
Type of Studies Included		
Randomized Controlled Trials	√	√
Analysis	√	√
Practice Guidelines	√	X
Reviews	√	√
Controlled Clinical Trial	√	√
Age		
New-Born	√	√
Infant	√	√
Pre-School Child 2-5 Years	√	X
1-6 Year	X	√

**Criteria for Literature Search**

**Results:** In the various setting in the developing countries it was observed that the seroconversion rates among those children receiving IPV/OPV sequential schedule was higher than OPV only schedule.

**Conclusion:** The rationales for use of sequential IPV/OPV schedule in current setting are (1) It will fill the existing immunity gap rapidly (2) It will reduce the excretion of vaccine virus and thereby the risk of cVDPV (3)The risk of VAPP will be reduced. (4)It will also allow strengthening of the routine immunization services by states.

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**Type: Poster Presentation**

Antibody persistence 22 months after vaccination of adolescents with the Novartis investigational meningococcal ACWY-CRM197 conjugate vaccine or Menactra®

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<sup>1</sup>Novartis Vaccines and Diagnostics, Cambridge, MA, USA, <sup>2</sup>Novartis Vaccines and Diagnostics, Sienna, Italy, <sup>3</sup>Novartis Vaccines and Diagnostics, Marburg, Germany

**Background:** Improved primary response, induction of immunologic memory and antibody persistence are key attributes of conjugate vaccines. We have previously presented immunogenicity data one month post-vaccination from a phase III, randomized, observer-blind comparative study of an investigational meningococcal ACWY-CRM197 conjugate vaccine (MenACWY-CRM, Novartis) or Menactra® (MCV-4, Sanofi Pasteur). Data from approximately two years post-vaccination in an ongoing long-term study of persistence of bactericidal antibodies are now available.

**Methods:** Subjects, who were aged 11-18 years when enrolled into the parent study, were approached for enrollment. Age-matched meningococcal vaccine-naïve subjects were enrolled to serve as additional controls. The primary objective was to assess the persistence of immune response for each serogroup using a serum bactericidal assay with human complement (hSBA), expressed as the proportions of subjects with a titer  $\geq 1:8$  and Geometric Mean Titers (GMTs).

**Results:** At a median of 22 months post vaccination, 278 MenACWY-CRM and 191 MCV-4 subjects, plus 128 naïve controls were enrolled. For serogroups A, C, W and Y respectively, the proportions of subjects with hSBA  $\geq 1:8$  were: 36%, 62%, 84%, and 67 % for MenACWY-CRM; 25%, 58%, 74%, 54% for MCV-4; and 5%, 42%, 51% and 40% for the naïve controls. Similarly hSBA GMTs for serogroups A, C, W and Y, respectively, were: 5.3, 10.0, 18.0 and 12.0 for MenACWY; 3.6, 8.7, 14, and 7.9 for MCV-4; and 2.4, 6.0, 7.8 and 5.1 for the controls. Compared with MCV-4 recipients, the proportions of MenACWY-CRM vaccinees with hSBA  $\geq 1:8$  was significantly higher for serogroups A, W and Y; GMTs were significantly higher for serogroups A and Y. Compared with naïve controls, GMTs were significantly higher for MenACWY-CRM for all four serogroups.

**Conclusion:** A majority of adolescents maintain bactericidal antibody titers above 1:8 against meningococcal serogroups C, W and Y two years after receiving either conjugate vaccine although more of the MenACWY-CRM vaccinees still had protective antibody titers against serogroups A, W, and Y than those who received MCV-4.

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KNOW ESSENTIALS – A novel algorithm for informed vaccine-related decision-making in developing countries.

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**Background:** In most developing countries, vaccine-related decisions are seldom evidence-based and health-care stakeholders (physicians/policy-makers/patients) usually do not have access to locally relevant health technology assessment (HTA), in contrast to developed countries.

The objective was to develop a decision-making algorithm facilitating informed vaccine-related decisions by various stakeholders in developing countries, using available information from literature.

**Methods:** *KNOW ESSENTIALS* is an acronym for 13 components to be evaluated for informed vaccine-related decisions. The first three defining criteria (acronym KNOW) are establishment of (i) Knowledge of need (KN) for the vaccine, (ii) Outcome of interest (O), (iii) Which stake-holder is involved (W). Subsequent components should be evaluated only when all three are clear. The other criteria (acronym *ESSENTIALS*) are: (iv) Evidence of effectiveness and/or efficacy, (v) Safety, (vi) Social quotient (consumer acceptability and ethical/legal/moral propriety), (vii) Economic issues viz cost and cost-effectiveness, (viii) Novelty (newness), (ix) Time to outcome of interest, (x) Integration with existing services/facilities, (xi) Alternate options, (xii) Likely impact of not choosing the intervention, and (xiii) Sustainability. Based on local and/or external data/information from literature, each of these is categorised as Red (unfavourable), Green (favourable), Yellow (insufficient/unclear data) or White (not-applicable). The basis for colour coding has been elaborately defined for each criterion to exclude subjectivity; but is not presented here for lack of space. Criteria (iv) to (vii) are mandatory criteria and stake-holders should proceed only if all are Green.

Table 1 shows practical application of the algorithm for a currently relevant, challenging decision: whether India should consider universal human papillomavirus (HPV) vaccination.

**Results:** The algorithm can be applied by multiple stake-holders (policy-makers/physicians/patients/health-care payers) to make objective decisions for their setting based on explicit criteria; thus saving time and cost. It enables each stake-holder to arrive at an informed decision appropriate for them. Pilot testing considering various vaccines as well as different stake-holders suggests that it facilitates objective, reproducible, and transparent decision-making. A randomized trial comparing it against formal HTA is planned.

**Conclusion:** *KNOW ESSENTIALS* is a practical algorithm facilitating informed vaccine-related decisions by various health-care stake-holders in developing country settings.

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vax-SPIRAL®: Cuban antileptospirosis vaccines for humans: Clinical and field assays and impact of the vaccine on the disease after 11 years of application in Cuba

**R. Martínez**

Instituto de Medicina Tropical "Pedro Kourí", Ciudad Habana, Cuba

**Background:** In Cuba, leptospirosis has shown an endemoepidemic behavior, with an important increase in morbidity between 1991 and 1995, that is why these years have been considered epidemic. According to what had been foreseen within the Leptospirosis Prevention and Control Program, a Russian vaccine was administered to persons at risk of getting the disease since 1983. In 1991, import of this vaccine was stopped and, in order to find an alternative solution to this health problem, it was decided to start the development of a trivalent Cuban vaccine with autochthonous strains (*Icteroahemorrhagiae*, *Canicola*, *Pomona*). The objective was to carry out the clinical evaluation and to observe the impact of the Cuban vaccine against human leptospirosis in persons exposed to the risk of getting this disease.

**Methods:** The first studies conducted were controlled clinical assays to evaluate both reactogenicity and immunogenicity of the vaccine in different groups of volunteers. Nine hundred and fifty-eight volunteers participated in the studies. To evaluate the vaccine efficacy, a controlled, randomized, double blind clinical assay was performed in Villa Clara province with 101 832 volunteers, who were followed up for one year. Another studied was carried out in 101 137 persons at risk in Holguin province with the same objective: to evaluate the vaccine effectiveness.

**Results:** The clinical evaluation showed safety and low reactogenicity. Immunogenicity was comparable to that of other bacterines. Efficacy was of 78.1% (IC 95%: 58.2 to 88.3) and the RR of getting sick of vaccinated individuals, with regard to non-vaccinated ones, was 0.22. So, a protective effect of the vaccine administration was considered. The vax-SPIRAL vaccine was registered in 1998 and included within the Leptospirosis Prevention and Control Program. Since the introduction of this vaccine, a marked decrease in leptospirosis cases has been observed. It has been possible to administered more than 8 million of doses and morbidity has been diminished to 84.1%.

**Conclusion:** vax-SPIRAL is safe, little reactogenic vaccine and has turned out to be an effective measure for leptospirosis control in individuals exposed to the risk of getting sick.

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Immunization with surface protein 2 (TcSP2) protects mice from acute experimental *Trypanosoma cruzi* infection: A potential candidate for vaccine development

**A. Carabarin**, C. González, L. Baylon-Pacheco, J. L. Rosales-Encina  
CINVESTAV-IPN, México, Mexico

**Background:** *Trypanosoma cruzi*, the causative agent of Chaga's disease, is a protozoan parasite that affects million of people in Latin America. TcSP2 is a surface protein expressed in amastigote, epimastigote and trypomastigote, and it is a promising molecule for a recombinant protein vaccine against American Trypanosomiasis.

**Methods:** The TcSP2 gene was amplified by PCR using as a template the plasmid pA83, cloned in the plasmid pRSETB and the his-tagged recombinant protein was purified by nickel affinity chromatography. Mice immunized (ip) with the His-TcSP2 protein were infected (ip) with bloodstream trypomastigotes H8, and parasitaemias and survival rates were monitored every other day. Serum samples from both immunized mice and challenged mice were assayed for anti-TcSP2 antibodies by ELISA (immunoglobulin isotype), and for the measurement of cytokines by flow cytometry. For histological studies, sections of fixed hearts were stained with hematoxylin and eosin, and evaluated by light microscopy.

**Results:** Mice were bleed 15 days after the last immunization and the anti-TcSP2 antibodies isotypes were determined. It was found a predominance of IgG1<IgG2b<IgG2a isotypes respectively. This isotype profile indicates a non polarized Th1/Th2 immune response. Immunized and unimmunized mice were infected with blood trypomastigotes, and the parasitaemia profiles showed a 70% reduction in parasite number in mice immunized with the recombinant protein TcSP2 as compared with unimmunized animals ( $p < 0.05$ ). Likewise, the surviving rate for both immunized and unimmunized animals was 100% and 25% respectively ( $p < 0.001$ ). The Cytokine profile at the end of the parasitaemia showed the presence of IL-10 and INF- $\gamma$  in immunized and infected mice, and IL-4 in unimmunized and infected mice. These results indicate that a Th1-type immune response was polarized after parasite challenge. Finally, histopathological analysis of tissue sections from unimmunized and infected mice showed an extensive infiltration of inflammatory cells in heart as compared with the absence of cell infiltration in immunized and infected mice.

**Conclusion:** The findings of this study indicate that immunization with the TcSP2 recombinant protein induces protection against infection with *Trypanosoma cruzi* in the mouse model. Therefore, TcSP2 recombinant protein may be an excellent candidate for further vaccine development.

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Long term immunogenicity following a booster dose of the inactivated Japanese encephalitis vaccine IXIARO®, IC51

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<sup>3</sup>Medical University Vienna, Vienna, Austria, <sup>4</sup>Medical University of Vienna, Vienna, Austria

**Background:** IXIARO (JESPECT® in Australia, IC51), Intercell's recently approved Vero cell-derived, inactivated Japanese Encephalitis vaccine, has been proven immunogenic and safe in a 0/28 Day primary immunization schedule in adults. Neutralizing antibody titers decline with time and booster doses are likely needed to obtain a longer lasting immune response.

**Objectives:** To assess the effect of a booster dose on neutralizing antibody titers for up to 12 months after the booster.

**Methods:** In this open-label phase III trial, 198 subjects, who had received their primary immunization in a preceding randomized trial, were boosted with IXIARO 15 months after the primary immunization and followed up for 12 months. Neutralizing antibody titers were assessed by plaque-reduction neutralization test, PRNT on the day of boosting and 1, 6 and 12 months later. A PRNT50  $\geq$  1:10 was the cut-off for seroconversion. Systemic and local tolerability were solicited with diaries for a 7 days period following the booster.

**Results:** Prior to the booster, 69.2% (137/198) of subjects had PRNT50 titers of  $\geq$  1:10. The seroconversion rate (SCR) was 100% (198/198) one month after the booster. Both at 6 months and 12 months after the booster, the SCR remained high at 98.5% (194/197 and 191/194 subjects, respectively). GMTs were 22.5 before the booster and 900, 487 and 361 at 1, 6 and 12 months after the booster.

During 7 days after the booster, 30.8% of subjects reported solicited local reactions, and 23.2% reported solicited systemic adverse events.

**Conclusion:** An IXIARO booster 15 months after primary immunization induced higher neutralizing antibody titers than seen immediately after primary immunization (a mean 5.3-fold increase). The GMTs and SCRs remained at high levels for 12 months after the booster. A booster dose of IXIARO was generally well tolerated with a safety profile in line with the primary immunization.

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Cost effectiveness of Pneumovax® 23 stockpile to prevent secondary pneumococcal infections among a high-risk population in the United States during an influenza pandemic

**P. Dhankhar**<sup>1</sup>, J. Grabenstein<sup>2</sup>, M. O'Brien<sup>2</sup>, E. J. Dasbach<sup>1</sup>

<sup>1</sup>Merck & Co. Inc., North Wales, PA, USA, <sup>2</sup>Merck & Co. Inc., West Point, PA, USA

**Background:** Recent literature concludes that secondary bacterial infections (especially pneumococcal infections) were the leading cause of death during prior influenza pandemics. One strategy to prevent pneumococcal infections in adults during a future pandemic is to stockpile 23-valent pneumococcal polysaccharide vaccines.

**Methods:** We developed a model to estimate the health and economic impact of a stockpile of Pneumovax® 23 to prevent secondary bacterial infections among high-risk adults in the age group 18-64 during an influenza pandemic. We used the model to project the number of pneumococcal cases, hospitalizations, deaths, and days of work loss averted. To measure the incremental cost-effectiveness ratio (ICER), we used the cost per quality-adjusted life year (QALY) metric. We used remaining life expectancy of the population following the pandemic as the analytic horizon. Two pandemic scenarios were examined to assess differing pandemic severities: 1918 and 1958/68. We included the impact of other interventions also, such as prepandemic influenza vaccines, antivirals, non-pharmacologic interventions, and herd immunity to pneumococcal disease from use of 7-valent pediatric pneumococcal conjugate vaccine (PCV7). Finally, we examined the impact of Pneumovax® 23 shelf-life, likelihood of a pandemic, and stockpile management on the cost-effectiveness ratio.

**Results:** Under a 1918-type scenario, based on a population of 20 million high-risk adults, a Pneumovax® 23 vaccination program is projected to avoid 185,000 days of work loss, 12,100 pneumococcal cases, 1,690 hospitalizations, and 3,592 deaths. Under a 1958/68 scenario, the Pneumovax® 23 vaccination is projected to avert 40,000 days of work loss, 7,431 pneumococcal cases, 143 hospitalizations and 461 deaths. The cost-effectiveness ratios (ICERs) for the Pneumovax® 23 stockpile compared to no stockpile under the 1918 and 1958/68 scenarios are \$4,661 and \$83,039 per QALY respectively. The ICER for Stockpiling Pneumovax® 23 increases as the likelihood of a pandemic decreases. Given a fixed shelf life of Pneumovax® 23, the proportion of stockpile that can be put in the mainstream use every year also affects the ICERs.

**Conclusion:** Stockpiling Pneumovax® 23 can be a cost-effective strategy for reducing the health and economic burden of pandemic influenza in the high-risk U.S. population.

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**Type: Poster Presentation**

Cost-effectiveness of the use 23-valent pneumococcal polysaccharide vaccine to prevent secondary bacterial infections related to pandemic influenza in Brazil

**M. O'Brien**<sup>1</sup>, P. Dhankhar<sup>2</sup>, J. Grabenstein<sup>1</sup>, E. J. Dasbach<sup>2</sup>

<sup>1</sup>Merck & Co. Inc., West Point, PA, USA, <sup>2</sup>Merck & Co. Inc., North Wales, PA, USA

**Background:** Secondary bacterial infections contributed to a significant number of deaths during prior influenza pandemics, and have also been shown to be associated with mortality during the 2009 H1N1 pandemic. The specific aim of this analysis was to assess the cost-effectiveness of using 23-valent Pneumococcal Polysaccharide Vaccine (PPV23) at the beginning of or during an influenza pandemic to prevent secondary bacterial infections among two cohorts of adults in Brazil.

**Methods:** We created an initial decision model to evaluate the cost-effectiveness of PPV23 used as part of a comprehensive pandemic plan in the United States. This model was adapted to reflect cost data from Brazil. Two cohorts of 6 million Brazilian adults, aged 20-64, were modeled: high-risk adults and critical workers. The model compares costs and disease outcomes associated with two vaccination scenarios: PPV23 vaccination and no PPV23 vaccination. Outcomes are based on attack rates from two prior pandemic types: severe (1918) and moderate (1957/1968). PPV23 effectiveness was assumed to be 59%. Vaccine costs consisted of dose and administration costs. Costs are reported in 2008 Brazilian reals. Cost effectiveness results are reported as incremental cost effectiveness ratios (ICER) using reals per Quality Adjusted Life Year (QALY).

**Results:** Under the assumption that no pandemic influenza vaccine is available, PPV23 could prevent approximately 15,000 pneumococcal disease cases in the high-risk population during a severe pandemic and 9,000 cases during a moderate pandemic. Among 6 million high-risk adults, the ICERs comparing the PPV23 vaccination scenario versus no PPV23 were estimated to be 1,700 reals per QALY for a severe pandemic and 18,000 reals per QALY for a moderate pandemic. Among 6 million critical workers aged 20-64, PPV23 could prevent 64,000 missed work days during a severe pandemic and 17,000 missed work days during a moderate pandemic.

**Conclusion:** The cost effectiveness ratios for mass vaccination of high risk individuals as well as critical workers using PPV23 in Brazil to prevent secondary bacterial infections related to pandemic influenza fall within the range generally considered to be cost-effective; however, the cost-effectiveness ratios depend on the severity of the pandemic.

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Impact study of hepatitis B vaccination in Sikkim - A north eastern state of India

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**Background:** Sikkim is a small state of India which introduced hepatitis B vaccination into its immunization schedule. Sikkim is the first state of India to introduce Hepatitis vaccination in the year 2004. Hepatitis B coverage for the state of Sikkim is approximately 10,000 children per year and immunization is done at 6, 10 and 14 weeks of age. Booster dose has not been incorporated in the immunization schedule.

Since the time of introduction of immunization no impact study has been done in the vaccinated children. Whether the children being vaccinated mount an adequate antibody response against the vaccine needs to be investigated.

