

Final abstract number: 14.001

Session: New Insights into Infectious Diseases (Oral Presentation)

Date/time: Friday, 20 June, 2008, 15:45-17:45 hrs

Room: 301

Human Chromosome 17q11.2-q22 Contains Typhoid Fever Susceptibility Genes

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The human chromosome 17q11.2-q22 region contains a number of interesting and biologically plausible candidate genes for infectious disease susceptibility. Evidence for the chromosome 17q11.2-q22 region being associated with tuberculosis and leprosy encouraged us to pursue this region in genetic studies of typhoid fever. Using a case control study we have tested for genetic association between polymorphic markers within this cluster of immune response genes which may be associated with typhoid fever.

Whole genome amplified DNA from 396 culture confirmed typhoid fever cases and 380 cord blood controls (Vietnamese Kinh) were genotyped at 37 SNPs spanning 14 genes (CRLF3, NOS2A, CCL1, CCL2, CCL3, CCL4, CCL11, CCL13, CCL15, CCL16, CCL18, CCL23, STAT3 and THRA1) using the Invader assay (Third Wave Technologies). 13 SNPs with minor allele frequency >10% and call rate >98% were subsequently analysed using unconditional logistic regression performed within STATA v8.0. The case/control genetic analysis showed 2 SNPs in the gene NOS2A encoding inducible nitric oxide synthase (iNOS), specifically NOS2A-277 and NOS2A/rs16949, were associated with typhoid fever (nominal P=0.02 for both SNPs). Individuals carrying these SNPs had an increased risk of typhoid fever (ORs=1.45 and 1.46, respectively). A higher disease risk was seen in individuals that have two copies of the minor alleles of these two SNPs [SNP NOS2A-rs16949 (OR=2.5, 95%CI 0.92-7.95, nominal P=0.05); SNP NOS2A-277 (OR=3.3, 95%CI 1.03-14.02, nominal P=0.03)].

Although the chromosome 17q11.2-q22 region has a large number of candidate immune response genes, only evidence to support a role of the NOS2A gene in typhoid fever was identified in this study. We are currently typing more SNPs in NOS2A with the aim to identify the disease causing mutation. Future work may involve functional studies based on the causative mutation to clarify the biological role of iNOS in typhoid fever.

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Role of ORF 1 Polymorphism, Viral Load and Cytokine Response in the Pathogenesis of Acute and Fulminant Hepatitis E Virus Infection

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Background: Viral hepatitis is one of the major health concerns worldwide. Amongst the hepatitis viruses, hepatitis E virus (HEV) can lead to acute as well as fulminant hepatitis. The viral and immune mediated factors possibly play a role in the immunopathogenesis of HEV related hepatic damage. However, the exact characterization of factors related to acute and fulminant hepatic damage has never been explored. Thus, the present study was carried out to understand the acute and fulminant HEV related hepatitis, in regard to viral load, cytokine production and genetic polymorphism.

Methods: HEV confirmed (by IgM and RT-PCR) acute viral hepatitis (AVH) and fulminant hepatic failure (FHF) cases were included as test group I & II. Asymptomatic HEV negative individuals were taken as controls. Viral RNA load was measured by real time PCR. Polymorphism in the ORF1 gene sequence of HEV strains obtained from acute and fulminant hepatitis patients was studied by RT-PCR followed by nucleotide sequencing. The level of Th1 and Th2 cytokines (TNF- α , IFN- γ , IL-4 and IL-10) were measured by quantitative micro ELISA.

Results: Mean viral load was found to be higher in AVH (83,625 copies/ μ l) as compared to FHF (3782.6 copies/ μ l). All the isolates from AVH and FHF patients were placed under genotype-1 and the isolates from AVH showed 98-99% homology to the North Indian strain. The level of TNF- α , IFN- γ and IL-10 in FHF patients were significantly higher as compared to that in AVH patients, whereas the level of all the 4 cytokines were higher in FHF patients as compared to healthy controls. Overall Th2 predominance was observed in both AVH and FHF patients.

Conclusion: Thus the present study highlights the immune mediated pathogenesis over the viral burden in HEV related acute and fulminant hepatic damage.

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Session: New Insights into Infectious Diseases (Oral Presentation)

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Genome Wide Search for Visceral Leishmaniasis Susceptibility Genes in Sudanese Population

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Sudan is one of the major foci of visceral leishmaniasis (VL).

Familial clustering and ethnic differences suggest genetic factors may be involved in the infection. In this study a two stages genome-wide scan was employed using two independent sets of families from two villages (El-Rugab and Um-Salala) inhabited by the Masalit ethnic group and located in the endemic area -eastern Sudan.

In the first stage (= scan1) 400 highly polymorphic microsatellite markers were typed in 220 individuals from 38 multicase pedigrees (= scan1 families).

In scan 1, the multipoint analysis performed provided evidence for linkage of VL susceptibility to 5 regions on chromosome 1p22, 1q31.3, 5q34-35.3, 6q27 and 13q 31 (logarithm of the odds LOD 0.0002 <p< 0.05) for linkage.

Stratification of scan1 families by village (after the grid tightening strategy was employed in which 35 additional markers were added close to regions that gave suggestive evidence for linkage of VL susceptibility in scan1) revealed village-specific peaks for linkage: in Um Salala at 1p22 and 5q34 ($p=1.6 \times 10^{-4}$, $p=0.047$ respectively), in El Rugab at 1q31.1, 5q35.3 and at 6q27 ($p=0.007$, $p=0.002$, $p=8.95 \times 10^{-5}$)

To confirm linkage; family set 2 (=scan2 families: 21 nuclear families, 48 affected sibs) were genotyped across these regions.

Analysis of scan1+2 families stratified by village demonstrated a major gene on 6q27 (LOD score 3.07; $p=8.6 \times 10^{-5}$) in El-Rugab only. A broad region of linkage on chromosome 1 also resolved into two clear peaks upon stratification by village: on 1p22 (LOD score 1.19; $P=0.009$) for Um-Salala and on 1q31.1 for El-Rugab (LOD score 1.25; $p=0.008$). These results indicate that VL susceptibility might be complex inheritance and that population substructure could be vital in the implication of the disease in different populations.

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Session: New Insights into Infectious Diseases (Oral Presentation)

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Microarray Analysis of Gene Regulation in *Streptococcus pneumoniae* Upon Penicillin Exposure

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Background: *Streptococcus pneumoniae* is a major cause of morbidity and mortality, with presenting invasive infection such as lobar pneumonia, bacteremia and meningitis. The emergence of penicillin resistant strains since the 1970s has been life threatening. In order to curb this problem, a better understanding of the mechanisms of antibiotic resistance is essential. In this study, the Affymetrix gene chip array was used to study a wider range of genes that may play a role in the development of antibiotic resistance.