An attempt was made to study the anti-HBs levels in a cross sectional study of children who received the vaccination against Hepatitis B.

**Methods:** 160 post vaccinated children and adolescents were included in the study. Only those who had completed three doses of the vaccination were included. Serum collected was tested using a commercially available sandwich ELISA kit. The quality control criteria were fulfilled and the data was analyzed. Anti-HBs Levels below 10mIU/ml were considered as non responders and levels above 10mIU/ml were considered as responders.

**Results:** 53.1% of the participants were males and 46.9% were females. Analysis of data revealed that 64.4% of the population under study were responders and 35.6% were non responders. Female participants who were non responders were greater than males (38.6%: 32.9%). No significant relation between age and response was seen.

**Conclusion:** The percentage of non-responders in the post vaccination children was significantly higher as compared to most studies conducted in other countries. No study has been done in India to see the impact of vaccination in children. Females seem to be non responders as compared to males contrary to some studies. Further studies need to be conducted to investigate the cause of this anomaly. Presuming that India is on the verge of introducing universal vaccination against HBV, preparedness to encounter the problem of non responders to vaccination needs to be addressed and a strategy developed to counter it.

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A vaccine derived poliovirus case in an immunocompromised argentinian child

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**Background:** Although poliomyelitis caused by wild-type poliovirus has been almost eradicated, especially in developed countries, vaccine associated paralytic poliomyelitis (VAPP) cases still continue to occur in Latin American countries. In Argentina, where OPV routine immunization policy is ongoing, the last wild poliovirus (WPV) case was registered in 1984. Since then, the several polio cases reported were VAPP and Vaccine Derived Poliovirus (VDPV) (first iVDPV case was detected in 1998).

**Methods:** To report a polio case caused by a VDPV in an immunodeficient patient diagnosed with polyclonal agammaglobulinemia.

**Results:** In May 2009 a 15 month-old patient was hospitalized in our institution for acute flaccid monoparesis in his left lower limb with areflexia, with residual paralysis 60 days after onset. The patient had a history of recurrent infections (sepsis meningitis and pneumonia). Vaccination calendar was completed for his age (DPt/Hib-HB & OPV: 3 doses). The child received OPV3, 8 months before.

LCR 8 cells / prot 25 mg/100 mL / Glu 50 mg/ml with negative cultures (bacteria - mycobacterium, fungi). EMG showed pre-ganglionic injury. Spine MRI showed focal intramedullary cone and lower thoracic spinal cord with hyperintense signal in T2 left hemicord. The major capsid protein (VP1) of the Sabin virus T1 isolated in feces showed a 96.5% genetic similarity to Sabin 1 vaccine strain (a 3.5% genetic divergence in this region).

**Conclusion:** The occurrence of iVDPVs appears to be very rare; the majority of patients stopped excretion of the virus after a certain period or have died. The overriding factor for the emergence of all VDPVs is the same as for WPV circulation, i.e., low routine OPV coverage rates in children. Thus, a community can become susceptible to the emergence of all types of VDPV producing an impact in the public health of the population. New polio vaccination strategies should be considered to avoid future VDPV cases and to be aligned WHO end- game strategies.

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Influenza vaccine: Immunization rates, knowledge, attitudes and practice of health care workers in Iran in 2008/09

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**Background:** Influenza is an important cause of excessive morbidity and mortality each winter. Its short incubation period and efficient transmission from person to person makes influenza hazardous to the patients and staff in healthcare facilities. The aims of this study were to determine influenza vaccine coverage and evaluate the knowledge, attitudes and practice about influenza and vaccine.

**Methods:** This study was a cross-sectional survey that was performed between October 2008 and February 2009 in 139 health care workers (HCWs) of health deputy of Tehran University of medical science, Tehran, Iran. They received a self-administered questionnaire and then the research assistant waited to collect them.

**Results:** The response rate to the questionnaire was 96.5%. The influenza vaccination coverage for the 2008–2009 seasons was 66.9 % (range, 45% to 62%). Most HCWs (80.6%) had received an influenza vaccination in the past, and 65.4% intended to receive vaccination in the future year. The main reason given for being immunized was effectiveness of influenza vaccine (51.4%). The main reason given for not being immunized was concerned about side-effects (23.1%).

The knowledge score for the 35 items ranged from 0 to 34 (mean 17.37). Mean knowledge scores differed between courses. There was no significant difference in mean knowledge scores between female and male ( $P > 0.05$ )

None of independent variables included age, sex, marital status, having children aged  $\leq 16$  years and courses were significant predictors of taking influenza immunization.

**Conclusion:** In our study, despite high coverage rate of influenza vaccination in comparison with other studies, we would expect higher rate because of free vaccine availability. The study results indicate that there is the need for on-going education of Influenza disease, vaccine action and Centers for Disease Control and Prevention (CDC) recommendation among HCWs to increasing vaccination rates.

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Variable serological response to PPV in HIV-positive patients – A need to review pneumococcal boost-prime strategies?

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**Background:** International guidelines recommend 23-polyvalent polysaccharide pneumococcal vaccination (PPV) for HIV-positive patients with CD4 >200 cells/mm<sup>3</sup>. Historical data shows suboptimal response to vaccination in HIV-positive patients when measured by pneumococcal-specific IgG and vaccine serotype-specific IgG2. There is little data to support the efficacy of vaccination in consistently producing serological response, or assessing the role of conjugate vaccine in adults.

**Methods:** In a cross-sectional study, 82 random samples were taken from HIV-positive outpatients. The samples were assessed for pneumococcal IgG and IgG2. IgG2 levels >69µg/L are considered good serological evidence of response in the absence of baseline titres with which to compare post-vaccination response. Demographic, vaccination and laboratory data was recorded. Results were analysed using Fisher's exact test and two-tailed p values with GraphPad InStat.

**Results:** Four patients were excluded with no prior history of pneumococcal vaccination, and two for incomplete data. The remaining 76 patients (M= 47, F= 29) were aged between 21 and 71 years (mean= 39.1yrs, SD 9.86) at the time of first vaccination. Twenty-six patients (34%) received booster. Sixty-one patients (80%) were taking ART at the time of sampling. Thirteen patients (17.1%) had vaccine serotype-specific IgG2 titre ≥69µg/L at the time of sampling. Achieving an IgG2 titre ≥69µg/L was not associated with any of the measured variables — age < vs ≥ 35 yrs at vaccination (p=0.54); male vs female sex (p=0.54); CD4 count <200 vs ≥200 cells/mm<sup>3</sup> at first vaccination (p=0.58); CD4 count <350 vs >350 cells/mm<sup>3</sup> at first vaccination (p=1.00); CD4 count <350 vs >350 cells/mm<sup>3</sup> at time of sampling (p=0.34); ART at sampling time vs no ART (p=1.00); single vs boosted doses (p=0.19); <234 weeks (4½ years) since vaccination vs ≥234 weeks (p=0.37).

**Conclusion:** In the HAART era only a minority of patients show adequate levels of vaccine serotype-specific IgG2 regardless of time since vaccination or booster dosing. Although immunoglobulin measurements are a surrogate marker of immunity, there is no reliable predictor of whether a HIV-positive patient will mount adequate serological response to PPV. Further studies are needed to assess the nature of response to PPV, alternative conjugate prime-boosting strategies, and the cost-effectiveness of mass-vaccination programmes.

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The generation of immortalized human B-lymphocytes secreting neutralizing monoclonal antibodies against Dengue virus

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**Background:** Dengue is an important re-emerging disease affecting humans in more than 100 countries worldwide. Presently, over 2.5 billion people live in risk areas and 50 to 100 million people suffer from dengue fever (DF) each year. The World Health Organization (WHO) estimates that currently 500,000 cases of Dengue Hemorrhagic Fever / Dengue Shock Syndrome (DHF/DSS) and more than 20,000 deaths occur per year. At the moment, there are no effective vaccines or drugs available to prevent or treat Dengue disease. Chimeric viruses, DNA, inactivated and subunit recombinant vaccines are also of interest but they are still in preclinical development. At present, vaccine may not be available for the next 3-5 years because the complex immune reactions that are involved in dengue immuno-pathogenesis needs further clarification. In this study, we propose a novel method of immortalizing and cloning Dengue virus specific B lymphocytes from convalescent patients secreting neutralizing antibodies. Upon which, DNA from these B lymphocytes were extracted and sequenced. Sequential cloning method was employed to clone in the variable light (VL) chain and variable heavy (VH) chain fragments into the IgG1 framework vector. This recombinant vector was transfected into suspension human embryonic kidney (HEK) 293 cells for transient expression of the dengue-neutralizing antibodies belonging to the various IgG subclasses. The antibodies isolated will prove useful tools for studying the immunology of Dengue virus infections and as possible future therapeutic reagents to prevent virus dissemination in infected individuals.

**Methods:** Ebv immortalization. ELISA. PRNT. Immunofluorescent Microscopy. Molecular Cloning. Antibody purification.

**Results:** Antibody generated.

**Conclusion:** Human antibody responses are important to resolve Dengue infections naturally and the best strategy is to isolate and produce the anti-dengue antibodies that resolve infections in human patients. These therapeutic antibodies have predictable pharmacokinetics properties and display low toxicological risk. A research collaboration involving the National University of Singapore (NUS), Novartis Institute of Tropical Diseases, Singapore (NITD) and Defence Science National Laboratories, Singapore (DSO) aims to produce human monoclonal antibodies (mAb), that can efficiently neutralize the Dengue virus. Each antibody will be fully cloned and expressed recombinantly as IgG class.

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**Type: Poster Presentation**

Factors Contributing to Uptake of the Publicly-funded HPV vaccine in Toronto

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**Background:** In August 2007 the Government of Ontario announced that, for the first time, it was offering the quadrivalent HPV vaccine to grade 8 females in all Toronto schools beginning September 2007. Uptake of the vaccine in the 2007/2008 province-wide program was much lower than anticipated. The provincial HPV vaccination rate was 58% for the first dose, which was lower than other school-based vaccination programs. For a vaccine with such far-reaching impact on a woman's health, this is a disappointing and not well understood outcome. We assessed parental factors for and against the HPV vaccine.

**Methods:** A random sample of parents of grade 8 females in Toronto who were eligible for the publicly-funded HPV vaccine in the 2007-2008 academic session were asked to respond to questions regarding the HPV vaccine program in Toronto. We conducted telephone interviews and used standardized questionnaires to capture data. We conducted bivariate statistics to compare the responses of parents who allowed their daughters to be vaccinated against HPV with those of parents who did not. Multivariate logistic regression statistics was calculated with SPSS to identify the predictive factors that were significantly associated with HPV vaccine uptake.

**Results:** Of the 138 respondents, 75.4% had vaccinated their daughters. Concern over safety of the vaccine (27.3%) and inadequate information (21.2%) were the most commonly reported reasons given by parents to not allow their daughters to be vaccinated. Religious affiliation was not associated with a difference in parental decision to vaccinate. Parents whose daughters were vaccinated were more likely to agree with the importance of vaccination prior to sexual activity onset (OR=10.46, 95% CI: 1.72-63.59, p<0.05) than parents whose daughters were not vaccinated. Parents whose daughters were vaccinated were less likely to agree that vaccination would encourage earlier sexual activity (OR=0.14, 95% CI: 0.02-0.91, p<0.05) than parents who did not approve vaccination for their daughters.

**Conclusion:** Parents need more information about safety and efficacy of the vaccine. Parental attitudes towards the importance of vaccinating before becoming sexually active and how the vaccine relates to sexual activity were the most significant predictors of vaccine uptake. Future public health HPV vaccine campaigns must address these issues.

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Effectiveness of influenza vaccine in elderly people with chronic pulmonary and cardiovascular diseases in Argentina

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**Background:** Influenza immunization proved to be effective in decreasing pneumonia hospitalizations in the elderly and in patients with high-risk conditions. The purpose of this study was to assess the effectiveness of influenza vaccine in persons <sup>3</sup> 65 years with chronic pulmonary and cardiovascular diseases during four immunization campaign seasons in Argentina.

**Methods:** Influenza vaccines containing the strains specifically recommended by the WHO were administered free of charge in immunization campaigns from 1994 to 1999. During campaigns, demographic data and disease status of each subject who was immunized was obtained through interview before vaccine was administered. Subjects with chronic pulmonary (CPD) and cardiovascular disease (CVD) were retrospectively evaluated for hospitalization due to pneumonia during the previous influenza season (June-October;1995-1998) and vaccination status.

**Results:** A total of 176.778 people with CVD and 82.385 with CPD were evaluated. Annual influenza vaccine effectiveness for reduction of pneumonia hospitalization was:

	1995 % (95%CI)	1996 % (95%CI)	1997 % (95%CI)	1998 % (95%CI)
CVD	43 (33-52)	36 (28-46)	29 (18-38)	37 (26-46)
CPD	41 (32-48)	35 (27-41)	27 (19-35)	27 (18-35)

**Conclusion:** Influenza vaccination is associated with a significant reduction in hospitalization rates due to community acquired pneumonia in the elderly population in Argentina, particularly in those with high-risk conditions. Widespread use of influenza vaccine should be promoted.

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Development and immunogenicity of a novel polyetherimine (PETIM) dendrimer based nanoformulated DNA rabies vaccine

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**Background:** Rabies is a fatal but preventable. In the past decade attempts have been made to develop an effective DNA vaccine against rabies. However, the effectiveness of plasmid DNA vaccines is limited due to ineffective intracellular gene delivery. Recently, a group of compounds called dendrimers have been shown to mediate effective gene delivery. In this study we have developed a novel, polyetherimine (PETIM) dendrimer-based nano-formulated plasmid DNA rabies vaccine and evaluated its immunogenicity.

**Methods:** The full length glycoprotein gene of rabies virus was cloned into the expression vector pIRES and its expression verified. Fourth generation amine-terminated polyetherimine (PETIM) dendrimer was synthesized and characterized. PETIM-pDNA complexes were prepared at varying ratios and their size, shape, transfection efficiency and cytotoxicity were studied. *In vitro* transfection experiments were conducted to identify the formulation possessing maximal transfection ability. The ability of the dendrimer to protect the encapsulated DNA was studied by deoxyribonuclease protection assay. The *in vivo* efficacy of the dendrimer-DNA complexes was evaluated by studying the virus neutralizing antibody responses in Swiss albino mice immunized with these formulations, in comparison with those produced by unmodified plasmid DNA.

**Results:** The PETIM dendrimer was found to be non-toxic to cultured mammalian cells upto 1mg/mL. It was also observed to protect the complexed DNA from nuclease degradation. The PETIM-DNA complexes were also observed to produce satisfactory expression of the encoded gene in transfected cells. The animals immunized intramuscularly with the dendrimeric formulations of plasmid DNA produced adequate titres of protective, rabies-virus neutralizing antibodies by 2 weeks post-vaccination, after a single dose. The mean titre of neutralizing antibody was higher (1:512) in mice immunized with the dendrimeric formulations than in the group immunized with the unmodified plasmid DNA (1:16) ( $p < 0.05$ ).

**Conclusion:** The fourth generation polyetherimine dendrimer is a promising nano-carrier for plasmid DNA rabies vaccines. Its utility in immunization of larger animals and humans needs to be evaluated further.

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Factors associated with DPT 1-3 vaccine dropout in Kabarole district, western Uganda

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**Background:** Among the top ten causes of poor health in the district are complications due to vaccine preventable diseases notably diphtheria, pertussis and tetanus (DPT). In 2008, the DPT dropout rate in Kabarole was high (18%). This study assessed the service, community and individual factors associated with DPT1-3 dropout in Kabarole District.

**Methods:** A cross sectional study using cluster sampling was employed. Two clusters at parish level (rural and urban) each from a county in the district were selected by simple random sampling and all villages therein were studied. A total of 230 children (115 from either cluster) were recruited and their parent or guardian interviewed. Cross-tabulations and chi-square tests were used to determine the strength of associations between independent variables and the outcome. Binary logistic regression was done to adjust for potential confounders and identify independent predictors.

Key informant interviews were held with in-charges of health units. Qualitative data was analysed manually using thematic approach and results presented in the form of text.

**Results:** Factors found to be associated with DPT1-3 dropout were; lack of caretaker knowledge about DPT dosage, (adj. OR=8.2; 95% CI: 3.12, 21.53); Child's Birth Order, 6th and above (adj. OR= 3.0; 95% CI: 0.80, 11.05); Child Birth Order 2-3 (adj. OR= 2.2; 95% CI: 0.70, 6.71); Child age group 31-36 compared to 12-18 (adj. OR=2.5; 95% CI: 0.81, 7.84). However, Rural residence (OR= 1.2; 95% CI: 0.56, 2.57); and Child without immunisation card (OR= 4.4; 95% CI: 0.35, 39.86) were not significantly associated with DPT dropout.

**Conclusion:** The current DPT1-3 dropout prevalence in Kabarole is still high but dropping (13.7%). DPT 1-3 dropout is associated with caretaker lack of knowledge of number of dosages a child should receive and involvement of religious leaders, long travel distance to point of accessing transport means, and convenient time for immunisation.

Findings from this study can be used to improve DPT immunisation services. Specific campaigns on DPT immunisation through home visits, involving community leaders and full day immunisation can help further reduce the dropout rate.

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Antibody persistence 10 years after 1st and 2nd doses of 23-valent pneumococcal polysaccharide vaccine (PN23), and immunogenicity and safety of 2nd and 3rd doses in older adults

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**Background:** In a clinical trial in ambulatory older adults, 1st and 2nd PN23 doses induced significant increases in IgG antibody and were generally well tolerated. We re-enrolled trial participants to study 10-year antibody persistence following the earlier doses, and immunogenicity and safety of 2nd or 3rd PN23 doses.

**Methods:** Ten years after receiving a 1st or 2nd PN23 dose, 143 trial participants (age 60-93 years, median 77) were re-enrolled and revaccinated (2nd dose n=72, 3rd dose n=71). Sera obtained before and 30 days postvaccination were analyzed by ELISA for IgG to vaccine serotypes 3, 4, 6B, 8, 9V, 12F, 14, and 23F. Participants recorded all adverse experiences (AEs) through 14 days postvaccination. Serious AEs were monitored through 30 days postvaccination.