Methods: 3 confirmed strains of *S. pneumoniae* (known MIC: Sensitive, Intermediate and resistant to Penicillin) were used. Total RNA of 3 strains were extracted using the hot acid phenol method. cDNA synthesis, fragmentation, labeling and hybridization were carried out according to manufacturer's protocol (Affymetrix). Labelled cDNAs were hybridized onto respective gene chip expression arrays masked with probes representing the known genome of *S. pneumoniae*. The strains were further treated with penicillin prior to extraction of RNA to elucidate the effect of antibiotic in the expression of genes. Scanning and analysis was done using the GCOS software. To further confirm that the induction of gene expression was specific for penicillin or other beta-lactam drugs which involves inhibition of cell wall synthesis, the strains were exposed to other antibiotics such as cefotaxime, and ceftriaxone, and the relative mRNA expression were measured using real-time PCR.

Results: Significant differences within the gene expression of the genome were observed among the 3 categories of strains; Penicillin Sensitive *S. pneumoniae* (PSSP), Penicillin Intermediate *S. pneumoniae* (PISP) and Penicillin Resistant *S. pneumoniae* (PRSP). Functional genes with significant expression levels include genes encoding transport, transcription regulation, two component signal transduction, ribosomal proteins and cell surface proteins. Genes which are involved in the biosynthesis of the cell wall envelope showed to have significant expression levels upon penicillin stress. These genes include the genes encoding the penicillin binding proteins, choline binding proteins and D-alanylation of cell wall. Exposure with the other beta-lactam drugs showed variation in the expression which was induced by the specific drugs.

Conclusion: The penicillin binding proteins (PBPs), encoding the formation of the cell wall proteins and the choline binding proteins (CBPs) had significant levels of expression, which correlated with the initial antimicrobial susceptibility of the strains. This shows that antibiotic stress has an effect on the bacterial physiology and gene regulation. Understanding the mechanisms of antibiotic resistance may lead to a proper management of pneumococcal infections.

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Session: New Insights into Infectious Diseases (Oral Presentation)

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Diversity of Coronaviruses in Bats: Insights Into Origin of SARS Coronavirus

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Background: Although the finding of SARS coronavirus (SARS-CoV) in caged palm civets suggested that wild animals are the origin of SARS-CoV, subsequent studies suggested that civet may have served only as an amplification host. In 2005, we identified a coronavirus closely related to SARS-CoV (bat-SARS-CoV) in Chinese horseshoe bats. However, it remains to be determined if bat-SARS-CoV or other coronaviruses in bats are the direct progenitor of SARS-CoV.

Methods: To understand the diversity and evolution of coronaviruses in bats, a 2-year surveillance study for coronaviruses was conducted in bats from various rural areas in Hong Kong. As coronaviruses are known to have high recombination frequency, the genomes of the identified novel coronaviruses were also sequenced and analyzed to determine possible recombination events responsible for interspecies transmission.

Results: Among 1389 bats of 16 species from 24 different locations, coronaviruses were identified from anal swabs of 132 (9.5%) bats by RT-PCR. Phylogenetic analysis revealed at least seven novel coronaviruses from seven different bat species, in addition to bat-SARS-CoV. Five of them belonged to group 1 coronaviruses while two belonged to group 2 coronaviruses. Besides bat-SARS-CoV, Chinese horseshoe bats were found to harbor another novel group 1 coronavirus. The genome of this virus represents the smallest coronavirus genome and possessed a unique spike protein evolutionarily distinct from the rest of the genome and containing a 15-amino acid peptide homologous to a corresponding peptide within the RBM of spike protein of SARS-CoV, suggesting a common evolutionary origin in the spike protein of this group 1 bat coronavirus, bat-SARS-CoV, and SARS-CoV.

Conclusion: Bats are important reservoir for a huge diversity of coronaviruses, including SARS-CoV-like viruses. The finding of another group 1 coronavirus in Chinese horseshoe bats with a homologous peptide to SARS-CoV warrants further investigations on the origin of the SARS-CoV spike protein.

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Session: New Insights into Infectious Diseases (Oral Presentation)

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Analysis of Putative Virulence Factors in *Enterococcus faecium* Isolated from Different Sources in Iran

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Background: Enterococci have emerged as a leading cause of nosocomial infections. The potential virulence factors include a cytolytic toxin, gelatinase, an aggregation substance, the enterococcal surface protein (Esp) and hyaluronidase. Our knowledge about the contribution of these virulence factors to the pathogenesis of enterococcal infections is still limited.

Methods: A total of 98 strains containing, 49 clinical Vancomycin-resistant *Enterococcus faecium* (cl-VREFm) and 49 environmental VREFm (en-VREFm), were included in this study. The phenotypical characterization was performed by their abilities to adhere to Vero cell line and to associate their phenotypes with the presence of known virulence genes detected within their genomes by PCR. The following genes were amplified by PCR: asa1 (aggregation substance), cyl A, B, M (cytolysin), hyl (hyaluronidase), gelE (gelatinase) and esp (enterococcal surface protein). The transferability of esp gene were examined by filter mating tests. The strains were typed by Pulsed-field gel electrophoresis.

Results: The genes that encode cytolysin and aggregation substance was never detected in isolates, whereas gelE and esp genes was detected in both clinical and environmental strains and hyl gene was only detected in clinical isolates (28%). The following data were obtained in this study: 81% of cl-VREFm and 80% of en-VREFm isolates were positive for esp gene, the gelE gene was detected in all of clinical and 47% of environmental isolates. Strong adhesion was observed only in clinical strains. None of the esp genes were transferable by conjugation tests. According to PFGE results the isolates were heterogeneous.

Conclusion: Environmental isolates were equipped with fewer virulence factors than clinical isolates and presence of virulence factors in environmental isolates demonstrates that they can be potentially virulent for human. There was a correlation between PCR and phenotypic tests in clinical strains. Phenotypic testing revealed the existence of apparently silent gelE and esp genes among environmental strains.

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Session: New Insights into Infectious Diseases (Oral Presentation)

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Immunogenicity of Novel Consensus-Based DNA Vaccines Against Chikungunya Virus

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CHIKV is an emerging arbovirus and is an important human pathogen that causes a syndrome characterized by fever, headache, rash, nausea, vomiting, myalgia, arthralgia and occasionally neurological manifestations such as acute limb weakness. It is also associated with a fatal haemorrhagic condition. CHIKV is geographically distributed from Africa through Southeast Asia and South America, and its transmission to humans is mainly through *Aedes* species mosquitoes. The frequency of recent epidemics in the Indian Ocean islands suggests that something else was carrying the virus, as *Aedes aegypti* are not found there. In fact, the relative Asian tiger mosquito, *Aedes albopictus*, was present and has raised concern in the world health community regarding the potential for a CHIK virus pandemic. Efforts to monitor the disease will only provide minimal warning in a global society, and steps must be taken to prevent the morbidity and mortality associated with a possible pandemic. There is no specific treatment for Chikungunya virus and there is no vaccine currently available. We propose a novel consensus-based approach to vaccine development, employing a DNA vaccine strategy that can provide more highly cross-reactive cellular immunity against CHIK virus. The vaccine cassette was designed based on Capsid (Cap) and Envelope (E1) specific consensus sequences with several modifications, including codon optimization, RNA optimization, the addition of a Kozak sequence, and a substituted immunoglobulin E leader sequence. The expression of Cap and envelope E1 was evaluated using T7-coupled transcription/translation and immuno blot analysis. A recently developed, adaptive constant-current electroporation technique was used to immunize mice (both Balb/C & C57BL/6 mice strain) with an intramuscular injection of plasmid coding for the CHICK-Cap and E1. We show such constructs can induce strong cellular immunity against CHIK-Cap and E1 antigens. The analysis of specific antibody responses suggested that CHIK-E1 could induce a strong E1 specific antibody response. Epitope mapping results indicated that there is an increase in the breadth and magnitude of cross-reactive cellular responses induced by both the Capsid and Envelope immunogen. These properties suggest that such a consensus immunogen deserves further examination for its potential to serve as a component antigen in a CHIK vaccine cocktail.

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Post-Exposure Vaccination with a Highly Attenuated Vaccinia Vaccine, LC16m8, for Protection of Nonhuman Primates from Monkeypox

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Introduction: A highly attenuated smallpox vaccine, LC16m8, is generated from parent strain, *vaccinia virus Lister*, by passages of Lister in primary rabbit kidney cells at a temperature of 30°C. LC16m8 lacks expression of the full-length B5R membrane protein, one of the most important viral membrane proteins for induction of immune response to vaccinia virus, due to a frameshift mutation in the gene. A single vaccination with LC16m8 protects non-human primates (NHPs) from monkeypox, monkeypox virus infection. In the present study, the efficacy of LC16m8 as a therapeutic vaccine was evaluated in protection of NHPs from monkeypox.

Materials and Methods: Twenty-two NHPs (*Macaca fascicularis*) were used. The NHPs were immunized with LC16m8 or mock followed by subcutaneous inoculation of monkeypox Zr-599. Three were mock-immunized and infected with the virus. Three were infected with the virus and then immunized with LC16m8. Three, 3, and 5 NHPs were infected with the virus 3, 7, and 14-24 days after vaccination, respectively. Clinical manifestations were monitored. Viremia level and antibody response were determined by the quantitative real-time PCR and IgG-ELISA, respectively. Pathological examination was carried out in these subjects.

Results: Post-exposure vaccination with LC16m8 for NHPs, which were infected with monkeypox virus, improved the clinical manifestation of monkeypox, while all the naive subjects were nearly lethal. Vaccination with LC16m8 7 days before monkeypox virus challenge completely protected NHPs from monkeypox. Viremia level in the post-exposure vaccinated subjects was significantly lower than that in the naive subjects.

Discussion: LC16m8 has been re-produced and stockpiled in Japan for the possible threat of bioterrorism with variola virus as a bioweapon. Based on these results, it is suggested that LC16m8 may protect humans from smallpox, if they were immunized with LC16m8 immediately after the event of variola virus infection.

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Distribution and Genetic Diversity of *Plasmodium falciparum* Erythrocyte Binding Antigen 175 and Clinical Outcome of Malaria in the Kassena-Nankana District

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The Erythrocyte Binding Antigen 175 (EBA 175) is a 175 kDalton *Plasmodium falciparum* antigen which plays a major role in erythrocyte recognition by the parasite. It also induces antibodies which inhibit merozoite invasion. EBA 175 has been sequenced from FCR-3 and CAMP strains of *Plasmodium falciparum*. The sequences were identical in most parts of the gene, differences were apparent in the 423bp segment in the FCR-3 strain, the F-genotype, and the 342bp segment, the C-genotype. Parasite strains possess either one or the other segment and never both. The functions and potential effects of this dimorphism remain unclear. This study therefore investigated the relationship between this dimorphism and clinical outcome of malaria. A nested polymerase chain reaction (PCR) was used to determine the genotypes of the parasite strains that exhibit this dimorphism in severe, mild and healthy controls of malaria from the Kassena Nankana District (KND), an area which had been earmarked for future vaccine trial. A total of 299 samples were analysed, 232 of these samples were positive with *Plasmodium falciparum* infections, these comprised 75 (32.2%), 76 (32.6%) and 81 (35.5%) samples of severe, mild and healthy controls of malaria respectively, were genotyped for the EBA-175 gene. The severe samples, had a distribution of 44 (58.66%), 24 (32%) and 7 (9.3%) of the F, C and CF EBA-175 genes respectively; the mild samples comprised, 42 (55.3%), 29 (38%), and 5 (6.6%) of the F, C and CF EBA-175 genes respectively; the healthy controls: 59 (72%), 21 (26%), and 13 (16%) of the F, C and CF EBA-175 genes respectively. A chi-square test revealed that the mixed genotype (CF) is significantly associated with severe malaria ($p=0.04$; OR= 8.23, 95% CI = 1.048 - 64.7), whereas the F genotype is significantly associated with protection to severe malaria ($p= 0.045$; OR = 0.909, 95% CI = 0.819- 1.008). The results therefore suggest that whilst the mixed genotype CF EBA-175 is associated with malaria severity, the F genotype of EBA-175 is protective.

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Session: New Insights into Infectious Diseases (Oral Presentation)

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Mycobacterium avium KatG protein (MAV_2753): Possible Role in the Pathogenesis of MAC disease

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Background: The dissemination of *Mycobacterium avium complex* (MAC) disease could be due to certain mycobacterial factors causing cytotoxicity of host cells, thereby leading to lysis of the cells and spreading of infection. Recently, in our lab, an 82kDa *M. avium* immunodominant protein was identified to be *M. avium* KatG homologue (MAV_2753). The BLAST search showed the N-terminal 40 amino acids of *M. avium* KatG to be 100% different from the N-terminal of *M. tuberculosis* KatG. Present study was designed to explore the role of *M. avium* KatG protein in the interaction with the host cells and its possible role in the pathogenesis of MAC disease.

Methods: Organ specific (human alveolar macrophages and A549 lung epithelial cell line) and systemic cells (human blood monocyte derived macrophages) were used to study the interaction of *M. avium* KatG protein in terms of various cytological, molecular and biochemical changes.

Results: *M. avium* KatG protein showed significant binding with the host cells and resulted into significant reduction in the percent cellular viability ($p < 0.001$) thereby depicting its cytotoxic influence. The fragmentation of cellular DNA of the KatG treated cells into smaller fragments demonstrating characteristic 'ladder like pattern' and the exposure of phosphatidylserine on the outer leaflet of the plasma membrane as represented by green halo and membrane blebbing further defined these cytotoxic effects as apoptosis. The apoptosis of the host cells, as an effect of KatG protein was mediated by ROS generation, caspase-3, caspase-7 and PARP activation but not by TNF-alpha production. An increase in the cellular viability following treatment with an antioxidant, further confirmed the role of ROS in KatG mediated apoptosis.