**Results:** Ten years postvaccination, geometric mean concentrations (GMCs) in 1st- and 2nd-dose recipients remained higher than prevaccination GMCs in 1st-dose subjects (when they were vaccine-naïve) for all but serotype 3. Second and 3rd doses induced significant increases in GMCs for 8 and 6 serotypes, respectively; GMCs for all 8 serotypes increased in participants < and ≥75 years old. Frequencies of injection-site and systemic AEs were lower after the 2nd than the 3rd dose. Among 3rd-dose recipients, injection-site pain, swelling, and redness were reported by 75%, 39%, and 30%, respectively, while fatigue, body aches, and headache were reported by 38%, 34%, and 25%, respectively. Fever (oral temperature ≥100°F [37.8°C]) occurred in 0% and 6% of 2nd- and 3rd-dose recipients, respectively; the maximum reported temperature was 100.2°F (37.9°C). AEs after either dose were generally mild, and >90% resolved within 1 week. No vaccine-related serious AEs were reported.

**Conclusion:** Although protective levels have not been established for adults, antipneumococcal antibody is known to protect against pneumococcal disease. In ambulatory older adults, 1st and 2nd PN23 doses induced IgG antibody to vaccine serotypes which still exceeded vaccine-naïve levels after 10 years. Moreover, 2nd and 3rd doses administered 10 years after the previous dose were immunogenic and generally well tolerated in those < and ≥75 years old. These findings are consistent with a beneficial effect of 1st, 2nd, and 3rd PN23 doses.

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**Type: Poster Presentation**

Public health approach after detection of an iVDPV case in Argentina

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Health, Buenos Aires, Argentina, <sup>6</sup>Ministry of Health, Buenos Aires, Argentina

**Background:** Argentine has been polio free since 1984 and has a sustained and active surveillance of the acute flaccid paralysis (AFP) that involves an epidemiology and laboratory approach, reaching all the PAHO indicators. In this context we describe the emergence of a case of AFP due to an VDPV in a 15-months old boy with polyclonal agammaglobulinemia.

**Methods:** The case was notified to the National Program, and a stool sample and a throat swab were sent to the Regional Reference Center for Polio Diagnosis. Samples were inoculated in Rd and L20B cells following the new algorithm recommended by WHO. In less than 2 days a virus was isolated in both samples which were characterized as a polio type 1. They were sequenced in the VP1 region, 5'NCR. As a result we found a 3,7% (stool sample) and 3,5% (throat swab) nucleotide difference in VP1 compared with the Sabin strain. These results confirm the presence of an iVDPV1. The sequences of 5'NCR showed the 480 nucleotide change, proving the reversion of the sabin strain to the infectivity. These findings were sent to the National Program in less than 15 days.

**Results:** After the detection of AFP notification was sent to the field, Epidemiology actions were made as in all cases. In the investigation three different locations were established as the child residence. All of them were visited and vaccination of all children under 18 years old was done. A national and international alert were sent Active community surveillance was made and contact and environmental samples were collected and sent to the Regional Lab. In none of them the iVDPV 1 was detected. Four serial samples from the case were taken each month, in all of them the same iVDPV1 was isolated.

**Conclusion:** The country has sustained the surveillance of AFP through 22 years based on the collaborative work between the laboratory and the epidemiologists. No other cases appeared although the vaccine coverage in one of the district was very low. As consequence of this finding a national vaccination campaign was made. Although poliomyelitis is a threat to the region Argentina is ready to face it.

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**Type: Poster Presentation**

Yellow fever vaccine (YFV) and events supposedly attributable to vaccination or immunization (ESAVIs): Argentina's experience

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**Background:** The acronym ESAVI defines any clinical picture after vaccination chronologically related to its use. Further analysis of the event determines the role of the vaccine in its causality. In the case of YFV, three categories of severe adverse events are described: anaphylactic reactions, YF neurotropic disease (YFV-AND), viscerotropic disease (YFV-AVD). YFV is included in Argentina's national immunization program for use in population older than one year of age in regions with transmission risk. It is also prescribed to travelers to endemic zones and can be required upon International Health Regulation allowance. We describe the clinical, epidemiological and laboratory profile of ESAVIs in the context of an extraordinarily increased YFV administration in Argentina in 2008, due to reported fatal cases involving humans and monkeys in risk zones.

**Methods:** This is a descriptive study encompassing the period between January and December, 2008. Complete YFV-ESAVI forms were included, after the expert committee evaluations. Adverse events were grouped using current PAHO/WHO classification. Samples (serum, CSF and liver biopsies) were processed at the INEVH through standard techniques. Vaccine shots: 1,806,400.

**Results:** Fifty ESAVIs were included:

Classification	Mild-Moderate	Severe
<b>1</b>	12	2
<b>2<sup>a</sup></b>	-	-
<b>2<sup>b</sup></b>	23	9
<b>3</b>	-	1

The 2b severe ESAVIs consisted of eight YFV-AND and one YFV-VD, whereas the two severe type 1 ESAVIs consisted of one urinary sepsis and a sepsis-like case without final diagnosis. The type 3 ESAVI was an ADEM.

Neither reactions nor programmatic errors were reported.  
YFV-VD rate was 0.5/1.000.000 doses; YFV-AND 4.4/1.000.000 doses.  
No particular vaccine lot was related to ESAVIs.

Global incidence of ESAVIs coincides with the heretofore published data. However, some of the authors knew of more clinically compatible YFV-AND and VD non-studied cases, and there is strong suspicion of underreporting.

**Conclusion:** An accurate surveillance system and a reference laboratory are fundamental for ESAVIs study.

Detailed reports for valid conclusions and opportune actions, plus a multidisciplinary work for rigorous analysis is needed.

A carefully managed risk-benefit balance when prescribing YFV alongside with updated epidemiological information for accurate guidance is critical.

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**Type: Poster Presentation**

Genetic characterization of *Mycobacterium bovis* BCG Mexico 1931

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**Background:** BCG vaccine is the only preventive measure against tuberculosis. At least two genomes from BCG, Pasteur and Japan, have been described. Evolutionary schemes establish by DU2 and other markers situated BCG Japan and Pasteur into group I and IV from genealogy of BCG vaccines, respectively, classified as early and late strains. Some BCG such as Mexico 1931 is not included in any comparative studies based on phenotypic, genotyping, immune response and drug resistance. Our aim was to characterize by full sequence BCG Mexico 1931 for using in the new develop vaccine.

**Methods:** The sequence of BCG Mexico 1931 was performed with 2X pyrosequencing method. BCG Mexico 1931 genetic profile was performed by multiplex PCR and PFGE. The multiplex PCR was performed to detect RD and DU regions in strains. The reference BCG strains used in this study were Pasteur 1173P2, Phipps, Tice and Danish 1331.

**Results:** The circular chromosome at 99.8% coverage of BCG Mexico 1931 was 4 350 386-bp, its analyses confirm the RD1, RD2, nRD18 absence and the presence of duplication DU2 group IV described by multiplex PCR. The comparison of BCG Pasteur1173P2 genome sequence with BCG Mexico 1931 showed differences between strains, including the presence of RD14, SNPs and the absence of DU1. In addition, three new RD regions of 53, 655 and 2847 bp were uncovered.

The comparison of profiles obtained by PCR multiplex showed that BCG Mexico 1931 profile was identical to BCG Phipps and Tice, with the lost of RD1, RD2, nRD18 and presence of DU2 group IV. PFGE was performed with *AsnI/DraI* enzymes, the analyses detected 18/12 fragments in BCG Danish 1331 and Mexico 1931, 17/12 in Pasteur 1173P2, 15/10 in Phipps and 14/10 in Tice, respectively. Interesting, the restriction pattern with *AsnI* showed the presence of 220kb fragment only in BCG Mexico, Tice and Phipps.

**Conclusion:** The BCG Mexico 1931 showed a close relation with BCG Phipps and Tice, strains situated into group IV from genealogy of BCG vaccines. However, in diverse studies these strains shown differences on the induction of immune response.

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**Type: Poster Presentation**

Pneumococcal vaccination - is hyporesponsiveness a problem?

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**Background:** Several studies in the past have demonstrated that the pneumococcal polysaccharide vaccine (PPV 23) will induce hyporesponsiveness in vaccinated patients over a long time period. The duration of this effect and the impact on a second vaccination with a conjugated vaccine (PCV 13) after at least 3 years is not known.

**Methods:** To address these open questions we investigated 55 persons (30 males, age between 68 and 87 years), who had a first PPV 23 - vaccination 3 - 8 years before and received a second vaccination with PCV 13. Immediately before and 4 - 6 weeks after PCV 13 vaccination blood specimens were taken and antibodies to 13 individual polysaccharides (1,3,4,5,6A,6B,7F,9V,14,18,19A,19F,25F) were determined by a validated enzyme-linked immunosorbent assay.

**Results:** 3 - 8 years after PPV 23 vaccination antibody concentrations were low but in 11 out of 13 serotypes still above 1,0 µg/ml (the assumed protection level for adults); only serotypes 3 and 23F had mean concentrations (+/- SD) of 0,66 +/- 0,38 µg/ml and 0,57 +/- 0,39 µg/ml. 4 - 6 weeks after vaccination with PCV 13 all serotypes showed a significant increase in antibody concentrations; however, a great response variability between the different serotypes was measured with small antibody increases in serotype 3 and high concentrations in serotypes 4, 5, 7F, 19A and 19F. The tolerance of vaccination with PCV 13 was excellent.

**Conclusion:** The results of this study demonstrate that a first PPV 23 vaccination has only limited impact on a second vaccination with a new conjugated pneumococcal vaccine (PVP13) 3 - 8 years later. Hyporesponsiveness was not a major problem in this study. However, these results have to be confirmed in a study with a greater number of elderly patients.

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**Session: Virology and Viral Infections (Non-HIV)**  
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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Intravenous immunoglobulin manufactured from selected Chinese donors protects mice from lethal Enterovirus 71 infection

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**Background:** Enterovirus 71 (EV71) has emerged as a significant cause of acute encephalitis and paralysis in China, resulting in hundreds of deaths in young children since 2006. Passive transfer of intravenous immunoglobulin (IVIG) from Chinese donors has been associated with reduced mortality in children with severe EV71 infections. The anti-EV71 neutralization antibodies in Chinese IVIG products may contribute to the protection from EV71 infection.

**Methods:** A modified high-throughput microneutralization assay was performed to screen for plasma units containing high titer (>100) anti-EV71 antibodies from Chinese plasma donors. Positive units were then pooled and processed into pharmaceutical grade EV71-specific IVIG (EV71-IVIG). Different doses (0.1 to 5 mg) of EV71-IVIG and commercial IVIG were administered following lethal EV71 infection in suckling mice, respectively, according to the specific experimental protocol. Mice were monitored daily for body weight and mortality.

**Results:** About 10% of the plasma units from Chinese plasma donors were selected to produce EV71-IVIG. *In vitro* neutralization assays showed that the anti-EV71 antibodies titer (>1024) in EV71-IVIG preparations was 10-fold higher than that in commercial IVIG preparations. Full protection was archived when the infected mice were treated with EV71-IVIG, while similar treatments using commercial IVIG had no protective effect. Recovery of the infected mice was dependent on the dose and time of IVIG administration.

**Conclusion:** These results demonstrated that IVIG with higher titer (>1024) neutralization antibodies conferred protection against lethal EV71 challenge in an animal model. This finding also suggested that blood from selected donors in EV71 endemic regions can improve the potency of IVIG and EV71-IVIG should be developed for the treatment of fatal EV71 infections.

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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Polio controlling Nigeria and other developing countries: A systematic review/meta-analysis of literature on the use of combined OPV & IPV measure as against OPV only

**S. I. EZIKEANYI**

Federal Ministry of Health Abuja, ABUJA, Nigeria

**Background:** Despite the overwhelming evidence-based studies across the world on the efficacy and effectiveness of poliomyelitis eradication using the combined OPV & IPV immunization schedule for children 0-59 months, Nigeria still adheres to her policy on monovalent 'Sabin' live attenuated oral polio vaccine (mOPV1) that is only targeting the type 1 strain among the three strains of the virus. The question here is what informs Nigeria health policy makers' decision to continue with the current policy that started since 2006 despite the persistent circulating Wild Polio Virus (WPV) that has had its toll on children and drains all efforts of achieving the MDGs 4 of reducing Child morbidity and mortality come 2015.

Empirical evidence from a number of countries suggests that a policy on combined Sabin live attenuated oral polio vaccine (OPV) and Salk killed inactivated polio vaccine (IPV) in polio control program by improving protection of children at individual levels and at community level by conferring herd immunity can make a major contribution to poliomyelitis control and possible eradication of the persistent circulating wild polio strain and its consequent disability and mortality.

**Methods:** A systematic literature review methodology was used to identify relevant publications that reported the use of OPV & IPV in the control of polio. Databases were searched using different search terms. Of the 100 publications initially selected, 38 met the inclusion criteria and had evidence level 1 and 2 ranking.

**Results:** The research revealed a remarkable reduction in polio incidence in those countries. OPV & IPV was found to reduce polio incidence or outbreak by 85%-90%, Acute Flaccid Paralysis (AFP) by 85% and cost per child of 5\$ for full immunization coverage.

**Conclusion:** OPV & IPV combination has been shown to be more effective than OPV alone and very potent in the eradication of persistent strains of the virus. The author recommends the adoption of OPV & IPV by Nigeria as the main dynamics of driving the polio immunization program towards elimination or even total eradication of the wild circulating polio virus in Nigeria and other neighboring sub-Saharan African countries.

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**Type: Poster Presentation**

Factors associated with outbreaks of measles in the highly immunized hilly areas of district Kangra, Himachal Pradesh, India, 2007

**S. N. Gupta**<sup>1</sup>, V. ramachandran<sup>2</sup>, N. N. Gupta<sup>3</sup>, M. D. Gupte<sup>2</sup>

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**Background:** Measles is the fifth killer disease among children under five years in the world. In Indian states, we are in the heterogeneity of measles vaccination. In Himachal Pradesh, despite high immunization coverage, the outbreaks are occurring. Based upon two outbreaks, we conducted a case control study to identify factors associated with outbreaks of measles and to recommend appropriate remedial measures to prevent further outbreaks.

**Methods:** We reviewed the factors under three heads: *programme related, health care providers and beneficiaries related*. We used case-definition of measles adapted by the WHO. We enrolled all 69 mothers of children with measles. Controls were matched for age and sex. We used a pre-designed pre-tested data collection instrument. We calculated the odds ratio and adjusted odds ratio with 95% Confidence Interval (C.I.) among women of children exposed and unexposed to selected characteristics.

**Results:** Only beneficiary related issues were associated with outbreaks in case area which were indicated by Focus Group Discussions and further confirmed during in-depth interviews in quantitative univariate analysis yielding statistical significance. *Important socio-economic variables* are Educational Status yielding to OR 27.63 (95% C.I. 9.46-85.16); Occupation with OR 0.35 (95% C.I. 0.16-0.75); Income with OR 5.49 (95% C.I. 2.36-13.00); mode of transport to the health care facility; OR 8.74 (95% C.I. 2.90-28.23); First help contact when the child falls ill OR 2.12 (95% C.I. 1.00-4.50). Knowledge about the child illness in the family, OR 5.03 (95% C.I. 0.95-35.17); Spread of illness from one person to other person OR 5.60 (95% C.I. 1.40-25.97) and Person for treatment, OR 2.85 (95 C.I. 1.27-6.46); Place of visit after recovery from the measles, OR 3.92 (95% C.I. 1.80-8.63). *Multiple Logistic Regression* yielded the significant variable as-educational status with AOR 438.72 (95% C.I. 17.0-11272.16).

**Conclusion:** Measles outbreaks were confirmed in high immunization coverage areas. We recommend (i) 2nd dose opportunity for measles (MMR) between 5 to 17 years; (ii) Improving access to health care facility and (iii) IEC activities for social behavioral change rather than informing the community.

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**Room: Poster & Exhibition Area/Ground Level**  
**Type: Poster Presentation**

Outbreak investigations of double measles in a two highly immunized hilly areas of district Kangra, Himachal Pradesh, India, 2007

**S. N. Gupta**<sup>1</sup>, V. ramachandran<sup>2</sup>, N. N. Gupta<sup>3</sup>, M. D. Gupte<sup>2</sup>

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**Background:** Measles is the fifth largest killer disease among children in the world. In September, 2006, a community leader/worker informed us about an increase in cases of fever and rash in some hilly villages of district Kangra. We investigated two sequential outbreaks of measles to confirm the diagnosis and to formulate recommendations for prevention and control.

**Methods:** We defined a case of measles as occurrence of fever with rash in a child aged six months to 17 years during 3rd September to 23rd November, 2006. We line listed cases and collected information on age, sex, residence, date of onset, symptoms, signs, traveling, treatment history and vaccination status. We described the outbreak by time, place and person. We estimated vaccine coverages and vaccine efficacies in the affected villages. We confirmed diagnosis clinically, serologically and through genotyping of the virus.

**Results:** We identified 69 case patients. Overall attack rates ranged between 4.2% and 6%. All cases were between 6 to 17 years. Age specific attack rate in 1st outbreak ranged between 1.7% to 13% in 6-15 years while in 2nd outbreak; it is 2.2 to 17.3%, highest in 11-17 years. No deaths or complications were reported. The epidemic curve was suggestive of typical propagated pattern. The 1st outbreak imported virus after an inter school game competition (Relative risk: 6.44%; 95% confidence interval: 3.81 – 10.91) followed by 2nd outbreak people exchanged foods in the festival in one infected village of 1st outbreak (Relative risk: 5.3; 95% confidence interval: 1.90 – 14.77; P <0.001). The calculated immunization coverage (93%) coincided nearly with administrative claims. We estimated vaccine efficacies to be 85% and 81% in 1st and 2nd outbreaks. 11/16 case-patients for measles IgM antibodies and 2/5 nasopharyngeal swabs were tested positive by PCR and D4 measles strain genotyped. Vitamin A supplementations were only in four villages.

**Conclusion:** Measles outbreaks were confirmed in high immunization coverage areas. We recommended second dose opportunity for measles and vitamin A supplementation to all the cases in Himachal Pradesh.