Conclusion: KatG protein of *M. avium* can bind to host cells, leading to their killing by apoptosis and might play a role in the cell to cell dissemination and pathogenesis of the disease.

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Session: Exciting Cases in Infectious Diseases (Oral Presentation)

Date/time: Saturday, 21 June, 2008, 10:1512:15 hrs

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Postpartum Onset of HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP): A Report of 7 Cases from Peru

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Background: HAM/TSP is a chronic progressive disease that causes impairment of the lower limbs and occurs in 1-5% of HTLV-1 carriers. The disease affects women three times more frequently than men.

Methods: We describe 7 cases of women in whom HAM/TSP initiated within 6 months after delivery. They are all participants of the HTLV-1 cohort at the Institute of Tropical Medicine Alexander von Humboldt in Lima. HAM/TSP diagnosis was based on WHO criteria.

Results: The age at HAM/TSP onset ranged from 24-39 years (mean: 31.4). The initial symptoms were noticed 3 to 16 weeks after delivery (mean: 8.8). Three of 6 women for whom information was available developed HAM/TSP after the birth of their first child. Four women were of Andean and 3 of mestizo origin. None of the women reported a family history of HAM/TSP. Information about the initial symptoms was available for 6 women: 3 had lower limb weakness, 2 reported sensory symptoms, and 1 woman had urinary incontinence. During the course of disease, all developed the classic clinical presentation of HAM/TSP. Finally, 2/7 presented rapidly progressive HAM/TSP, defined as the inability to walk unaided within 2 years after HAM/TSP onset.

Conclusion: This is the first report of several cases of postpartum HAM/TSP. Several reports have shown that HAM/TSP patients have a higher proviral load than asymptomatic carriers. In one study, this difference was significant only among women. It has also been described that clinical progression is faster in women than in men, especially in pre-menopausal women. We suggest that pregnancy could cause a state of immunosuppression with diminished cytotoxic T-cell response, expansion of HTLV-1-infected cells, and increased proviral load. After delivery, the immune system returns to normality and the high proviral load might lead to an intense inflammatory response in the central nervous system causing HAM/TSP.

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Session: Exciting Cases in Infectious Diseases (Oral Presentation)

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Clinico-Epidemiological Study of Pure Neural Leprosy From a Tertiary Hospital in Delhi, India

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Background: Pure neural leprosy (PNL) continues to be common in India. This form of disease is least studied and very little information exists in literature. We analyzed the demographic profile and clinical aspects of PNL at our center.

Methods: Retrospective analysis of confirmed PNL registered in leprosy clinic in our institute between January 2003 and July 2007 was undertaken. Demographic and clinical profile including onset of disease and presenting complaints, pattern of nerve involvement, presence of nerve abscess and deformities were analyzed. Investigations such as slit-skin smear (SSS), skin biopsy, electrophysiological study (EPS) and nerve biopsy were done.

Results: Of 1975 leprosy cases seen during this period, 188 (9.5%) had PNL which included 160 (85%) males and 28 (15%) females, with 121 (64.36%) cases within 20-40 years. Presenting symptoms were paresthesia, pain, sensory loss and motor weakness. Majority of the patients i.e 119 (63.3%) had 2-5 nerve involvement while 49 (26%) had > 5 nerve involvement. Ulnar nerve was most commonly involved in 130 (69.14%) cases followed by common peroneal in 91 (48.4%). Deformities included claw hand in 50 (26.6%), foot drop in 21 (11.17%) facial palsy in 2 and wrist drop in one patient only. Skin biopsies were non-specific in all cases. Nerve biopsy (n=17) revealed features ranging from normal, to infiltration with epithelioid cell granulomas, fibrosis, lymphohistiocytic infiltrate and AFB positive foamy histiocytes. EPS showed features of sensory/ motor axonal neuropathy, demyelination, denervation with poor to moderate reinnervation or decreased sensory nerve action potential.

Conclusions: PNL is a distinct type of leprosy in India. Men are more commonly affected and ulnar nerve involvement is the most common manifestation. Sensory complaints are early and more common. Early diagnosis and treatment is helpful in preventing sequel due to nerve damage.

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Session: Exciting Cases in Infectious Diseases (Oral Presentation)

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Brucellar Epididymo-Orchitis: Review of 45 Cases in Babol North of Iran

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Background. Brucellar epididymo-orchitis is frequently seen in endemic regions of brucellosis. The purpose of this study was to assess the clinical manifestations and outcome of treatment on 45 cases of brucellar epididymo-orchitis.

Methods. From September 2000 to September 2007, 45 cases of epididymo-orchitis were treated and followed at the Research Center of Infectious Diseases of Babol Medical University in north of Iran. The clinical manifestations and outcome of treatment were recorded.

Results. The mean age of the patients was 32.5 ± 14 years. The mean duration of the onset of disease to diagnosis was 21.5 ± 12 days. Simultaneous involvements of other organs were seen in 19 (42.2%) cases. Thirteen cases (28.8%) were treated by co-trimoxazole + rifampin for two months and relapse was seen in 1 (7.7%) case and orchitomy was performed in 2 (15.4%) cases due to destruction of the testis. Thirteen cases were treated by co-trimoxazole + doxycycline for two months and relapse was seen in 1 (7.7%) case. Nineteen cases were treated by gentamicin for seven days and doxycycline for 45 days and relapse was seen in 1 (5.3%) case. The efficacy of gentamicin plus doxycycline (94.7%) or cotrimoxazole plus doxycycline (92.3%) were higher than cotrimoxazole plus rifampin (76.9%) ($p < 0.05$).

Conclusion. The results of this study show that simultaneous involvement of other organs are frequently seen in subjects with epididymo-orchitis. Treatment with gentamicin plus doxycycline or cotrimoxazole plus doxycycline both are recommended for therapy of brucellar epididymo-orchitis.

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The Expanded Access Program to HAART in Chile: Baseline Characteristics and Primary Outcomes of Patients Receiving Treatment, Evaluated Through the Chilean AIDS Cohort (ChiAC)

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Background: Chile, a middle-income country with a population of 16.2 million, has a HIV prevalence of $\approx 0.3\%$ in adults. Since 2001 there is an expanded access program (EAP) for HAART that reached 100% coverage in 2003; around 13,000 patients are under health care, 85% in the public health system (PHS). 70% of the patients under control required and received treatment, according to the national guidelines. Distribution of therapy is centralized and each request is reviewed and approved on an individual basis. The system provides for free: HAART, CD4, viral load (VL) and genotype tests as well as additional budget for diagnosis and treatment of AIDS related diseases. The outcomes of the EAP in the PHS, have been evaluated by ChiAC, which has enrolled almost 95% of patients on HAART since 2001.

Methods: ChiAC database was analyzed for baseline (BL) characteristics and primary outcomes: mortality, maintenance of initial regimen and AIDS free survival. Descriptive statistics was used.

Results: In 3649 treatment naive patients (15% female) on HAART, with a follow up (f/u) of 9442 patient-years: BL CDC status: A:23%, B:23.6%, C:53.4%; BL CD4 below 200 cells/ μL :83.4% (51% $<$ 100). Most common first HAART regimen was Combivir® plus efavirenz. Less than 10% received protease inhibitors initially.

Outcomes at 3 years of f/u: 57.5% remained on initial therapy, 20.6% on second HAART, 4.9% had ≥ 3 HAART regimen. Mortality by BL CD4 count: CD4 0-99: 12.5%, CD4 100-199: 3.5%, CD4 $>$ 200: 3.1%. Change of HAART due to toxicity: 47%. VL $<$ 400 and $<$ 80 cps/mL at 3 years were 77.4% and 54.3%, respectively.

Overall survival at 3 and 5 years of HAART was 90.5% and 88% respectively; AIDS free survival at 3 and 5 years was 86% and 83% respectively.

Conclusions: The EAP to ART in Chile is succeeding in achieving high viral control and survival of HIV patients. These results are comparable to those of high income countries. ChiAC is a useful tool for evaluating the impact of the program.

Final abstract number: 32.005

Session: Exciting Cases in Infectious Diseases (Oral Presentation)

Date/time: Saturday, 21 June, 2008, 10:15:15 hrs

Room: 301

Ocular Toxocariasis in Peru: A Report of 16 Cases

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Background: Toxocariasis is a worldwide parasitic zoonosis caused mainly by *Toxocara canis*. This helminth invades the eye and cause blindness. We aimed to describe clinical features and complications among patients with ocular toxocariasis evaluated at a referral center.

Methods: Charts, retinographies and ocular ultrasound studies of patients with ocular toxocariasis were reviewed from 2000 to 2007 at Hospital Nacional Cayetano Heredia, Lima, Peru. The diagnosis of ocular toxocariasis was based on the presence of typical clinical features and the absence of an alternative diagnosis. Demographic and clinical features were evaluated.

Results: We report 16 patients, 9 females, the ages ranged from 7 years to 29 years (mean, 14.2 years). A history of contact with a puppy was reported by 92%. The principal symptoms were visual loss (87.5%) and strabismus (37.5%), 2 cases (12.5%) presented leukocoria. The mean time between the first symptom and the first consult was 1.6 year. The eye fundus exam showed the presence of peripheric granuloma (50%), posterior pole granuloma (37.5%) and vitreous retinal bands (50%). Fifteen cases (94%) had unilateral involvement. None of the patients had systemic manifestation. The complications were tractional retinal detachment in 6 patients (37.5%), 5 of them were detected by ultrasonography, 1 case (6%) glaucoma and 1 (6%) choroidal neovascularization. Serum ELISA for *T. canis* was positive for 12 patients (75%), negative 1 patient (6%), and unknown 3 patients (19%). Eosinophilia was found in 2 patients (15%). Four patients were treated with albendazole.

Conclusion: Ocular toxocariasis affects mainly children and young adults. This condition is unilateral in most cases and presents as a granuloma in the peripheral retina or in the posterior pole. Serum ELISA for *T. canis* could help to support the diagnosis. The most frequent complication was retinal detachment with significant visual loss.

Final abstract number: 32.006

Session: Exciting Cases in Infectious Diseases (Oral Presentation)

Date/time: Saturday, 21 June, 2008, 10:15:15 hrs

Room: 301

RNA Viruses Are an Important Cause of Community-acquired Pneumonia in Nepalese Children Living in a Semi-urban District in Kathmandu Valley

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Background: Pneumonia is among the main causes of illness and death in children <5 years of age. Viral etiological data based on molecular diagnosis of community acquired pneumonia (CAP) in developing countries are scarce.

Methods: We examined nasopharyngeal aspirates from 2226 two-35 (mean 13.4) month old Nepalese children with CAP over a three-year period. The specimens were evaluated for viral pathogens using RT-PCR.

Results: We identified 901 virus isolates in 872 (39.2%) of the 2226 specimens, of which 325 (14.6%) yielded respiratory syncytial virus (RSV), 7.3% influenza (Inf) A, 5.8% parainfluenza virus (PIV) 3, 4.4% PIV1, 4.0 % human metapneumovirus (hMPV), 3.7% InfB and 0.7% PIV2. The children infected with RSV had more severe illness than other children (including those infected with any of the other viruses); RSV being identified in 28.4% of the 127 children with lower chest indrawing, in 17.9% of the 671 children with hypoxia (spO₂<93%) and in 20.3% of the 625 cases with crepitations. Crepitations were observed in 31.4% of children who tested positive for virus, in 27.9% of children who were negative, and in 40.6% of RSV cases. There were two yearly incidence peaks of pneumonia during the two first years of our study, one coincided with the end of the monsoon season in August-September, the other occurred during winter. There was an end-monsoon, but no winter peak, during the 3rd year of our study. The incidence of viral pneumonia followed the same seasonal pattern. We observed three distinct epidemics with RSV, all coinciding with the highest peaks of pneumonia.

Conclusion: RNA viruses are an important cause of CAP in our study children. The most commonly isolated virus, RSV, yielded a more severe clinical presentation than the other viruses and occurred in epidemics, both during the winter and in the end-monsoon period.

Final abstract number: 32.007

Session: Exciting Cases in Infectious Diseases (Oral Presentation)

Date/time: Saturday, 21 June, 2008, 10:15:15 hrs

Room: 301

East African Trypanosomiasis in Travellers

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Background: Although there has been a resurgence of trypanosomiasis in many countries in sub-Saharan Africa since the early 1970s, the risk to short-term travellers and expatriates remains small. However, East African trypanosomiasis (EAT) is an acute life-threatening disease, and diagnosis and treatment are challenging.

Methods: NICD is a centre for surveillance of communicable diseases and operates an advisory service for clinicians and public health practitioners. Serious and unusual imported cases therefore come to our attention. We describe the epidemiological features, clinical presentation and outcomes of 18 travellers with EAT managed in South Africa between 2001 and 2008.