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Higher prevalence of norovirus than rotavirus as cause of acute gastroenteritis in hospitalized infants of Concepcion, Chile

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**Background:** Norovirus (NoV) are the main cause of acute gastroenteritis (AG) worldwide because of their contagiousness, low infectious dose, environmental stability, rapid secondary transmission and genetic variability. In Chile, the role of rotaviruses as causative agent of AG in children is well established but the prevalence and clinical significance of Norovirus is unknown, because its clinic similarity with rotavirus and lack of diagnosis methods. We investigated the prevalence of NoV and Rotavirus (RV) in children with AG.

**Methods:** We collected stools samples from Dec.07 to Dec. 08 from 145 hospitalized children in the Regional Hospital GGB of Concepcion. As controls, we collected stools from 57 healthy infants from a day care center. A 71% of children with AG were infants (103) and 42 were children 2-14 yr old. For detection and typing of NoV genogroups GI and GII, we used a RT-PCR with 3 specific TaqMan probes. RV presence were determined with a commercial VIKIA™ "Rota-Adeno"kit Biomerieux.

**Results:** In 103 infants we found 34% of NoV and 17.8% of RV. A 48% of them were hospitalized for AG and 52% were initially hospitalized for other causes. In the first group we found 32.7% NoV and 24.4% RV. The second group presented 35.2% NoV and 13% RV. In children 2-4 yr old we found 9.5% of NoV and 11.8% RV. Children older than 4 yr old were negative for NoV and had 12% of RV. Only 2 healthy infants were positive for NoV (3.5%). Summer prevalence was the highest for RV at 27.3% and the lowest for NoV at 3%. Average prevalence was 48.5% for NoV and 13.4% for RV.

**Conclusion:** Main cause of AG in hospitalized infants in Concepcion was due to NoV. Prevalence of infection caused by NoV greatly exceeded that of RV, except in Summer. Younger children, less than 2 year old suffered most for NoV infection as compared with RV infection that equally affected all age groups. Norovirus were more likely to facilitate nosocomial infections (35.2%) as compared with Rotavirus (13%).

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**Type: Poster Presentation**

Lessons that human virology can acquire from studies on avian circo- and tumor viruses

**I. Davidson**

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**Background:** Economically-poultry-significant avian viruses can also provide scientific insights in un-experimentable human topics. Virological studies in poultry reflect natural phenomena, as they replicate in commercial flocks, causing natural infections, natural stress, large populations, allowing rare events understanding.

This presentation will review long-term studies on oncogenic viruses (1) and on circovirus infections (2) elaborating on human infections with similar virus-families.

1. Davidson, I. & Silva, R.F. (2008). *Virus Genes* 36: 1-10.

2. Davidson, I. & Shulman, L. M. (2008). *Virus Research* 137: 1-15.

**Methods:** Molecular integration were assessed by the detection of chimeric molecules in vivo.

**Results:** Avian tumor viruses include one herpes- and four retroviruses. Molecular recombination between DNA and retroviruses was created in vitro, resulting in a recombinant MDV with altered properties (Drs. Kung and Witter, USA).

We now questioned multiple-virus-infections in commercial flocks, examining whether inter-viral molecular recombinations occur also in vivo, and found 25% double-virus-infected commercial flocks and 5% samples with molecular integrations. Spontaneous inter-viral recombination occurred also between retroviruses in commercial birds, emerging in the avian leukosis-subgroup-J, that caused great economic losses. Avian tumor viruses could provide animal models to human dual infections with herpesviruses and retroviruses.

We also reviewed similarities between human Anellovirus and avian *Circoviridae*, to examine whether knowledge acquired from studies of natural and experimental avian infections with could reflect on human Anelloviruses.

**Conclusion:** Studies on avian circoviruses, specifically chicken anemia virus (CAV) can add to current understandings on Anellovirus infections, directed towards finding associated diseases. The health burden imposed by *Circoviridae* and Anellovirus infections may be underestimated because lack of awareness for search beyond the predominant clinical effects of identified pathogens. Their immunomodulatory contribution by co-infecting *Circoviridae* and, by analogy, human Anelloviruses necessitates consideration.

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Optimization of IgG-ELISA and molecular analysis of Reston-ebolavirus among swine in Northern Luzon, the Philippines

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**Background:** In late 2008, Reston-ebolavirus (RES) in swine was first reported in the world from 2 provinces in the Philippines, and those were also affected with Porcine Respiratory and Reproductive Syndrome (PRRS). The aims of this study are 1) to establish the detection of anti RES IgG by ELISA and 2) to analyze the extent of transmission and spread of RES in swine in the affected farm.

**Methods:** Swine samples collected in Bulacan Province and Pangasinan Province, where the RES infection was reported, were examined in this study. 1) From the lymph node of RES infected swine, RES-Nucleoprotein (RES-NP) and RES-Glycoprotein (RES-GP) gene were amplified and nucleotide sequences were determined. 2) RES-NP and GP were expressed in insect cells by *recombinant baculovirus* and then purified. IgG-ELISA was compared with different antigens: purified recombinant RES-NP and GP purified recombinant Zaire Ebola (ZAI)-NP, RES-infected cell antigens (authentic-RES, prepared by US-CDC), ZAI-infected cell antigens (authentic-ZAI, prepared by US-CDC). Immunofluorescent (IF) test using Hela cells expressing the recombinant RES-NP, GP and ZAI-NP were also conducted.

**Results:** 1) Multiple mutations were detected in *variable region* of GP, compared with the RES from the monkeys in 1989, 1992 and 1996. 2) IgG-ELISA using purified recombinant RES-NP, GP and authentic RES showed the highest sensitivity, followed by ZAI-infected cells and lowest with purified recombinant ZAI-NP. The serum samples being positive in IgG-ELISA with RES-NP and GP were confirmed as such in IF test. Approximately 20% of the swine serum from Bulacan Province showed positive.

**Conclusion:** It is still unclear if RES is pathogenic in swine and how PRRS is involved in infection and spread of RES among swine. Further seroepidemiological survey in swine in other farms is still necessary to reveal the actual situation of RES in the Philippines. RES antibody detection system will be very useful in augmenting the RES detection systems currently available in the Philippines.

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A newly discovered viral enzyme capable of alteration of nucleic acid structure via phospho-triester and phosphodiester bonding complex: An event leading to a new frontier of research and development for viral diseases

**D. Pan**

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**Background:** Viruses are interesting because many cause serious illness in humans, animals, and damage crop plants. During the last century, progress in the control of infectious disease through using new vaccines and drugs have reduced the threat to human. The advance of new knowledge/ technology relevant to viruses provide a better way to control viral diseases. We report a newly discovered virus associated enzyme capable of altering nucleic acid structure through the formation of phospho-triester/phosphodiester bonding.

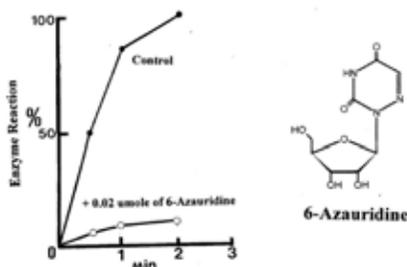
**Methods:** Enzyme was partially purified from plant/animal sources by combining (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> Fractionation, Gel Filtration, Ion Exchange Chromatography. Virions were gifts from laboratories of the following professors: Roland Rueckert (poliovirus and influenza virus); Paul Ahlquist (Brome mosaic virus), Molecular Institute of Virology; Thomas German (Southern Bean mosaic virus), Department of Entomology; Virginia Hindshaw (avian virus), and Mouse retrovirus from the late Prof. Howard Temin, UW; and University of Laval, Canada, respectively. The phospho-bonding complexes were determined with phosphodiesterase/phospho-triesterase, and by gel filtration of molecular mass.

**Results:** The enzyme:

- was widely distributed in animal, plant tissues, and in microorganisms.
- was associated with poliovirus, influenza virus, brome mosaic virus, avian virus, southern bean mosaic virus and mouse retrovirus
- 6-azauridine inhibits enzyme activity and the infectivity of influenza virus on HeLa cells.

**Conclusion:** - An evolutionary significant enzyme.

- We speculate the enzyme is associated with the following viral families: Flaviridae, Herpesviridae, Coronaviridae, Picornaviridae, Papillomaviridae and Nipahviridae to name a few.
- 6-azauridine inhibits on soluble form and virus bound enzymes (Fig.3).
- This investigation has been carried out on/off over two decades in Department of Genetics, UW-Madison, US.
- This presentation is dedicated to remember the late professor Oliver E. Nelson, member of National Academy of Science.
- We are anxiously looking forward to collaborate with any laboratory, institution, organization and industry in worldwide to demonstrate quickly and precisely the existence of this enzyme on surface of virions, i.e; mump, smallpox, yellow fever, rubies, rubella, particularly HIV and H1N1 viruses and many others. Since we believe undoubtedly that the results will contribute to understanding the prevention, treatment and infectivity of viruses



**6-Azauridine inhibits soluble enzyme activity**

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Infectious mononucleosis in Albanian children

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**Background:** To study some of the epidemiological data, clinical manifestations, diagnosis and treatment of the children hospitalized in the pediatrics infectious diseases ward.

**Methods:** In the study are included 47 children from 1-4 years hospitalized in Pediatrics Infectious Diseases Ward during 2003-2008. From the childrens cards we studied important data as: distribution according to age group, gender, residence, season and important clinical manifestation such as fever, fatigue, pharyngitis, generalized lymphadenopathy and splenomegaly. Confirmation of the diagnosis is done by specific serological tests (antibody anti-EBV).

**Results:** According to age group 1-4 yr 30 cases (64%), 4-8 yr 11 cases (23%), 8-14 yr 6 cases (13%); gender male 35 cases (74%), female 12 cases (26%); residence city 36 cases (76%), village 11 cases (24%); season spring 12 cases (25%), summer 6 cases (13%), autumn 11 cases (23%), winter 18 cases (39%). Clinical manifestations: fever 47 cases (100%), Pharyngitis (hyperemic exudativ) 47 cases (100%), lymphadenopathy 37 cases (73%), cervicolateral predominantly 25 cases (68%), splenomegaly 15 cases (32%), hepatomegaly 7 cases (15%). In 85% of cases was found leucocytosis (10000-26000 cel/mm<sup>3</sup>), atypical lymphocytosis in peripheral blood (>10%), in 37 cases (79%) and elevated SGPT. Specific serological tests for anti body anti-EBV (anti-VCA-IgG and anti-VCA-IgM) were positive in all cases. 12 cases (25%) resulted with pulmonary infections, 5 cases (10%) with urinary tract infections and 3 cases (6%) with biliary tract infection.

**Conclusion:** Infectious mononucleosis is a clinical syndrome caused by Epstein-Bar virus which is not rare and must be considered in pediatric population. This syndrome expressed clinically by the triad; pharyngitis, lymphadenopathy and splenomegaly must make us suspect the diagnosis of infectious mononucleosis which is confirmed by specific serological tests. Therapy with corticosteroids for short periods (<2 weeks) is indicated only in complications as airway obstruction, massive splenomegaly, myocarditis, hemolytic anemia, seizure, meningitis. Therapy with acyclovir decreases viral replication and oropharyngeal shedding but does not reduce the severity or durarion of symptoms

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**Type: Poster Presentation**

Symptomatic, long-term Parvovirus B19 infection in otherwise healthy adults

**R. Manfredi**

University of Bologna, Bologna, Italy

**Background:** Parvoviridae are part of air-, parenteral- and perinatal-transmitted ubiquitous viruses, whose associated signs and symptoms strongly depend on patient's age and immune defence.

**Methods:** All cases of symptomatic Parvovirus B19 infection in otherwise healthy adults which came to our attention since spring 2006 were prospectively investigated and followed-up.

**Results:** In a 21-month period, 11 patients (7 females and 4 males), with a mean age of 36.9 (range 27-46) years with a symptomatic Parvovirus B19 infection were recorded and followed-up. Intrafamilial exposure and occupational (health care) exposure were identified in two cases each. Clinical signs and symptoms included fever (100% of cases), polyarthralgia (90%), followed by headache (80%), anemia (70%), and rash. A mild-to-moderate myelosuppression of all hematological lines characterized 8 cases of 11 (72.7%), while increased serum transaminases were associated in 63.6% of patients. Three patients of ours deserved hospitalization (mean 10.8 days of admission), and five more cases were followed on Day-Hospital basis (for a median 75-day period); in four patients a treatment with i.v. high-dosage human immunoglobulins was performed. Elevated levels of specific serum anti-Parvovirus B19 IgM antibodies were detected in all cases. In a 33-year-old female a severe anemia and a persisting headache, vomiting, and neck stiffness, led to RBC transfusion and a diagnosis of meningoencephalitis, with positive search of IgM antibodies and Parvovirus B19 viremia (detected by RT-PCR) in the cerebrospinal fluid which lasted up to three months, despite treatment with i.v. serum immunoglobulins.

**Conclusion:** Parvovirus B19 infection may play a significant role also in adult, immunocompetent subjects, and the disease sometimes is not mild and self-limiting, requiring admission and/or frequent outpatient interventions in a significant number of cases. The causes supporting a persistent infection in immunocompetent subjects have not been investigated to date, as well as the pathogenesis of myelosuppression and severe polyarthralgia. Symptomatic Parvovirus B19 infection is still an underestimated clinical condition, and therapeutic perspectives are extremely limited until now.

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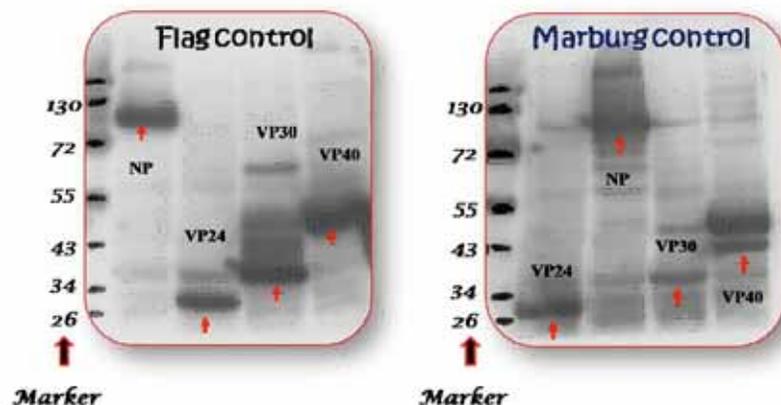
Detection of *Sudan ebolavirus* (strain Gulu) epitopes that are targets of the humoral immune response in survivors

**A. sobarzo**<sup>1</sup>, A. Groseth<sup>2</sup>, O. Dolnik<sup>2</sup>, S. Becker<sup>2</sup>, J. J. Lutwama<sup>3</sup>, V. Yavelsky<sup>1</sup>, R. S. Marks<sup>4</sup>, L. Lobel<sup>1</sup>

<sup>1</sup>Ben-Gurion University of the Negev, Beer Sheva, Israel, <sup>2</sup>Institute of Virology, Philipps-University Marburg, Marburg, Germany, <sup>3</sup>Uganda Virus Research Institute, Entebbe, Uganda, <sup>4</sup>Ben-Gurion University of the Negev, Beer-sheva, Israel

**Background:** Epidemics and pandemics have had a great impact on the course of human history. As such emergence and re-emergence of diseases of high risk to the individual, and/or the community, are of specific interest and concern to public health systems, whether they be in developed or developing countries. Ebola viruses represent prime examples of such emerging pathogens. Ebola outbreaks are unpredictable, with high severity and fatality rates. This high case fatality rate of Ebola virus infection and the lack of approved vaccines and therapeutics resulted in classification of Ebola viruses as biosafety level 4 pathogens and potential bioweapons by the WHO and the CDC. The main goal of this work is to identify epitopes within the viral proteins of *Sudan ebolavirus* (strain Gulu) that are targets of the humoral immune response in survivors. Epitope specific antibodies are important indicators of survival from this deadly disease and can serve as templates for the development of synthetic or vectored vaccines

**Methods:** Using standard cloning techniques the *Sudan ebolavirus* (strain Gulu) genes for all seven structural proteins were cloned and sequenced. For viral protein 24 (VP24), 30 (VP30), 40 (VP40) and NP further expression constructs were generated containing a Flag tag epitope for detection. These proteins were expressed in 293T cell using standard techniques and transfection efficiency was controlled by using a VP40-GFP fusion protein construct. Following expression, viral proteins were isolated and tested by western blot analysis.



**The expression of the recombinant viral protein of Ebolavirus (strain Gulu) by western blot (WB) analysis**

**Results:** Initial results comparing immunoreactivity between survivor and non survivor serum samples have revealed different recognition patterns to the individual viral proteins; VP24, VP40 and NP.

**Conclusion:** Identification of specific targets of the successful adaptive immune response during Ebolavirus infection will hopefully be useful for development of next generation diagnostics and development of synthetic or vectored vaccines.

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Full genome sequence analysis of Group B human rotaviruses

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**Background:** Rotavirus is a major causative pathogen of severe diarrhea in humans and animals. On the basis of VP6 antigen, Rotaviruses are classified into seven groups (A-G), among which only groups A-C rotaviruses cause infection in humans. Group B rotavirus (GBR) was first detected in China in 1982 as a cause of adult diarrheal outbreaks. Although the detection of GBR had been limited in China, GBR has been found in India since 1998, in Bangladesh since 2000, and in Myanmar in 2007. Because of limited data, genetic characteristics of GBR have not been well known so far.

**Methods:** GBRs detected recently in India (IDH-084 in 2007, IC-008 in 2008), Bangladesh (Bang117 in 2003), and Myanmar (MMR-B1 in 2007) were analyzed genetically. Full genome sequences of these strains were determined by RT-PCR and direct sequencing methods. Sequence data was analyzed phylogenetically by MEGA4 program with those of GBRs reported previously.

**Results:** Sequences of all genes of GBRs, including those of animals, were classified into three clusters, i.e., Chinese lineage, India-Bangladeshi-Myanmar lineage, and animal (bovine and murine) lineages. Each strain showed high sequence identity among the same lineage (e.g., 95.6-100% among India-Bangladeshi-Myanmar lineage). The evolutionary rate of all segment genes of GBRs was estimated to be  $1.89-2.05 \times 10^{-3}$  nucleotide substitutions per site per year, using the synonymous substitutions between CAL-1 (1998 in India) and IDH-084, CAL-1 and IC-008, and Bang373 (2000 in Bangladesh) and Bang117.