Results: Most patients acquired the infection in Malawi, notably in the Kasungu National Park, with the remainder presenting after visiting game parks in Kenya, Zimbabwe, Uganda and Tanzania. Thirteen patients were tourists, one was a Zambezi valley farmer and 4 were involved in conservation or army field exercises. Incubation periods were generally less than 10 days, and disease was typically acute with fever and headache, and trypanosomal chancres in at least 50%. Malaria was the most frequent misdiagnosis and several patients received malaria treatment despite persistently negative laboratory tests. Thrombocytopenia and varying degrees of renal dysfunction were typical laboratory findings. Three patients had central nervous system involvement (presence of trypanosomes, and/or leucocytosis and/or raised protein on examination of cerebrospinal fluid) and required melarsoprol treatment. Suramin treatment was generally well-tolerated with renal dysfunction in only one patient. Three patients died; one with myocarditis and arrhythmia, one with multi-organ failure, and one with a likely melarsoprol-induced encephalopathy. Two patients were reported to have relapsed after treatment.

Conclusions: East African trypanosomiasis should always be considered in the differential diagnosis of a febrile syndrome in travellers from countries where the disease is endemic. Disease is frequently complicated and expert laboratory support and clinical management is generally required.

Final abstract number: 32.008

Session: Exciting Cases in Infectious Diseases (Oral Presentation)

Date/time: Saturday, 21 June, 2008, 10:15:15 hrs

Room: 301

Is Pregnancy Outcome Influenced by Chikungunya Infection? A Case-Control Study in 1401 Pregnant Women Enrolled in the CHIMERE Cohort

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Background: In 2005-06 a Chikungunya virus outbreak infected 38% of Runion Island population. Forty-two cases of mother to child transmission were described for the first time. The purpose of the CHIMERE cohort study was to determine the consequences of Chikungunya infection on pregnancy outcomes.

Methods: In 2006, 1401 pregnant women were enrolled in the CHIMERE cohort. The diagnosis of Chikungunya was based either on serology (IgM & IgG Chikungunya specific serology) planned at inclusion and at delivery, or RT-PCR performed in case of symptoms. We determined that 584 women were not infected by the virus at delivery (IgM- and IgG-), 648 were infected antepartum (IgM+, RT-PCR+, or IgG seroconversion) and 27 before pregnancy, date of infection was imprecise for 50 and assessment was incomplete for 92. We compared pregnancy outcome (prenatal hospitalization, fetal-loss and stillbirth, premature delivery, mode of delivery, birth weight, fetal malformations, newborn hospitalization) between the 584 women free of Chikungunya infection and the 648 infected during pregnancy. This prospective multicentric study has been approved by IRB (CPP de Tours, France).

Results: For the 648 women infected by Chikungunya during pregnancy, the infection occurred during the first, second, and third trimester for 15, 59 and 26%, respectively; 50% presented fever, 94% arthralgia and 75% skin rash. The only difference between non-infected and infected women was the number of hospitalization during pregnancy (28 versus 42%, p=0.0001). Other outcomes, fetal loss and stillbirth (2.2 versus 2.0%), premature delivery (12 versus 10%), cesarean rates (18 versus 16%), birth weight (3067 versus 3121g), fetal malformations (5.0 versus 5.7%) and newborn hospitalization (6.7 versus 7.4%) were similar.

Conclusion: We do not find any impact of Chikungunya infection on pregnancy outcomes except for the number of prenatal hospitalizations.

Final abstract number: 32.009

Session: Exciting Cases in Infectious Diseases (Oral Presentation)

Date/time: Saturday, 21 June, 2008, 10:15:15 hrs

Room: 301

Pilot Study of Magnesium Sulphate in Adults with Tetanus

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Background: Recent data suggests that Magnesium sulphate alone improves clinical outcome in tetanus, but this has not been confirmed.

Aim: To examine the efficacy and safety of intravenous magnesium sulphate for control of rigidity, spasms and autonomic instability in tetanus.

Methods: This was a pilot prospective clinical study of intravenous magnesium sulphate in 35 consecutive adult patients with tetanus over a period of two years in a tertiary teaching hospital. All patients received human tetanus immunoglobulin, tetanus toxoid and parenteral antibiotics. Intravenous magnesium sulphate 20mg/kg was administered followed by 1.0 mg/hr infusion. The infusion rate was increased by 0.5 mg/hr every two hours until cessation of spasms or abolishment of patellar tendon jerk, whichever occurred earlier. The primary outcome measure was efficacy determined by control of spasms (defined as less than two brief spasms within 60 minutes). Secondary outcomes included frequency of autonomic instability, duration of ventilatory support, hospital stay and mortality.

Results: At presentation, the frequency of severity of tetanus was as follows: Grade I: 5 (14%), Grade II: 13 (37%), Grade III: 16 (46%) and Grade IV: 1 (3%). Rigidity and mild spasms were controlled with magnesium therapy 6 patients (17%), all were Grades I and II. Grading worsened in 22 patients (63%), and remained static in the rest. 17 patients developed autonomic instability while on magnesium infusion. The average duration of ventilatory support required was 18.3 + 16.0 days whereas the mean hospital stay was 30.8 + 16.7 days. The overall mortality was 22.9%. Asymptomatic hypocalcemia was a universal finding.

Conclusion: Magnesium sulphate therapy alone cannot be considered efficacious for the treatment of tetanus.

Final abstract number: 57.001

Session: Hot Topics (Oral Presentation)

Date/time: Sunday, 22 June, 2008, 10:15 - 12:15 hrs

Room: 301

Role of the C-terminal Domain of OmpA Receptor, Ecgp in Stat3 Interaction During *Escherichia coli* K1 Invasion of Brain Microvascular Endothelial Cells

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E. coli K1 is a major causal agent of neonatal meningitis. Earlier studies from our lab demonstrated that outer membrane protein A (OmpA) of *E. coli* contributes to the invasion of brain microvascular endothelial cells (BMEC) by interacting with a receptor, Ecgp, a gp96 homologue. We have also shown that *E. coli* entry into BMEC requires Ecgp interaction with activated Stat3. Here, we demonstrated that overexpression of full length Ecgp in BMEC increased the invasion by two-fold, whereas overexpression of C-terminal 200 and 400 amino acids-truncated Ecgp showed no increase in *E. coli* invasion of BMEC. Of note, immunoprecipitation studies using anti-Ecgp antibodies have revealed that Stat3 phosphorylation increases between 15 and 30 min post infection with OmpA+ *E. coli*, whereas, infection with OmpA- *E. coli* did not show such an increase. BMEC overexpressing C-terminal truncated forms of Ecgp revealed no increase in Stat3 phosphorylation despite infection with OmpA+ *E. coli*. Inhibition of Stat3 activation by overexpressing a dominant negative form of Stat3 significantly abrogated the *E. coli* invasion, suggesting that Ecgp-Stat3 interaction is critical for the invasion process and that the C-terminal portion of Ecgp is necessary for activation of Stat3. Studies are in progress to determine the mechanisms of cross talk between Stat3 and other signaling molecules necessary for the invasion for *E. coli*.

Final abstract number: 57.002

Session: Hot Topics (Oral Presentation)

Date/time: Sunday, 22 June, 2008, 10:15 - 12:15 hrs

Room: 301

Molecular Epidemiology of H5N1 Avian Influenza Virus: Correlations between Antigenic Drift, Geographical Migration and Expansion of Viral Diversity

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Background: H5N1 highly pathogenic avian influenza virus (H5N1-HPAIV) has seriously impacted poultry industries. Nevertheless, the evolutionary and epidemiological dynamics of H5N1-HPAIV were not fully understood.

Methods: Maximum likelihood (ML) phylogenetic tree was reconstructed using hemagglutinin (HA) genes of 1266 H5N1-HPAIV isolates in 1996-2007, to study the global viral epidemiology in avian population. By enforcing the molecular clock, an evolutionary time-scale for worldwide H5N1-HPAIV was established, and was utilized to estimate the rate of HA antigenic drift and viral migratory history in the last decade, using ML joint method and parsimony optimization method respectively. The viral genetic diversity over time was estimated using Bayesian coalescence method. Since all these estimations were grounded on the real time-scale, their temporal correlations could be assessed.

Results: Our analyses suggest H5N1-HPAIV first emerged in China in 1995-1996. The occasions of viral dispersions from China to Thailand, Vietnam, Indonesia, and other Asian and European countries temporally coincided with the rapid expansion of global H5N1 viral genetic diversity which started in 2001-2002. The HA antigenic drift rate of H5N1-HPAIV circulating in China remained slow, at 0.1-0.2 amino acid substitutions on 25 antigenic sites per total amino acid substitutions (a.t.) throughout 1998-2007. In contrast, the drift rates were high (0.5-0.3 a.t.) when the H5N1-HPAIV initially emerged in Indonesia, Thailand and Vietnam in 2001-2003, but gradually declined to 0.1-0.2 a.t. when the virus progressed to endemic maintenance after 1-2 years following the invasion.

Conclusions: The temporal coincidence of viral dispersions between countries and expansion of global H5N1 genetic diversity suggests the geographical spread might expand the ecological niche and species distribution for H5N1-HPAIV. Furthermore, our study suggests that the mutations on antigenic epitopes of H5N1-HPAIV are essential for their adaptation and immune evasion in bird populations, particularly at the early stage of invasion.

Final abstract number: 57.003

Session: Hot Topics (Oral Presentation)

Date/time: Sunday, 22 June, 2008, 10:15 - 12:15 hrs

Room: 301

Pathogenicity of Lagos Bat Virus - An African Rabies-Related Lyssavirus

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Lagos bat virus (LBV) constitutes genotype (gt) 2 in the Lyssavirus genus and the principal hosts are fruit bats. Members of this genus cause fatal rabies encephalitis. Based on phylogeny, serologic cross-reactivity and peripheral pathogenicity to mice, lyssaviruses were divided into two phylogroups. Phylogroup I viruses are pathogenic for mice when inoculated via the intracerebral (i.c.) and intramuscular (i.m.) routes. Phylogroup II viruses (LBV and Mokola virus (MOKV)) were shown to be pathogenic for mice only when inoculated via the i.c. route. This study compared the pathogenicity of several isolates of LBV in a murine model. Amino acid substitutions along the glycoprotein, previously suggested to be important for peripheral pathogenicity of lyssaviruses, were also analysed.

Four-week-old mice were inoculated with lyssavirus isolates using different routes of inoculation and different doses of inoculum. Mice were observed for 56 days. The direct fluorescent antibody test (FAT) was performed on mouse brain collected from succumbed or euthanized mice. The nucleotide sequence of pathogenic domains of LBV isolates was determined and amino acid sequences were compared using multiple alignments.

The peripheral pathogenicity of some representatives of LBV in the murine model were found to be as high as the corresponding pathogenicity of rabies virus. Domains on the glycoprotein that has previously been implicated in virulence, were found to differ between LBV strains that demonstrated a difference in pathogenicity.

Previous studies suggested that LBV were not pathogenic to mice when introduced peripherally. We demonstrated that representatives of LBV caused rabies in mice when introduced i.m and therefore the pathogenicity had been underestimated previously. The surveillance and public health precautions for LBV must be enhanced and this is particularly important since commercially available rabies biologicals do not protect against this virus.

Final abstract number: 57.004

Session: Hot Topics (Oral Presentation)

Date/time: Sunday, 22 June, 2008, 10:15 - 12:15 hrs

Room: 301

Design and Development of a Novel Electrochemical DNA Biosensor for Rapid Molecular Identification of *Enterococcus faecium*

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Enterococci have emerged as prominent nosocomial pathogens that cause a variety of clinical infections in many parts of the world over the last decade. The most common enterococci strains isolated from clinical samples are *E. faecium* and *E. faecalis*. Enterococci are known to have acquired resistance to vancomycin (glycopeptide) antimicrobials, resulting in the rapid increase of vancomycin resistant enterococci (VRE) strains in human. The conventional culture methods are time-consuming and laborious. Alternative molecular techniques polymerase chain reaction (PCR) and agarose gel electrophoresis utilize harmful elements such as carcinogenic ultraviolet light and ethidium bromide. In addition, optical-based techniques such as real-time PCR are expensive and require specialized equipments. Recently, interest has been increasing in the development of simple, inexpensive and disposable DNA biosensors for field and clinical assays. In the present study, an electrochemical DNA biosensor was designed and developed for detection of *E. faecium*. Design, fabrication and electrochemical characterization of screen-printed carbon electrodes (SPCEs) were carried-out. Optimization of the PCR and biosensor protocols such as PCR hapten labeling, washing step and peroxidase oxidation signal were performed. Under the optimized conditions, the oxidation signal threshold value was determined at $2.00 \pm 0.02 \mu\text{A}$. The analytical specificity of the biosensor assay was evaluated with reference *E. faecium* and non-*E. faecium* strains and was found to be 100%, while analytical sensitivity of the assay was 10 CFU/ml. The biosensor assay gave quantitative results rather than qualitative results when compared with agarose gel and DNA-chromatography based tests. In this study, the biosensor was optimized using *E. faecium* as a model organism and proved to be sensitive and specific. Hence in future, it will be possible to use this biosensor for antimicrobial resistant determinants, other microorganisms or mutant gene detection in hospital and environmental settings.