**Conclusion:** Full genome sequences of recent group B human rotaviruses were determined and revealed the presence of two major lineages in human GBRs by phylogenetic analysis. Compared to the strains detected in different years, the substitution rate was estimated for the first time for all the gene segments. It was similar to those from partial sequence data reported previously and was comparable to the rate of other rapidly evolving RNA viruses. Further accumulation of genetic data is needed for resolution of ecological features of group B rotaviruses.

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**Type: Poster Presentation**

Diagnostic criteria for *Herpes simplex* encephalitis: 5 year analysis in Mofid Children's Hospital  
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<sup>1</sup>Pediatric infectious Research disease, Tehran, Iran, Islamic Republic of, <sup>2</sup>Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of, <sup>3</sup>PIRC, tehran, Iran, Islamic Republic of

**Background:** To define the criteria for diagnosis of Herpes Simplex Encephalitis, (HSE), in children.

**Methods:** Charts of all patients, admitted to Mofid Children's Hospital in Tehran, Iran, from 1999 to 2005, with clinically suspected Herpes Simplex Encephalitis or meningo-encephalitis were reviewed. We documented relevant variables, including demographic data, signs and symptoms at presentation, and laboratory investigations such as CSF analysis, PCR and the results of neuro-imaging. Patients were categorized into three groups according to the diagnosis of HSE being definite, probable or possible. Diagnosis was defined as 'definite' in the presence of clinical symptoms compatible with HSE and detection of the pathogen, or antigen-detection from the cerebrospinal fluid by PCR. Diagnosis was designated as 'probable' in the presence of clinical symptoms of HSE and abnormal analysis of CSF, or abnormalities in neuro-imaging compatible with HSV infection.

Clinical symptoms compatible with HSE with normal CSF, or clinical manifestations not characteristic of HSV infection but with abnormal analysis of CSF, were placed in the category of 'possible' HSE.

**Results:** According to above criteria, definite HSE was diagnosed in seven; probable HSE in nine, and possible HSE in two patients.

**Conclusion:** We think every patient who has encephalitis with symptoms suggestive of HSE, should be graded into one of the following diagnostic categories: definite, probable, or possible HSE; otherwise the diagnosis should be rejected. This classification would help the clinician in decision-making as regards treatment with Acyclovir. We recommend a multicenter epidemiological study to confirm the efficacy of these criteria.

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Problems Associated with Community-Based Parenteral Anti-Infective Therapy with Intravenous Ganciclovir for *Cytomegalovirus* Prophylaxis in Allogeneic Hematopoietic Stem Cell Transplant Recipients

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**Background:** Our center utilized Cytomegalovirus (CMV) prophylaxis with intravenous ganciclovir (IV GCV) in allogeneic hematopoietic stem cell transplant (allo HSCT) recipients starting from engraftment to day +100. Most of this regimen was administered as community-based parenteral anti-infective therapy (CoPAT). Surveillance CMV DNA detection by hybrid capture assay was done weekly through day +100 and at varying intervals thereafter.

**Methods:** Retrospective review of the CoPAT database and electronic medical records to evaluate adherence to this protocol. Events were followed for 6 months from HSCT.

**Results:** 66 patients underwent allo HSCT from 5/07 to 6/08. Demographics were similar in patients who received any prophylactic IV GCV (21 [32%]) and those who did not (45 [68%]). 36 patients did not receive CoPAT for appropriate reasons: CMV IgG donor negative & recipient negative (19), delayed engraftment (5), relapse of underlying disease (5), death within 100 days of HSCT (5), and refractory disease (2). Protocol deviation occurred in 23 patients (35%): IV GCV was not started due to physician or patient choice (5), was started appropriately, but stopped before D +100 due to physician or patient choice (4), was started for treatment of CMV viremia in a patient who should have been on prophylactic IV GCV before that point in time (8), and replacement of prophylactic IV GCV with oral valganciclovir (6). Other reasons for early discontinuation of prophylactic IV GCV before D +100 were leucopenia (6) and renal insufficiency (2). Median time from HSCT to starting CoPAT was 41 days. Median CoPAT duration was 45 days. Of the 30 appropriate candidates for prophylaxis, only 3 (10%) received the complete prophylaxis course from engraftment through day +100. In this nonrandomized study, the incidence of CMV viremia, peak viral load (VL) and graft-versus-host disease (GVHD) were not significantly different between prophylaxed, partially prophylaxed, and unprophylaxed groups.

**Conclusion:** In practice it proved to be difficult to administer and complete the IV GCV prophylaxis course. Reasons for this included delayed engraftment, relapsed or refractory underlying diseases, physician or patient individual choices, leucopenia, and renal insufficiency. This study illustrates the importance of assessing prophylaxis strategies in the context of actual clinical care. Valganciclovir; the L-valyl ester of GCV is 60% orally bioavailable; representing a more convenient and effective preventive alternative. Thus, in 1/09 we changed our CMV preventive strategy from universal prophylaxis to preemptive therapy with valganciclovir.

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A description of the pathogenesis associated with pirital virus (an arenavirus) infection in the Syrian golden hamster

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**Background:** Arenaviruses are rodent-borne agents and some are capable of causing hemorrhagic fever in humans. These viruses cause acute or persistent infection in a diverse range of animals. Research involving arenaviruses that induce hemorrhagic fever is normally performed in a BSL-4 laboratory environment. However, BSL-2/3 arenaviruses that cause hemorrhagic fever in animals may be used as a surrogate model system to screen potential vaccines and/or therapeutics. Pirital virus (PIRV) is a BSL-3 New World arenavirus that induces hemorrhagic fever manifestations in the Syrian golden hamster model. This model can be utilized to screen and test vaccines and therapeutics.

**Methods:** Female Syrian golden implanted with telemetry units measuring temperature and activity were followed for 14 days post-challenge. Clinical signs of disease were monitored. Viral titers in tissues, viremia, clinical chemistry, hematology, and coagulation parameters and analyzed.

**Results:** Intraperitoneal infection of female Syrian golden hamsters with PIRV led to a specific disease progression involving fever, loss of weight, lethargy, petechial rashes, huddled posture, ruffled fur, epistaxis, and orbital hemorrhaging. Animals surviving to the later stages of disease exhibited whole body tremors, loss of balance, shaking, hind limb paralysis, and rectal hemorrhaging. PIRV infection in hamsters results in 100% mortality in 6-8 days after challenge and analyses of tissues resulted in measureable viremia and viral titers in the adrenals, lymph nodes, intestines, kidney, liver, and brain. Changes in clinical chemistry parameters suggest liver and kidney damage while coagulation parameters demonstrate a disruption of coagulation and clotting. Hematology data demonstrate an increase in white blood cell (WBC) and an increase or stabilization of thrombocytic counts.

**Conclusion:** In all, this has led to a description of a hemorrhagic fever model to study arenavirus hemorrhagic fever pathogenesis and can be used as a cost effective, time-efficient BSL-3 surrogate to screen various antiviral therapeutics and/or vaccine strategies.

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**Type: Poster Presentation**

Modification of the type I interferon and proinflammatory cytokine responses by enterovirus 71  
**Y.-P. Lee**<sup>1</sup>, S.-W. Huang<sup>1</sup>, Y.-F. Wang<sup>2</sup>, C.-K. Yu<sup>1</sup>  
<sup>1</sup>National Cheng Kung University, Tainan City, Taiwan, R.O.C., <sup>2</sup>National Health Research Institute, Tainan City, Taiwan, R.O.C.

**Background:** Enterovirus 71 (EV71) is a neutropic virus targeting the brain stem that may result in severe sequelae including paralysis and pulmonary edema (PE). Type I interferons (IFNs) represent an essential innate defense mechanism for controlling EV71 infection. Inflammatory response with induction of toxic inflammatory cytokines has been considered as an essential component of the pathogenesis of EV71, especially the development of neurogenic PE.

**Methods:** In this study, the role of the type I IFN and proinflammatory cytokine responses on the disease development and mechanisms of viral evasion were investigated by using a murine model with mouse-adapted EV71 strain.

**Results:** After EV71 inoculation, the animals developed viremia, limb paralysis, pulmonary dysfunction, and emphysema with a significant increase of interleukin (IL)-6, monocyte chemoattractant protein-1, tumor necrosis factor and IFN- $\gamma$ , but not IL-10, IL-12, IL-13, and type I IFNs in serum and brain. We assumed that sufficient amounts of PE-associated cytokines (i.e., IL-6, IL-13, and IFN- $\gamma$ ) may be required for the development of EV71-induced PE. The results showed that EV71-infected mice with post-treatment of IL-6, IL-13, and IFN- $\gamma$  developed PE and severe emphysema accompanying with a more severe pulmonary dysfunction than EV71-infected, cytokine non-treated mice. Furthermore, we observed that mice inoculated with EV71 produced a significant lower amount of serum type I IFNs than those inoculated with poly (I:C), adenovirus type V, or Coxsackie B3 virus (CB3). EV71 pre-infection abolished both poly (I:C) and CB3 induced-type I IFN production, and decreased the percentage of IFN-producing plasmacytoid dendritic cells in blood of CB3-infected mice. In addition, a pre-incubation of EV71 also reduced poly (I:C) induced-type I IFN production of murine monocyte/macrophage cell line, RAW264.7. The inhibitory effect of EV71 on type I IFN production was contributed by 3C protease that was proven using over-expression systems either in RAW264.7 cell or adult mouse.

**Conclusion:** In conclusion, EV71 may block type I IFN synthesis through 3C protease to interfere with host innate defence, and on the other hand, trigger proinflammatory cytokine responses to promote PE development.

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The study of epidemiological data of varicella and its complications in Albanian children

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**Background:** Varicella is a serious diseases especially for its complications and even death, may occur in healthy children. Complication have been estimated at a rate of 29.2 per 10000. The serious problem of varicella are reduced after introduced of the vaccine against varicella. The aims of this study was to show epidemiological data ,risk factors and complications of varicella in the Albanian children.

**Methods:** This was a retrospective study and in this study were included 68 children aged 0 to 14 years old admitted in University Hospital Center during January 2004 to December 2008. Epidemiological data analysed were: sex , age, origin, length of hospitalization, risk factors and complications.

**Results:** The mean length of hospitalization was 5.2 days. Age-group most affected was 1-6 years old with 30 cases or 44.12%, followed by children aged 7-14 years old with 22 cases or 32.3%. 47 cases or 69.1% were male and there were no difference for origin. We observed this complications : bacterial overinfections: 22 cases or 32.36% (skin and soft tissue infectious caused by *S. aureus* and *S. pyogenes*) 4 osteoarthritis, neurological complications : 6 cerebellitis, 2 encephalitis, 3 status epilepticus, 1 neuritis, 12 varicella pneumonia, 1 hepatitis, 1 thrombocytopenic purpura , 1 hemolytic anemia. 18 cases or 26.45% under one years old presented enteritis. Acyclovir was used in 26 cases or 38.2%. (mainly complicated). We observed risk factors in 8 cases or (11.8%) as : 3 leukemia , 5 atopic dermatitis.

**Conclusion:** Varicella still remains a frequent infection of childhood , which often is followed by serious complications. The results of this study can contribute to evaluating the options for varicella vaccination.

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Latent manifestations in the US Congenital Rubella Syndrome (CRS) Population

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**Background:** Since rubella and congenital rubella syndrome (CRS) have not been globally eradicated and populations who do not vaccinate continue to persist, increasing knowledge about adults with CRS remains of great importance. This study investigated the latent CRS manifestations of 174 adults with CRS in the United States, born before 1987

**Methods:** Cross sectional design. In this study, Helen Keller National Center's (HKNC) registry was used to identify individuals born with CRS in the United States. Established through an Act of Congress, HKNC is mandated to maintain a national registry of persons who are deaf-blind. The national database, located at HKNC in New York contains personal (race, marital status, residential status, education and employment) and medical (etiology, diagnostic and comorbidities) information on more than 10, 000 deaf-blind individuals with birth dates ranging from 1930-present. According to HKNC, there are approximately 1000 deaf blind individuals 21 and older who are listed as having CRS (HKNC, 2006). According to the CDC, this cohort is the result of the 1963-1965 rubella epidemic. In addition, the registry lists 113 CRS children, ages birth to 21.

**Results:** The survey reported the prevalence of medical disorders including diabetes, cardiac and thyroid dysfunction and glaucoma. Additionally, psychological symptoms such as autistic-like behavior, mood disorder and aggressive behavior were reported

**Conclusion:** CRS is characterized by multiple defects, particularly to the heart, eyes, ears and brain. In utero, the rubella-virus can damage the immune system and vessels which is a harmful starting point for several diseases once the CRS-individual ages. CRS individuals are developing both new medical and psychological symptoms as they age. However, there has been very little information written about this phenomenon, although late onset medical problems have been documented, particularly in the Australian Rubella population born in the 1940s. The results of this study are helpful in understanding both medical and psychological symptoms of the CRS adults, and anticipate potential diseases and behaviors.

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Inhibition of high risk HPV-31 in human cervical epithelial cells *in vitro* by the PC-PLC inhibitor LMV-601

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**Background:** Expression of early genes and episomal DNA replication of human papilloma virus (HPV) is dependent from an active AP1 complex. Activation of AP1 was shown to be precluded by inhibition of phosphatidylcholine specific phospholipase C (PC-PLC). We studied the effect of the PC-PLC inhibitor LMV-601 on HPV-31 infected 9E cervical epithelial cells (CIN 612 9E). LMV-601 is (-)-exo/exo-O-Tricyclo-[5.2.1.0(2,6)]-dec-9-yl-dithiocarbonate potassium salt. Tricyclo-[5.2.1.0(2,6)]-dec-9-yl-dithiocarbonate potassium salt consists of 8 isomers (4 diastereomers, each having 2 enantiomers) and became known under the code D609, first synthesized in 1984 by Merz and Co in co-operation with the German Cancer Research Centre (DKFZ). The pure (-)-exo/exo isomer was first isolated in 2006 and is developed by Lumavita AG as an antiviral drug.

**Methods:** 9E HPV-31 infected cervical epithelial cells were from L.A. Laimins, Chicago.

(a) Short term study: After 72 h treatment, effect on cell growth, HPV-31 specific DNA (Southern Blotting) and RNA (Northern Blotting) was assessed.

(b) Long term treatment (9 passages): After each passage, viral RNA and DNA levels, and cell morphology were assessed.

**Results:** (a) Short term study: LMV-601 displayed a dose dependent inhibitory effect on cell growth (IC<sub>50</sub> 16 µg/mL), HPV-31 specific RNA expression (IC<sub>50</sub> 10.69 µg/mL) and DNA content (62.5 % reduction at the highest dose tested, *i.e.* 32 µg/mL).

(b) Long term treatment: The number of passages required to reduce the amount of HPV-31 specific RNA by 50% (T<sub>50</sub>RNA) was 2.23 at 3.3 µg/mL LMV-601 and < 1 at 10 µg/mL LMV-601. The corresponding T<sub>50</sub>DNA values were 3.28 and < 1, respectively.

After six passages the growth rate of the cells was reduced and the morphology of the cells changed from the spindle form to a normal phenotype. After passage 9, cells were enlarged, became senescent (identified by expression of the senescence marker beta-gal), and ceased to grow.

When human, non-HPV immortalized HaCat keratinocytes were treated with the same concentrations of LMV-601, neither cumulative inhibition of growth rate nor induction of senescence could be observed.

**Conclusion:** LMV-601 inhibits HPV-31 specific RNA expression and DNA replication. Furthermore, these results support the hypothesis that chronic treatment with LMV-601 "cures" pre-cancerous 9E keratinocytes by elimination of HPV genomes.

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The pain topography caused by misdiagnosed zoster

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**Background:** In Herpes Zoster, the pain precedes dermatome manifestation from 10–12 hours to 2–5 days even to 10 days. Zosterian pain last, however it's intermittent.

Our goal in this study is in highlighting the topographic variety of Zosterian pain and initial misdiagnose related with it.

In this study we have included 202 cases of Herpes Zoster. 97 of the them were initially not identified as Herpes Zoster. The group age was 19–78 years old, time period from 1998–2009. 37 of them were HIV positive. In our cases pain precedes exantematic manifestation from 18 to 68 hours.

**Methods:** The cases were assessed based on correlation between neurotics zosterian pain and initial nosology.

**Results:** According to pain location we distinguished these initial misdiagnoses:

Location	Diagnosis	Number of cases
Head	migraine	8
sinusitis frontal	2	
otitis	2	
ophthalmitis	3	
arthritis temporo – mandibular	1	
odontalgia	2	
Thorax	angina pectoris	3
pericarditis	2	
pleuritis	13	
pneumonia	11	
Abdomen	abdominal colic	6
kidney colic	4	
hepatic colic	4	
cholecystitis	2	
mezenterial thrombosis	1	
orchitis	2	
Upper extremities	cervical racialgia	5
cervical spondylarthrose	7	
thorax racialgia	6	
scapulo-humeral bursitis	2	
Lower extremities	ischialgia	7
discal hernia	1	
angiopathies	2	
polymialgia rheumatica	1	
coxo-femoral arthritis	2	

**Conclusion:** 1) Zoster cases identified wrong initially were 48.02 %.

2) Incorrect nosologies conditioned by pain syndrome were 25.

3) Misdiagnoses depends on dermatome affected by zoster.

4) Neuritic pain that precedes zoster dermatomes needs to be considered in diagnosis of pathologies with pain syndrome.

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Study on the prevalence of human bocavirus among children with acute respiratory tract infection in Guangdong, China

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**Background:** To investigate the prevalence of human bocavirus (HBoV) among children with acute respiratory tract infection (ARTI) in Guangdong,China.

**Methods:** 447 nasopharyngeal aspirates or swabs from children with acute respiratory tract infection in Guangdong were collected from Jun.2007 to May.2008. HBoV capsid protein VP gene fragments were detected by using PCR. Positive PCR products were sequenced. The DNA and the translated amino acid sequences were aligned with known HBoV sequences in GenBank and were done phylogenetic analysis.

**Results:** 23 (5.1%) specimens were positive for HBoV, among which 43.5% 10/23 were codetected with other respiratory virus. The mainly diagnosis for HBoV positive children were wheezing pneumonia, bronchiolitis and bronchial pneumonia. HBoV positive children ranged from 43days to 6 years old, mainly aged  $\leq$  1year, among which 43.5% 10/23 were aged 1~6 months and 39.1% 9/23 were aged 7~12 months. HBoV were mainly detected in summer, early autumn and late spring. Through sequence alignment and phylogenetic analysis, The DNA and translated amino acid sequences of VP gene fragments of HBoV positive strains showed 97.8~98.8% and 98.5%~99.2% identity with ST1, respectively.

**Conclusion:** HBoV was the important pathogen of ARTI children in Guangdong and was more prevalent in infants  $\leq$  1year. Although VP gene fragments of HBoV were conservative, there were still some mutant strains leading to amino acid change.

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Deciphering the infectious entry process of human enterovirus 71  
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National University of Singapore, Singapore, Singapore

**Background:** Enterovirus 71 (HEV71) is one of the most clinically significant enteroviruses known to cause severe morbidity and mortality and is most frequently presented as hand, foot and mouth disease (HFMD) in children, although infected individuals could also develop neurological complications. In the absence of anti-viral agents or vaccines, urgent emphasis is therefore being placed on developing anti-viral strategies against this viral pathogen. As yet, little is known of the initial interaction between HEV71 and host cells, which may represent potential anti-viral targeting sites.

**Methods:** A targeted small-interfering RNA (siRNA) screening platform assay was established and validated to identify and profile key cellular genes involved in processes of endocytosis, cytoskeletal dynamics and endosomal trafficking essential for HEV71 infection. Screen evaluation was conducted via the expression of well-characterised dominant-negative mutants, bioimaging studies (double-labeled immunofluorescence assays, transmission electron microscopy analysis), secondary siRNA-based dosage dependency studies and drug inhibition assays.

**Results:** The infectious entry of HEV71 into RD cells was shown to be significantly inhibited by siRNAs targeting genes associated with clathrin-mediated endocytosis (CME), such as AP2A1, ARRB1, CLTC, CLTCL1, SYNJ1, ARPC5, PAK1, ROCK1 and WASF1. The functional role of CME was verified by the observation of strong co-localisation between HEV71 particles and clathrin as well as dose-dependent inhibition of HEV71 infection upon siRNA knockdown of CME-associated genes. HEV71 entry by CME was further confirmed via inhibition by dominant-negative EPS15 mutants and treatment of CME drug inhibitors, with more than 80% inhibition observed at 20 $\mu$ M chlorpromazine. The involvement of other entry pathways, such as caveolae-mediated endocytosis and macropinocytosis, was also found to be minimal, based on the failure of associated drug inhibitors in hampering HEV71 infection. Furthermore, HEV71 infection was shown to be sensitive to the disruption of human genes in regulating early to late endosomal trafficking as well as endosomal acidic pH. The importance and involvement of actin dynamics in mediating the infectious entry of HEV71 was also investigated.

**Conclusion:** The identification of clathrin-mediated endocytosis as the entry pathway for HEV71 infection of susceptible host cells contributes to a better understanding of HEV71 pathogenesis and enables future development of anti-viral strategies against HEV71 infection.

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The clinical severity of Puumala hantavirus-induced nephropathia epidemica and partial complement protein C4 deficiencies

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**Background:** Hantaviruses are rodent-and insectivore-borne zoonotic viruses that are found worldwide. Hantaviruses cause two diseases: hemorrhagic fever with renal syndrome (HFRS) in Eurasia, and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. In Finland, Puumala hantavirus causes nephropathia epidemica (NE) that is referred as a mild form of HFRS with 0.1-0.2% mortality. The pathogenesis of NE is inadequately understood. Previous studies have shown that severe NE is associated with HLA-B8, DR3, DQ2 alleles and this haplotype invariably carries a complement protein C4 null allele. Accordingly, it is speculated that the immune system of the host is involved in the pathogenesis of Puumala virus infection. The role of the complement system in the pathogenesis of NE is poorly studied. The aim of the present study was to evaluate whether the complement protein C4 phenotype associates with the clinical severity of NE. C4 protein, which exists as two isotypes (C4A C4B), is an essential component of the classical complement pathway and deficiencies of C4 are associated with defective processing of immune complexes, impairment of B-cell memory and persistence of bacterial and viral infections.

**Methods:** Complement protein C4 phenotype was determined in 61 hospitalized patients with NE. Phenotyping was performed by immunofixation electrophoresis. To study the clinical relevance of C4 deficiencies, a number of laboratory parameters and clinical findings reflecting the clinical severity of NE were evaluated with regard to C4 phenotype.

**Results:** Thirty-eight of 61 (62%) patients had either C4A or C4B null allele. Partial deficiency of C4B was found in 19 patients (31%) and of C4A in 17 patients (28%). No patient had homozygous deficiency of C4A but two patients (3%) had total deficiency of C4B.

**Conclusion:** The C4 deficiency was not statistically significantly associated with any of the clinical variables measured during acute NE. However, all the 6 patients who had abnormalities in chest X ray, indicating a more severe form of the disease, had C4 null allele ( $p=0.068$ , Fisher's exact test). This observation indicates a trend that C4 deficiency may be associated with more severe forms of NE although statistical significance is not obtained possibly due to too small sample size.

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Diagnosis of congenital cytomegalovirus infection by detection of viral DNA in urine of neonates in Honduras--Advances

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**Background:** Human cytomegalovirus (HCMV) is one of the most commonly found agents of congenital infections, with an incidence of 0.5–3% of live births worldwide. Most of the infections are asymptomatic but may be detected from urine positivity for human cytomegalovirus deoxyribonucleic acid in the newborn period. Epidemiology and clinical outcomes are known to vary with socio-economic background, but few data are available from developing countries, where the overall burden of infectious diseases is frequently high.

Due to non existing data on congenital HCMV infection in the country, we determined the prevalence of congenital HCMV infection in a defined population in Tegucigalpa, Honduras by urine polymerase chain reaction (PCR) in the babies, and assessed the seroprevalence and activity of the virus in the mothers.

**Methods:** Urine samples were collected at birth and tested by PCR for the presence of HCMV DNA in newborns; at the same time, mothers' blood samples were tested for the virus by PCR and by ELISA for IgG/IgM antibodies; the IgG avidity test was performed to determine IgG maturation and evolution of infection. Furthermore, antigenemia for CMV pp65 was assessed in the mothers.

**Results:** Two of the 20 newborns (10%) were found to have HCMV DNA in their urine, consistent with congenital CMV infection. In the mothers, 40% were positive for HCMV DNA. Cytomegalovirus IgG antibodies were detected in 95% of the 20 mothers' samples investigated; 14 (70%) women had both IgG and IgM antibodies at delivery. Two (10%) women presented low avidity IgG antibodies with positive IgM, suggesting primary infection. Among the 20 pregnant women, 50% presented IgM antibodies with a high avidity IgG index, implying recurrent infections. Five percent of the women presented positive pp65 antigenemia.

**Conclusion:** Although we have not established the prevalence of HCMV congenital infection, these preliminary results permit evaluate the frequency of congenital CMV among the population studied.

By using the urine PCR screening method, we were able to evaluate the magnitude of the problem in a defined population. Therefore, an accurate diagnostic method such as PCR is highly advisable for the management of congenital infection in newborns.

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Epidemiology of human rhinovirus C (HRV-C) in Hong Kong reveals a potential HRV-C subgroup  
**C. Y. Yip, S. K. P. Lau, K. H. Chan, P. C. Y. Woo, K. Y. Yuen**  
The University of Hong Kong, Hong Kong, China

**Background:** A novel human rhinovirus species, HRV-C, was recently identified, but its epidemiology, compared with HRV-A and HRV-B, remains poorly understood, especially in adults.

**Methods:** One thousand two hundred nasopharyngeal aspirates (NPAs) from hospitalized children and adults during a 12-month period were collected and subjected to HRV detection by reverse-transcriptase polymerase chain reaction. The epidemiology of HRV-A, HRV-B and HRV-C was analyzed.

**Results:** Of 600 NPAs from children, 178 (29.7%) were positive for HRVs, while of 600 NPAs from adults, 42 (7%) were positive for HRVs. HRV-A showed the highest prevalence (n = 111), followed by HRV-C (n = 91) and HRV-B (n = 18). Although upper respiratory tract infection was the most common presentation in children, 8 (62%) of the 13 adults with HRV-C infection had pneumonia, compared with 6 (27%) of the 22 adults with HRV-A infection (P<.05). Wheezing episodes were also more common among individuals with HRV-C (37%) and HRV-A (20%) infections than among those with HRV-B (0%) infection (P<.05). Clinical and molecular data analysis indicated HRV-C as a frequent cause of community and institutionalized outbreaks. A possible distinct HRV-C subgroup was revealed among a highly diverse array of HRV-C strains that was circulating throughout the year.

**Conclusion:** HRV-C is associated with pneumonia in adults and outbreaks of respiratory tract infections requiring hospitalization. A potential distinct HRV-C subgroup was identified.

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The role of human papillomavirus Infection in prostate carcinoma

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**Background:** Human papillomavirus (HPV) infections are frequently associated with benign, pre-malignant and malignant lesions of the female genital region and male anogenital tract. One potential etiological factor of interest for prostate carcinoma (PCa) in light of the associations with sexual practices is exposure to HPV. Currently the possible role of HPV infections in prostate carcinogenesis is a subject of great controversy. In this study we aimed to investigate the role of HPV infection in prostate carcinoma.

**Methods:** Formalin-fixed and paraffin-embedded tissue samples of 104 primary prostate adenocarcinoma cases with varying degrees of differentiation and 104 control tissues of benign prostatic hyperplasia (BPH) were provided for analysis. HPV-DNA was purified and amplified through MY09/MY11 and GP5+/GP6+ primers and subsequently subjected to sequencing.

**Results:** HPV-DNA was found in 11 of 104 (10.58%) PCa and 7 of 104 (6.73%) BPH samples. There was not any significant difference between PCa and BPH specimens regarding HPV-DNA presence. High-risk HPVs (type 16&18) were detected in all of the PCa and 6 of 7 (85.7%) BPH samples (not significant) and low-risk HPV (type11) were detected in none of the PCa and 1 of 7 (14.2%) BPH specimens (not significant). HPV-16 was the predominant HPV type in both PCa and BPH samples.

**Conclusion:** Our study showed that there was not any significant difference between PCa and BPH specimens regarding HPV-DNA presence. Our data do not support the hypothesis that HPV infection is related to the development of prostate cancer. However, as with other HPV-associated diseases, attributing a role to the virus in the etiology of prostatic carcinoma will require further detailed studies.

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Rotavirus diarrhea in Kashmir: Detection of genotype G12P[6] strains

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**Background:** Rotavirus genotype G12P[6] was detected among children with acute gastroenteritis in Kashmir Valley-India. The study was conducted in two major hospitals of the Valley from Jan 2006 to Jan 2007. Six (6) cases of G12 infection were identified having P[6] specificity. Three (3) cases of G12 P[6] were detected by PCR and other three (3) by gene sequencing. Sequence analysis of the VP7 gene from three neonatal strains indicated a high level of aminoacid homology (98-99%) to other G12 strains reported world wide, suggesting introduction of novel rotavirus strain serotype into the community. The G12 strains were detected sporadically and were not associated with any outbreak or nosocomial infection. Genetic characterization of VP7 gene of these strains revealed one lineage and a very little aminoacid divergence  $\leq 2\%$ . The 6 cases represented clinical etiology of watery diarrhea, severe vomiting and dehydration and only one patient with low grade fever. The study documents the first detection of G12 strain from Kashmir-India.

**Methods:** Detection by RNA-PAGE and reverse transcription and amplification of the VP4 and VP7 genes. Non-typeable samples were subjected to sequencing of VP7 gene and the genotype determined by comparison. Phylogenetic tree was constructed by clustal X (version 1.81) software.

**Results:** (45%) prevalence in the age group of <5 years. Majority of the positive cases were suffering from loose motion with watery stools, persistent vomiting, dehydration and fever. The 6 cases of G12P[6] were all children between 6 months to 10 months of age, collected in a months time between 30th of May 2006 to 29th of June 2006. The G12 strains were detected during summer season. The phylogenetic analysis of VP7 gene sequence shows high degree of amino acid conservation with a South African strain and other globally reported strains.

**Conclusion:** In conclusion present study documents' first detection of G12 strains in Kashmir India. Rich diversity of rotavirus strains circulating in India poses a question for evolution of more novel strains. Hence efforts for carrying surveillance on large scale population should be carried in order to access the percentage prevalence of this strain in the community. Infants ( $\leq 8$  months) under 2 years of age are susceptible to rotavirus infection as has been seen in this study

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Surveillance of rotavirus strains reveals evidence of emerging G12 and unusual human-animal reassortant strains in Manipur, North-eastern India

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**Background:** The rotaviruses are the major cause of severe gastroenteritis and account for an estimated 100,000 deaths and 400,000 hospitalizations in children under 5 years of age in India.

To date, 23 G and 31 P genotypes have been reported in Group A Rotaviruses on the basis of differences of the outer capsid VP7 and VP4 encoding genes. North-Eastern states of India are geographically distinct with temperate weather compared to rest of the India. Few outbreaks were reported during the year 1979 to 1988 in Manipur with evidence of zoonotic transmission but systematic surveillance study was not done till date.

**Methods:** The samples were collected from children (<5 years) hospitalized with acute diarrhea at the Regional Institute of Medical Sciences (RIMS), Manipur between December 2005 to March 2008. Rotavirus positivity was screened by EIA or PAGE. RT-PCR was used to determine specific genotypes. Sequencing and blast analysis was also performed to analyze evolutionary and genetic diversity of the circulating strains.

**Results:** The globally common genotypes G1P[8] and G2P[4] constituted 58% of the total positive strains, while 3% and 8% strains were of the emerging genotypes, G9P[6] and G12P[6]. G12 strains have been reported from Manipur for the first time. The G12 strains clustered with lineage III strains and had >98% identity with Bangladeshi, Thailand and USA strains. Few unusual G-P combinations like G4P[4], G4P[6], G10P[6] and G9P[19], along with some G-P non-typables were also found. G4 and G10 strains clustered with the porcine and bovine strains, indicating zoonotic transmission.

**Conclusion:** High frequency of rotavirus infection ( $\approx$  50%) and predominance of the common strains G1P[8] and G2P[4] among children with acute diarrhea emphasizes the need for implementation of currently available vaccines to reduce the burden of rotavirus induced diarrhea in high risk regions of developing countries.

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The nucleocapsid protein of measles virus and other morbillivirus blocks host interferon signaling pathway

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**Background:** Interferon (IFN)- $\alpha/\beta$  and IFN- $\gamma$  are potent antiviral cytokines that are induced in direct response to viral infection and have important roles in the inhibition of viral replication and regulation of host immunity. Against these functions, viruses have developed many mechanisms to escape the antiviral action of IFN. Measles virus (MV) belongs to the genus *Morbillivirus* of the family *Paramyxoviridae*, which contains canine distemper virus and rinderpest virus, and has negative-sense RNA genome and consists of six tandemly linked genes that encode eight proteins. A number of paramyxoviruses contribute to viral evasion of the host IFN response by various mechanisms involving one or more their nonstructural accessory proteins. In the case of MV, it has been reported that the accessory V and C proteins inhibit host IFN signaling pathways. However, the mechanisms are unclear in detail. In this study, we confirmed the effect of not only the accessory proteins of MV-HL strain but also other proteins on the IFN signaling pathways.

**Methods:** HEK-293T cells were transfected with IFN- $\alpha/\beta$  or IFN- $\gamma$ -inducible reporter plasmid together with expression plasmid encoding various viral proteins and then treated with each IFN. To clarify the mechanism of the inhibition, we further analyzed details using western blotting and indirect immunofluorescence.

**Results:** We found that the MV nucleocapsid (N) protein (MV-N) inhibits both IFN- $\alpha/\beta$  and IFN- $\gamma$  signal transduction like the accessory proteins. In the results of western blot analyses, MV-N neither prevents the phosphorylation of STAT and Jak nor induces STAT degradation. On the other hand, MV-N interferes with the nuclear import of activated STAT. We confirmed that the N protein of the other morbilliviruses also disturbs the IFN signaling.

**Conclusion:** The N protein is the most abundant of the viral proteins and enwraps the genomic RNA into helical filamentous structures referred to as nucleocapsids. In this study we revealed a novel function of the N protein of morbillivirus that acts as an IFN-antagonist. The presented here give insight into understanding how the viral proteins of morbilliviruses perform to inhibit IFN signaling.

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Analysis of phosphorylation residues on Nipah virus nucleoprotein and role of the phosphorylation  
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The Institute of Medical Science, Tokyo, Japan

**Background:** Nipah virus (NiV) is a recently emerged zoonotic virus that causes encephalitic and respiratory illness in humans and livestock, with a high mortality rate (40-70%) in humans. NiV is a negative single-stranded RNA virus that belongs to the family *Paramyxoviridae* and is composed of six structural proteins. The viral nucleoprotein (N) encapsidates the genomic RNA and forms a nucleocapsid. This serves as a template for viral replication and transcription, which is catalyzed by an RNA-dependent RNA polymerase that is composed of viral phosphoprotein (P) and large (L) proteins. NiV is related closely to Measles virus (MV), which has a well-characterized N protein and also belongs to the family *Paramyxoviridae*. It has been reported that MV-N undergoes phosphorylation modification. Recently, we identified the phosphorylation sites of MV-N, and the phosphorylation was required for efficient viral transcription. However, phosphorylation of NiV-N has not been reported. In this study, we investigated that if NiV-N also undergoes phosphorylation.

**Methods:** NiV-N was expressed in COS-7 cells in the presence of <sup>32</sup>P and immunoprecipitated, followed by SDS-PAGE and autoradiography. Alternatively, after expression in COS-7 cell, cesium chloride density-gradient centrifugation was performed to purify the nucleocapsid complex. The phosphorylation site of NiV-N was subsequently determined by ESI-Q-TOF MS analysis. To evaluate the role of the phosphorylation for viral transcription and replication, NiV minigenome assay was utilized.

**Results:** We demonstrated that the NiV-N is phosphorylated and dephosphorylated in a short-span turnover in steady-state cells. Furthermore, we succeeded in identification of the phosphorylated site on NiV-N. In the minigenome assay, introduction of mutation on this site resulted in the reduction of reporter activity by approximately 45% as compared with the wild-type protein. In addition, a mimic of phosphoserine mutation, showed more reduction. Northern blotting showed that the transcription level was affected more than replication level by mimic of phosphoserine mutation. On the other hand, mutation of the phosphorylation site of NiV-N did not affect N-P complex formation.

**Conclusion:** In this study, we revealed that NiV-N undergoes phosphorylation in a short-span turnover. In addition, the instant phosphorylation of NiV-N was important for efficient replication, and rapid dephosphorylation for transcription.

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Regulation of poly(A) binding protein during herpes simplex virus infection

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**Background:** The herpes simplex virus (HSV) ICP27 protein is an essential IE protein, conserved through the Herpesviridae family, that constitutes a target for the development of antiviral drugs and vaccines. ICP27 is a key regulator of viral and cellular gene expression, with most of its activities in the nucleus.

**Methods:** The regions of ICP27-PABP1 interaction were mapped by GST pull down and immunoprecipitation, using a panel of recombinant HSV. PABP1 phosphorylation was studied by 2D-electrophoresis, while shuttling activity was shown by confocal microscopy. Tether function assay in *Xenopus* eggs was used to study ICP27 effects in translation, independently of its nuclear activities.

**Results:** ICP27-PABP1 interaction is partially affected by RNase I treatment *in vivo*, which could be explained by the ability of both proteins to form multimers with RNA. Both purified proteins are able to interact directly *in vitro* in the presence of RNase I. The C-terminal of ICP27 is required for this interaction, while PABP1 interacted through RRM1-2 and C terminal. We described here a novel role for ICP27 in the cytoplasm, where it stimulates translation. We showed that PABP1 activity is regulated early in HSV infection by phosphorylation, mediated by HSV UL13 kinase. These events may cause redistribution of ICP27 from the cytoplasm to the nucleus late in infection.

**Conclusion:** Our results showed that PABP1 activity is regulated during HSV-1 infection by phosphorylation and relocalisation, events that seems to be linked to translation regulation. Experiments to elucidate the mechanism of translational control exerted by ICP27 are currently under investigation.

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Complications of varicella in healthy children in Izmir, Turkey

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**Background:** Varicella is usually a benign and self-limited childhood illness. Complications are thought to be quite rare in immunologically healthy children. However, studies report that varicella epidemiology is changing and that the rate of hospitalization in immunocompetent children are more frequent than previously thought.

The purpose of the study was to describe complications of varicella requiring hospitalization in a defined population (Izmir, Turkey) and to compare the complication of varicella with our published study in 1997-2001.

**Methods:** Between 2005 and 2009, hospital records of patients admitted with complications of varicella to three children's hospitals in Izmir (Ege University Children's Hospital, Behçet Uz, and Tepecik Children's Hospital) were reviewed. Incidence and clinical spectrum of complications were analyzed.

**Results:** From 2005 to 2009, 262 cases (median age, 3.8 years) were hospitalized for varicella complications, whereas 178 cases (median age, 3 years) were hospitalized between 1997 and 2001. This resulted in a crude incidence of 8.7/100 000 population at risk (6.3/100.000 previously). There was a seasonal distribution of complications with a peak in January. The most frequent complications were infectious complications, which were observed in 96 children (36.6%). Pneumonia was observed in 48 children (18.5%). Superinfections of the skin were present in 39 patients. A total of 93 (35.5%) neurologic complications were observed. Cerebellar ataxia was most frequent neurologic complication. Infectious complications occurred more frequently in younger children (median age: 2.5 years), whereas neurologic complications occurred at an older age (median age: 6.3 years). Hematologic complications were seen in 9 children (3.4%). Three cases (0.1% of hospitalized patient) were dead from complications of varicella (2 from encephalitis and 1 from pneumonia) between 2005 and 2009, whereas no fatality has been reported during previously study period (1997-2001).

**Conclusion:** The rate of hospitalization of children with complications of varicella is higher than described in our previously study.

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Para influenza type 3 epidemic in intermediate care nursery

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**Background:** Para influenza virus (PIV) causes more than 30% of all acute respiratory infections in infants and children. PIV type 3 accounts for the majority of infections in newborn infants. The objective was to describe an epidemic of PIV infection in the intermediate care neonatal unit (IMCNU) of king Fahad National Guard Hospital, Riyadh-KSA

**Methods:** Prospective descriptive report of viral epidemic occurred in the IMCNU

**Results:** During the period April 26 to May 6, 2008; a total of 7 infants proved to be infected with PIV type 3, The attack rate was 12.7% (7 infants affected out of 55 infants exposed), median birth weight of 1850 gms (range : 735-2750gms), and median gestational age of 30 weeks (range : 26-39 weeks) and median postnatal age of 37 days (range: 17-123 days). Five infants presented with apneas and desaturation, 4 infants were observed coughing and 4 infants showed excessive nasopharyngeal secretion. Four infants were having chronic lung disease as a predisposing factor. Infants positive for PIV were cohorted together in 2 isolation rooms, other contacts infants were also nursed together in a different zone of the unit and the unit continued to receive new admission. One infant needed nasal intermittent ventilation and 4 babies responded to oxygen therapy by nasal cannula. All infants survived and discharged in good conditions

**Conclusion:** PIV infection of little infants in IMCNU may cause destabilization of their clinical conditions and can precipitate serious episodes of cardiorespiratory instabilities. Conservative management of affected infants usually adequate. PIV epidemic can be controlled by utilizing basic infectious barriers precautions and cohorting infected infants together

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Introduction of a new lineage VP7 of rotavirus G1 in the Venezuelan population

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**Background:** G1 is the most widespread rotavirus type associated to acute gastroenteritis in children from many countries. Genetic and antigenic heterogeneity seem to be the reason for this consistent predominance of G1 serotype rotaviruses throughout the world over the years. Although studies previously conducted in Venezuela indicated a broad diversity in circulating rotavirus types, more recent data show that G1P[8] rotaviruses is the most widely circulating type since November 2007 in Caracas. In an attempt to investigate the impact of currently available vaccines, human rotavirus isolates obtained from clinical samples in Venezuelan cities over time and identified as G1P[8], were studied to acquire information about the pattern of genetic evolution of this type, and to understand the mechanisms of distribution and selection of rotavirus strains and lineages.

**Methods:** Sequences of amplicons of 850 bp from VP7 rotavirus genes of one G1 strain isolated in Valencia during year 2003, and 3 strains obtained during the 2008 in Caracas were compared with the homologous regions of 43 reference G1 strains obtained from GenBank. Phylogenetic analysis was made using the neighbor-joining method.

**Results:** VP7 gene analysis demonstrated that Venezuelan G1 rotavirus strains segregated in two different genetic lineages, with the three most recent isolates of 2008 clustering into lineage I and the strain of the 2003 into lineage II, the latter closely related to the recently introduced monovalent G1 *Rotarix*® vaccine. Analysis of deduced amino acid VP7 sequences of the three G1 rotavirus strains isolated during the 2008 revealed amino acid changes located on B (94S→N) and E (217T→M) regions.

**Conclusion:** The increase in circulation of G1 rotaviruses observed in Caracas since November 2007 may be associated with the introduction of novel G1 strains that exhibited amino acid changes in antigenic regions involved in rotavirus neutralization. It is important to continue the surveillance to understand how these changes might affect the effectiveness of the vaccine.

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The nucleocapsid protein of SARS CoV interacts with PIAS1 and affects the NFkappaB pathway  
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**Background:** A leading cause of morbidity, mortality and economic loss amongst viral diseases are acute viral respiratory tract infections. Today, even after the chain of spread of disease has been broken successfully, there remains a frightening prospect of a future outbreak which may be more contagious or virulent than ever encountered before.

**Methods:** Protein inhibitor of activated signal transducer and activator of transcription (STAT) 1(PIAS1) was identified in yeast two hybrid screens as an interacting partner for STAT1. The interaction of N with PIAS1 was validated in mammalian cells using co-immunoprecipitation.

**Results:** Our results of time-course based co-localisation shows that the localization of N shifts from cytoplasmic to nuclear in presence of PIAS1. This, not only points towards interaction between N and PIAS1 but also one that is of major physiological consequence. PIAS1 acts as a key regulator of NKkB signaling pathway in the nucleus where it inhibits its DNA binding activity and NKkB mediated gene activation. NF-kB is a critical regulator of the immediate early pathogen response, playing an important role in promoting inflammation and in the regulation of cell proliferation and survival. Gene activation analysis have shown that PIAS1 selectively regulates a subset of NKkB dependent genes, with a notable preference for proinflammatory cytokines and chemokines. Here, we found that in presence of N, the inhibition induced by PIAS1 to DNA binding activity of NFkB and NFkB mediated gene activation is lifted. Not only that, our results reveals that both DNA binding activity and NFkB mediated gene activation is enhanced in presence of N.

**Conclusion:** We hypothesize that N translocates into the nucleus where it, enhances the DNA binding activity of NFkB and NFkB mediated gene expression by lifting up the inhibition imposed by PIAS1. Since many protein products of the NFkB sensitive genes have been reported to be upregulated in fatal clinical cases of SARS, we further hypothesize that N protein plays an important role in excessive expression/secretion of cytokines and chemokines thereby contributing significantly to the lung injury seen in cases of SARS.

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Molecular epidemiology of rhinoviruses among children diagnosed as severe pneumonia in the Philippines

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**Background:** Rhinovirus (Rhino) is one of the major viruses of respiratory illness. There is a report that rhinoviruses had the highest prevalence among diseased cases among children under 5 years old who are hospitalized by acute respiratory illness. The novel group of rhinovirus (rhino C) was found recently. However, details of epidemiological features, including rhino C, in tropical climate are still unknown. In this study, we tried to assess the importance of rhinoviruses among severe respiratory infection of children in the Philippines.

**Methods:** 816 nasopharyngeal swabs which were collected from May 2008 to May 2009 from children (age: 7days -14years old) who were diagnosed as severe pneumonia were used. RNA was extracted and subjected to RT-PCR targeting 5'Non Coding Region and VP4/VP2 region. Sequencing analysis was carried out and rhinoviruses genogroups (A~C) were determined.

**Results:** 243 out of 816 samples (30%) were positive for rhinoviruses. Among rhinoviruses positive samples, 131 (54%) were positive for rhino A, 25 (10%) were for rhino B, and 86 (35%) were for rhino C. The nucleotide sequence of Rhinoviruses (A,B,C) in VP4/VP2 showed that variable types of rhinovirus are co-circulating in one area, and were related closely to the ones reported from various countries and year. No etiological correlation was seen between any genogroups or subspecies and severity. Rhinoviruses were detected all the year. However there was a peak for Rhino A in July to September, Rhino B in September to November, and Rhino C in February to March. Rhinovirus infection in tropical zone may have different seasonality from the one in temperate zone and each genogroups may have different seasonality.

**Conclusion:** Rhinoviruses were detected with high prevalence among severe pneumonia which may suggest their importance among children in the Philippines.

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Ferrovir in treatment of viral infectious diseases

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**Background:** Viral diseases occupy 1-st place in infectious pathology and agents having pluripotent activity against different viruses are welcomed. Previously, it was found that Ferrovir (FV) (sodium salt of DNA from salmon's milt conjugated with Fe<sup>3+</sup>) possess antiviral activity against DNA- and RNA-viruses with lipid membrane: Herpes Simplex Virus (HSV), Cytomegalovirus (CMV), Human Immunodeficiency Virus (HIV), Tick-borne encephalitis virus, Human and Avian Influenza Virus in vitro studies. The mechanism of FV action is connected with induction of inflammation cytokines and oligopeptide antiviral factors. FV was effective in immunocompromised patients (pts) with HIV-infection and Hepatitis C infection. The aim of the study was to analyze the efficiency of FV against HSV-, CMV- and Human Papillomavirus infection (PVI).

**Methods:** Clinical study enrolled 29 pts (15 female and 14 male) with recurrent genital HSV-infection who received FV 75 mg once in 2 days as i/m injection for 20 days and 30 pts received standard therapy. Group of 11 pts (female) with reactivated and persistent CMV-infection received 75 mg FV twice daily for 10 days. Group of 63 pts with latent form of PVI received course of FV of 10 injections every 72 hours.

**Results:** After FV treatment in 80% cases of pts with HSV-infection not a single relapse was observed during 6 months, lack of Ig M anti-HSV antibodies, negative cytological results. In pts group with standard therapy of HSV-infection lack of relapse was in 34% cases and in 50% cases in cytological substrates HSV-1,2 was found. In 81,8% pts with CMV-infection virus DNA in saliva, urine and cervical secret was not found by PCR after treatment and 1 month later. FV treatment resulted in 100% correction of immunological indices in untreated previously PVI pts and 93,8% cases who previously received therapy. 100% elimination of virus was observed in newly treated pts and in 77,5% pts with previous therapy. FV administration was well tolerated and no side effects were observed.

**Conclusion:** Ferrovir demonstrated good antiviral properties, is well tolerated by patients, is useful in case of HSV-, CMV and HPV-infection, and the low price makes it accessible to population in limited resources context.

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Initial poor prognostic factors of echovirus 6 and 9 infections in children

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**Background:** Echovirus 6 and echovirus 9 have been found to cause deaths and critical infections in children but their initial poor prognostic factors and clinical courses were not well known.

**Methods:** Echovirus 6 or 9 was identified by immunofluorescent staining with virus-specific monoclonal antibody in cultures of children admitted in a tertiary hospital in Taiwan from 2000 to 2008. The medical records of all cases without any co-infection were extensively reviewed. Clinical presentations of 6 severe cases (including those were fatal, with intensive care or long-term sequel) and 168 non-severe cases were analyzed. Initial prognostic factors were compared severe cases with non-severe cases by Fisher's exact test.

**Results:** Six children had severe clinical presentations. Two children with echovirus 6 infections and one child with echovirus 9 infection were fatal. They died of multi-organ failure, brain stem infarction and brain herniation, individually. One boy with echovirus 6 infection was rescued by extra-corporeal membrane oxygenation (ECMO). All live patients had full recovery except 2 children left long-term sequel of convulsion. Among 168 non-severe cases, the disease spectrums were similar for echovirus 6 (n=96) and 9 (n=72) infections, with aseptic meningitis being the most common syndrome, followed by meningismus, upper respiratory tract infection, pneumonia, and herpangina. The unfavorable outcomes were more frequently encountered in cases with initial presentations of seizure with hyperglycemia (serum glucose > 150 mg/dl:  $p=0.001$ ), diagnosis of myocarditis ( $p=0.034$ ), abnormal serum aspartate aminotransferase level (AST >80 U/l:  $p=0.006$ ), and higher serum alanine transaminase level (ALT >45 U/l:  $p=0.001$ ), but did not correlate with C-reactive protein level (CRP >50 mg/l:  $p=0.653$ ), white blood cell level (WBC level >15000 /ul:  $p=0.657$ ), or young age (<1 month-old:  $p=0.444$ ; <3 month-old:  $p=0.319$ ; <6 month-old:  $p=0.135$ ) (Table 1).

Table 1. Analysis on risk factors of severe echovirus 6 or 9 infections by comparing 6 severe cases with 168 non-severe cases ( $p$  was calculated by Fisher's exact test)

Initial presentation of patients	Percentage of severe cases		Odds ratio	95 % confidence interval	$p$
	Cases with this presentation	Cases without this presentation			
Seizure with initial hyperglycemia (sugar >150 mg/dL)	100 (2/2)*	2.3 (4/172)	44.0	16.7-115.9	0.001
Myocarditis	100 (1/1)	2.9 (5/173)	34.6	14.6-82.1	0.034
AST > 80 U/L	50 (2/4)	2.4 (4/170)	41.5	4.6-373.3	0.006
ALT > 45 U/L	100 (2/2)	2.3 (4/172)	43.0	16.3-113.3	0.001
CRP >50 mg/l	2.9 (1/35)	3.6 (5/139)	0.8	0.1-7.0	0.653
WBC >15000 /ul	3.5 (1/28)	3.4 (5/146)	1.0	0.1-9.3	0.657
<3 month-old	6.1 (2/33)	2.8 (4/141)	2.2	0.4-12.6	0.319

\*Percentage (Number of severe cases/Total number of cases in this condition)

Abbreviations: AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; CRP, C-reactive protein; WBC, White blood cell count

**Conclusion:** The most common manifestation of echovirus 6 and 9 infections in children was aseptic meningitis. Initial biomarkers of poor prognostic factors, such as sugar, AST and ALT, are recommended to be checked. Fatal outcome or long term sequel in survivors may be encountered in young children with specific presentations, including seizure with hyperglycemia

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Please note that the official publication of the International Journal of Infectious Diseases 2010, Volume 14, Supplement 1 is available electronically on <http://www.ijidonline.com/> or organ (liver, brain or heart) damage. Early intensive care must be considered in patients with these initial poor prognostic factors.

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Detection and clinical characterization of WU polyomavirus with acute respiratory tract infection in children, Guangdong of China

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**Background:**

In 2007, Gaynor et al. discovered a previously unidentified human polyomavirus in respiratory secretions obtained from human patients with symptoms of acute respiratory tract infection. This new virus was designated WU polyomavirus. To investigate the frequency of its infections in Guangdong, China, we sought to describe the detection and clinical characterization of WU Polyomavirus with infection in children.

**Methods:**

From July 2008 through June 2009, nasopharyngeal aspirates were collected from 771 children who were hospitalized with acute lower respiratory tract infection in Second Affiliated Hospital of Shantou University Medical College, and 82 were asymptomatic who visited the wellbeing clinic. WU Polyomaviruses were detected by using PCR technology and were identified by using DNA sequences. All WU polyomavirus positive specimens were screened for 9 common viruses (influenza A and B; RSV; PIV 1 and 3; human metapneumovirus; human bocavirus; adenovirus; rhinoviruses) by using PCR or RT-PCR.

**Results:** In this study,

fifteen of the 771 tested specimens with acute lower respiratory tract infection were positive for WU polyomavirus, the positive rate was 1.95%. and all of the asymptomatic children who visited the well-

being clinic were negative. Positive specimens were noted for patients 2 months to 48 months of age, the median age was 18.8 months. Of the 15 WU Polyomaviruses positive patients, 9 (60%) were male, 6 (40%) were female. WU polyomavirus was the sole virus detected in 9 specimens (60%) from patients with acute respiratory tract infection. WU polyomavirus were associated with the co-

infection of another respiratory virus in 6 of 15 (40%), most frequently with RSV (n = 4), followed by adenovirus (n = 1) and rhinovirus (n = 1). The most common clinical findings in the patients with WU polyomavirus are cough, fever, wheezing. The most frequent diagnoses were pneumonia (n=8), bronchiolitis (n=4), upper respiratory tract infections (n=2) and bronchitis (n=1). A severe case was complication with viral encephalitis.

**Conclusion:**

We suggest that WU Polyomaviruses may be a respiratory pathogen because WU polyomavirus was the sole virus detected in 9 specimens from patients with respiratory illness and all of the asymptomatic were negative. The most common clinical findings are cough and wheezing. Young children are the main target. And the pathogenetic conditions are generally mild. More comprehensive studies are required to prove these viruses are agents causing respiratory disease.

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Amplification of early genes of Human Papilloma Virus targeting nine virus genotypes. Mérida, Venezuela

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**Background:** Subtyping of Human Papilloma Virus (HPV) by molecular biology tools may enhanced cervical cytological assessments information for patient's management and for prognosis of cervical cancer evolution. The **aim** of this study was to subtype HPV from cervical samples of sexually active women (15-69yr-old) seeing in cervic-gyn public clinic (IHAULA) of Merida State, Venezuela.

**Methods:** DNA-collection device (Digene®) were used to collect and transport 250 cervical smears, DNA were isolated using QIAamp® DNA Mini Kit (Qiagen®) and storage at -20°C until used. A Nested-PCR-Multiplex assay was standardized for amplification of early E6/E7 HPV virus. A first run included amplification of a 630bp-length region of E6/E7 gene and a second run allowed to genotype HPV virus in a multiplex format. Amplicons sizes varied from 151-457bp for HPV-16,-18,-31,-45; and HPV-33,-6/11,-58,-52,-56. Simultaneously, HPV detection assays were performed using Hybrid-Capture II (Digene®) technology and an end-point PCR assays for L1 and E6/E7 regions.

**Results:** HPV were detected in 27.2% samples, 94.12% (64/68) were positive for at least one of the genotypes assayed. High risk HPV were identify in 98.44% (63/64) samples; where HPV18 (50/63) was the most common genotype isolated, along with HPV16 (24/63). One or several types were detected in 56.25% (36/64) of the cases, being VPH-18-6/11 (14/64) combination the most common one.

**Conclusion:** we found a high frequency of cervical infection with high-oncogenic-risk HPV genotypes, specially caused by HPV18, alone or in multiple types infection.

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Development of a new genotyping system for predicting TTV genotype using evolutionary restriction map and artificial neural network

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**Background:** TT virus is belonging to the family Circoviridae and was discovered in 1997. On the basis of sequence variation in both coding and noncoding regions, many classification systems have been proposed. Several genotyping technique , including DNA sequence analysis as well as primer specific PCR are used. None of the reported systems was able to determine the TTV genotype in all major types and the common subtypes on the basis of the TTV sequence. To date, TTV genotypes have been primarily determined by sequence-based PCR products analysis techniques, including direct sequencing. Here we proposed a new simple method for genotyping based on neural network method and restriction map.

**Methods:** This method classifies genes into groups which are made distinct from each other by evolutionary changes in their restriction site. Our objective was to accurately predict, from complex restriction site patterns, TT virus, by use of artificial intelligence. Neural network models were constructed based on changes at restriction site in the untranslated region of virus. Models were trained, validated, and tested with 330-UTR sequence. A procedure of determining the optimal neural network parameters was proposed to speed up the training processes.

**Results:** The results suggested that the restriction site map is a more accurate predictor of TTV genotype. To show the ability of the model in genotyping, the internal representations developed by the networks are analyzed using principal component analysis (PCA). This analysis shows that the networks are able to discover relevant features on the basis of the association between the restriction map and the virus genotype.

**Conclusion:** The proposed predictor shows vigorous clusterization of both predicted and observed TTV genotype. The current study is designed to find the algorithm for genotype prediction using the restriction site information in combination with artificially created neural network. The applied properties for the model must be mighty and low time consumption. After the pattern recognition model is constructed the algorithm can be used to determine the repertory of restriction site properties required for each genotype. This complex can be defined as genotypic pattern. The pattern can be used for further correlation analysis with known and unknown viral sequence.

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**Type: Poster Presentation**

Seroprevalence of human cytomegalovirus infection in Singapore  
**H. N. Leong**, B. H. Tan, S. H. Lim, K. P. Chan  
Singapore General Hospital, Singapore, Singapore

**Background:** The human cytomegalovirus virus (HCMV) is a ubiquitous *betaherpesvirus*, common in all human populations, with seroprevalence of 80-100% in adults across the world. Most infections are harmless, but it can cause congenital HCMV infection in neonates or disseminated disease in the immunocompromised hosts. Till date, there has been scarce seroprevalence data of this disease in our country.

**Methods:** A retrospective analysis of the HCMV IgG assays performed in our laboratory was done. The period of study was from 2004 to 2007.

**Results:** A total of 3582 patient data were available. Duplicates and patient with missing variables (age, sex) were removed, leaving 2042 patients (median of 707 patients per year) for analysis. Across the 4 years of data, there was no significant difference seen in across the age groups, sex or race. An overall seropositivity rate was 40% for the 1-10 year age group. This rises gradually to 44%, 73%, 82%, 90%, 97% and 100% for each decade increase. The males and females have a similar rate of acquisition of HCMV seropositivity at age group 1-10 of 40%. However, seropositivity in females increases rapidly compared to the males in the age groups 10-20 to 40-50 before being similar again in the older age groups. This difference becomes statistically different in the 20-30 year age group ( $p=0.0024$ ). Amongst the races, the predominant Chinese race group has a lower seropositivity in all age groups from 1-10 to 40-50 compared to the minority races of Indians and Malays combined. This difference was statistically different in the 20-30 year age group ( $p=0.001$ ). The differences are likely related to the social habits of each race, and sex.

**Conclusion:** The result show a HCMV seroprevalence of 73% in the adult age group of 20-30. This prevalence is higher for females and in the minority races. This data has implications in estimating the risk for congenital CMV disease in neonates born to mothers acquiring their primary infection during pregnancy, and risk of CMV disease in transplant programmes.

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**Type: Poster Presentation**

Viral load and genome integration detection: Two molecular markers for HPV persistent infection  
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**Background:** Detection and quantification of Human Papillomavirus (HPV) are predicting factors associated with cervical cancer progression. Molecular biology techniques based upon amplification are the most sensitive methods for the detection of the viral DNA, especially in patients without apparent lesions, colposcopic changes or pathologic PAP smears. The aim of this study was to determine HPV infection, genotypes and viral load, in a cohort of 250 women in Mérida State (2008-2009) comparing a novel SYBR Green® quantitative real-time (q-RT) polymerase chain reaction (PCR) technology targeting the late-L1 viral genes with an end-point-PCR assay. Additionally, in order to detect HPV integration, we amplified viral E6/E7 ORFs as the target region by end-point PCR. In this assay, consensus primers including HPV18 and HPV16 genotypes were tested.

**Methods:** Samples were collected in transport medium device (Digene®) and DNAs were isolated using QIAmp® DNA isolation Kit (QIAGEN®). Pap smear was performed by Papanicolaou tech.

**Results:** End-point PCR of L1 region was applied to 125 samples, 20% (71/125) were positive for HPV and there was a moderate agreement ( $\kappa = 0.46$ ) with the SYBR Green® q-RT PCR assay. Amplification of early E6/E7 region identified 19.2% (24/125) more positive cases, 50% (12/24) were HPV18, 37.5% (9/24) HPV16 and in 12.5% (3/24) amplification of more than one genotype occurred.

**Conclusion:** In conclusion, target region for amplification of HPV DNA is a determinant factor in the molecular diagnosis of cervical infection. Moreover, quantification of the viral load by qRT-PCR SYBR Green-L1 assay allows to determine HPV infection, viral load, genotyping especially in women with high risk lesions by PAP smear.

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**Type: Poster Presentation**

Rabies cases in dog markets in Kaduna state, northern Nigeria

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**Background:** The study was conducted to determine the potential role of dog markets in the epidemiology of rabies in Kaduna.

**Methods:** Thirty dog brain samples were collected from different dogs brought to the dog markets in Kaduna State, Nigeria. The dog brain tissues were tested using several assays: Direct Rapid Immunohistochemistry Test (dRIT); Direct Fluorescent Antibody Test (DFA); the Mouse Inoculation Test (MIT); and a Reverse Transcriptase- Polymerase Chain Reaction Test (RT-PCR). Both apparently healthy and clinically ill dogs were brought to the markets.

**Results:** Fifteen (50%) samples tested positive for rabies virus antigens. All the samples tested positive by the dRIT and DFA tests, with +4 staining intensity and antigen distribution. The inoculated mice developed signs of rabies by day 9 post inoculation via the MIT. All positive samples were sequenced after RT-PCR detection. Detected rabies viruses were similar to other variants reported previously from Nigeria.

**Conclusion:** In this preliminary study, the presence of rabies was confirmed in the local dog markets, and may play an important role in the spread of rabies. Both the butchers and the buyers are at risk during the processing and sale of the dog meat. Additional work should be done at other animal markets throughout Nigeria. Greater awareness is needed on the danger rabies may pose on both human and animal populations from such activities.

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Clinical and epidemiological aspects of mumps virus infection  
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**Background:** Mumps is an acute viral infection characterized by a non-specific prodrome, followed by acute onset of unilateral or bilateral tender swelling of parotid or other salivary glands. The aim of the study is to describe an epidemic form of mumps viral infection in the region of Strumica.

**Methods:** Retrospective clinical study of 1248 mumps cases diagnosed in the last 2 years in the Department of Infectious Diseases in Strumica.

**Results:** Between 1 March 2008 and 31 March 2009, a total of 1248 mumps cases were notified in our department. In total, 533 cases were hospitalized for clinical indications. The majority of patients had epididymo-orchitis 253 (47%). Unilateral involvement was found in 20-30% of patients, whereas bilateral involvement occurs in fewer than 2% of cases. Other localizations of the mumps infection were unilateral parotitis 225 patients (42,2%), meningitis 43 (8,1%), and pancreatitis 12 (2,25%). The peak incidence of mumps in 2008 was in June 290 patients, July 225 and May 187 patients.

Of the 1248 reported cases of mumps, 49,6 % were between 15-19 years old, and 416 (79%) were male. Vaccination data were available and 96 % of them had been vaccinated. Mumps vaccination coverage has been high since its introduction in 1983, except for a period in the 1992 and 1993. Individuals born in 1992-1993 had coverage only with one dose of mumps vaccine. Patients who had documentation of receiving only one dose of vaccine were at greater risk than those who received two doses.

Treatment was supportive, symptom-based. No deaths have been reported.

**Conclusion:** The main strategies for controlling a mumps outbreak are to define the risk population and transmission setting, identify and isolate suspected cases, and to rapidly identify and vaccinate susceptible persons. Vaccination is the cornerstone of mumps prevention.

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Spatial and temporal trends of the Lassa fever epidemic in Nigeria 2001 – 2009, with particular reference to the Edo State experience

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**Background:** The initial interest following the report of the Lassa fever (LF) epidemic in Nigeria in 1969 waned considerably unlike in the Mano River Basin countries of Sierra Leone, Liberia and Guinea. The NLFSFN was formed in 2007 to meet the need for a structured and sustained response to the control of the epidemic in Nigeria. We describe the spread of the epidemic from 2001-2007, the change in its seasonality, the rise in prevalence and the decline in case fatality rate (CFR) among hospital patients.

**Methods:** Review of surveillance data at the Federal Ministry of Health and the Institute of Lassa Fever Research and Control, ISTH, Irrua and review of the case load and outcome of suspected LF at the ISTH. The diagnosis of LF was based on defined criteria.

**Results:** From 2001 – 2006, 1-3 (average 1.7 or 5%) of the 36 States and the Federal Capital Territory reported LF compared to 7-12 (average 9.7) States in 2007-2009 (OR (95% CI) = 0.26 (0.13, 0.53),  $p < 0.01$ ). Suspected LF constituted 0.53% of 41,440 admissions in 2001-06 compared to 2.16% of 35,484 admissions in 2007-09 (RR (95% CI) = 1.7 (1.64, 1.76),  $p < 0.001$ ). Ninety (76%) of 118 cases of suspected LF managed at the ISTH in 2001-04 were seen in January to March compared to 49/103 (48%) in 2004-05 (RR (95% CI) = 1.9 (1.32, 2.62),  $p < 0.001$ ) and 271/765 (35%) in 2007-09 (RR (95% CI) = 1.56 (1.09, 2.24),  $p = 0.02$  for 2007-09 versus 2005-06). CFR from suspected LF was 97/221 (43.9%) in 2001-06 versus 102/768 (13.3%) in 2007-09 (RR (95% CI) = 0.61 (0.53, 0.7),  $p < 0.001$ ). Overall, the diagnosis of LF was confirmed in 16-25% of cases of suspected LF in 2008-09. In each of the 3 Senatorial Districts in Edo State, the prevalence of suspected LF as seen at the ISTH increased by >149% from 2008 – 2009 while that of confirmed LF increased by >73%.

**Conclusion:** Both awareness of the epidemic and the index of suspicion in clinical diagnosis have increased as immediate gains from the LF control activities of the NLFSFN. The seasonality of LF has changed while the prevalence in Nigeria may have increased, perhaps in part due to the increased awareness and index of suspicion. Assistance of the international community is required to scale up the control initiatives, particularly with regard to capacity building in surveillance, laboratory diagnosis and case management.

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Immunocompromised patients with shingles - Therapeutic approach

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**Background:** The complexity of the therapeutic approach of the immunocompromised patients is determined by the capacity of their immunoresponse corresponding with the arising infection. Among the patients with shingles with a comorbide immunodeficiency syndrome such as primary hypogammaglobulinaemia or secondary lymphopenia as a result of a long lasting immunosuppressive therapeutic treatment that is even more complex, due to threatening viral generalization, including visceral generalization of the illness as well as dermal and pulmonary bacterial superinfection.

**Methods:** During the 6 year long period, 20 immunocompromised patients with shingles were hospitalized and healed in our department. Dermal eruptions were spreaded on several dermatoms, in 8 patients with dermal generalization. Visceral generalization appeared in 4 cases with all consecutive disorders. Diagnostic criteria, including complete blood count (CBC), sedimentation, chemistry profile of renal and liver function tests, bacteriological (inducted expectorated) sputum sample testing, blood culture, pleural fluid analysis, chest x-ray and tomography, were determining moments in the diagnostic procedure and therapeutic treatment.

**Results:** Appearance of the Acyclovir (for oral or i.v. application), as a systemic virostatic with high efficiency, dramatically changed the evolution of the illness in this group of patients, their clinical symptomatology and expression, with the drastic reduction of the sequels and the epilogue of the illness. Due to the effect of viral replication blocking, its efficiency is evident, especially if it is applicated within the first 3 days of the illness (24-48-72 hours after the appearance of the first symptoms) at the period of the most intense viral replication. These patients were parenterally/peroral treated with Acyclovir (Virolex) and antibiotics - Penicillines, II and III generation Cephalosporines, Aminoglycosides, Macrolides, Quinolones, Clindamycin, Lincomycin, Co-trimoxazol.

**Conclusion:** All patients showed satisfying resolve of the dermal lesions, resolution of the pulmonary inflammation and correction of the chemistry profile of renal and liver function as well as abrogation of all clinical symptoms and subjective difficulties. Is it necessary to point out the importance of the substitutional and symptomatic therapy that have to be implemented simultaneously with antiviral and antibiotic therapy, as the only possible and satisfactory way of treatment of this group of patients along with necessary attention and care.

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Peculiar case of herpetic viral encephalitis

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**Background:** Herpetic viral encephalitis is a severe disease, especially in neonates and immunocompromised hosts. HSE with HSV 2 appears more frequently in persons younger than 20 and older than 50. Immunossupresion may be the cause of herpes simplex viral encephalitis.

**Methods:** The case presentation is supported by the information found in this patient's files from three medical services where he was admitted: Clinic of Internal Medicine, Neurosurgery, and finally Clinic of Infectious Diseases of the University of Oradea.

**Results:** A 16 years old rural male patient, presented starting with September 2009 headaches, night sweating, lack of appetite, vomiting, abdominal pain, diarrhea and weight loss, diagnosed as digestive disorder. In October 2009 he was sent to the Clinic of Neurosurgery for persistent headaches, fever, paresthesia of the upper limbs, with the suspicion of a cerebral tumor. At the admission into the hospital, a massive elimination of round worms was observed in his feces. Physical examination revealed tremor of the upper limbs, herpetic vesicles in the genital area and hepatomegaly. The patient declared that he had recently started his sexual life.

Cell blood count and CSF analysis were normal. Cerebral CT revealed a hypodense area in the right frontal lobe and MRI indicated a possible viral/parasites encephalitis. Serological test was positive for HSV 2 IgM antibodies (14.5 UI) and negative for HSV 2 IgG antibodies. Stool test was positive for *Enterobius vermicularis* and *Giardia lamblia*. The patient was transferred to the Clinic of Infectious Diseases. Treatment with Acyclovir 10 mg/kg i.v. was started for 14 days, every 8 hours, associated with Manitol, neurotrophic drugs, and vitamins. The patient was also administered Albendazol 400 mg b.i.d for 14 days, repeated 3 times. The evolution was favorable, without complications.

**Conclusion:** HSE with HSV type 2 is a rare condition. Localization in the frontal lobe is infrequent, with the lesions usually occurring in temporal lobes; CBC and CSF in normal range are also rare for HSE. The association with enterobiasis and lambliasis might have produced the immunodepression in our case, other favorizing factors being pubescent age, and the debut of sexual life.