Final abstract number: 57.005

Session: Hot Topics (Oral Presentation)

Date/time: Sunday, 22 June, 2008, 10:15 - 12:15 hrs

Room: 301

Development of a Thermostabilized Cholera-NASBA-ELISA Assay for the Specific Detection of *Vibrio cholerae*

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Cholera is a diarrheal disease caused by *Vibrio cholerae*. It is potentially lethal if not diagnosed early. Conventional culture and biochemical tests for cholera are laborious. Although molecular-based methods are rapid, offer better sensitivity and specificity, they require expensive equipments and cold storage of reagents. Furthermore, DNA-based tests such as PCR do not distinguish between viable and non-viable cells. Nucleic acid sequence-based amplification (NASBA) is an isothermal amplification technique that specifically amplifies RNA, hence detecting viable cells only. In this study, a cholera-NASBA-ELISA assay was developed for detection of *lo/B* gene of *V. cholerae* and the feasibility of thermostabilizing the NASBA mix was explored. NASBA conditions were optimized and its amplicons were detected using ELISA. The assay was tested with 41 reference strains comprising *V. cholerae*, *Vibrio* species and enteric pathogens. The clinical evaluation of the assay was carried out using spiked stool samples. The cholera-NASBA mix was thermostabilized by lyophilization and its stability was evaluated at different temperatures periodically. In addition, the ability of the assay to detect only viable cells was investigated by subjecting *V. cholerae* cultures to various lethal treatments and detecting their NASBA signal. The cholera-NASBA-ELISA had an analytical sensitivity of 10 CFU/ml at the bacterial level and 10² molecules/ μ l RNA transcript at the gene level. Diagnostic evaluation with spiked stool samples gave 100% sensitivity, 84.52% specificity, 89.92% positive predictive value and 100% negative predictive value. The thermostabilized NASBA mix was stable for 2 months at 8°C and -20°C. In the viability assay, *lo/B* mRNA was detected even after 48 hours post-treatment, therefore precluding its use as a viability indicator. Hence, we have for the first time developed a thermostabilized cholera-NASBA kit that reduces multiple pipetting steps and is highly sensitive.

Final abstract number: 57.006

Session: Hot Topics (Oral Presentation)

Date/time: Sunday, 22 June, 2008, 10:15 - 12:15 hrs

Room: 301

Epidemiology and Pathogenicity of Methicillin-Resistant *Staphylococcus aureus* Isolates from Pediatric Patients in China

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Background: Our study was to investigate the genetic differentiation and pathogenicity of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from the five biggest pediatric hospitals, located in four different regions in China from 2005 to 2006.

Methods: Seventy-three MRSA isolates were analyzed by a combination of different genotyping methods, including multilocus sequence typing (MLST), SCCmec typing and *spa* typing.

Additionally, Pantone-Valentine Leukocin (PVL) gene was detected. Susceptibility tests were performed for 14 antimicrobial agents. Clinical information about these MRSA isolates was also collected.

Results: Among 73 MRSA isolates, 14 sequence types (STs) of MLST were identified, including two novel types. The SCCmec types of most MRSA strains were type III (42.5%) and type IV (34.2%). Seventy-one strains were differentiated into 19 *spa* types, including three novel types. Also, 30.1% of MRSA isolates were found to carry the PVL gene. The prevalent strains were ST239-MRSA-III and ST1-MRSA clones in the northern region; ST239-MRSA-III, ST910-MRSA-IV and ST88-MRSA in the eastern region; and ST59-MRSA in the southern region. Only the ST910-MRSA-IV clone (PVL gene-positive) has been found in China until now, and it is closely related to ST30-MRSA-IV. All MRSA isolates were found to be resistant to penicillin and azithromycin, and susceptible to vancomycin. Resistance to other antimicrobial drugs tested was relatively higher and multidrug resistance was also observed. The cases of necrotic pneumonia, severe skin and subcutaneous tissue infection and cervical lymphadenitis resulted from PVL gene-positive MRSA.

Conclusions: A combination of different genotyping methods proved useful for studying the endemic clones of MRSA isolated from children in China. There was a obviously geographical variation in the prevalence of MRSA strains. Antimicrobial susceptibility tests showed high resistance of many antimicrobials and multiple drugs. PVL gene-positive MRSA was likely to be associated with the necrotic process in clinical infections.

Final abstract number: 57.007

Session: Hot Topics (Oral Presentation)

Date/time: Sunday, 22 June, 2008, 10:15 - 12:15 hrs

Room: 301

Sero-Epidemiological Results in the Human Population Exposed to Highly Pathogenic Avian Influenza H5N1 Outbreak in a Large Poultry Farm in the East of England

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In February 2007, the presence of highly pathogenic avian influenza H5N1 of Eurasian lineage (Clade 2.2) was confirmed in turkeys at one of 22 sheds of a large poultry farm located in the east of England. A multi-agency Incident Management Team assessed the risk to human health and instituted appropriate control measures. This is a summary of results of investigation into associations between risk-factors and outcomes as described below.

A total of 482 individuals received chemoprophylaxis with Oseltamivir, of which 187 (39%) consented to blood testing for evidence of infection with H5N1 at 8-10 weeks following exposure.

A supervised questionnaire was administered at the same time to investigate associations between exposure, occupational and demographic variables, and outcome.

After excluding people with no exposure, 162 were available for analysis. 29 (18%) reported one or more symptoms of influenza like illness (ILI) experienced only after exposure, of whom 21 had received Oseltamivir before possible exposure to H5N1. 157/162 (97%) reported 90-100% compliance with taking prescribed Oseltamivir, while 73% reported total compliance with personal protective equipment (PPE). 141 (87%) individuals received seasonal influenza vaccination, of whom 16 (10%) had received the vaccination prior to the incident.

There was no evidence of sero-conversion to H5. Multivariable logistic regression modeling failed to find any statistically significant associations between above-mentioned variables and ILI symptoms at 5% significance level. However, there was a suggestion that 'workers with no specific occupational activity' may be at elevated risk (OR=3.93; 95%CI: 1.00-15.54; p=0.059). Interestingly, amongst others, there was no statistically significant association between 'always wear complete PPE' nor 'always wear FFP2 or FFP3 respirator' (both binary variables), and symptom development (p=0.95, 0.9 respectively).

The public health response included the largest ever mass provision of Oseltamivir for 'at-risk' individuals in the UK, the assessment framework for which needs review.

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Session: Hot Topics (Oral Presentation)

Date/time: Sunday, 22 June, 2008, 10:15 - 12:15 hrs

Room: 301

Paediatric Community-Associated Methicillin-Resistant *Staphylococcus aureus* Infection at Chris Hani Baragwanath Hospital, Soweto, South Africa

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Background: Community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infection has increasingly been reported from developed countries with little information from developing countries, especially those with a high HIV prevalence.

Methods: A retrospective chart review was done on subjects admitted to general paediatric wards with *S. aureus* identified from blood culture between January 2005 and December 2006. Cases from intensive care, oncology and burns units were excluded. Isolation of *S. aureus* within 48 hours of hospital admission was defined as community-associated (CA) *S. aureus* infection, ≥ 48 hours as hospital-associated (HA).

Results: 248 *S. aureus* isolates were identified, of which 182 (74.0 %) fulfilled criteria for CA. Of CA infections, 71 (39.0%) were caused by MRSA and 111 (61.0%) by MSSA, compared to HA infections, where 79.7% were caused by MRSA ($P < 0.001$). Forty-nine (70.0%) of CA-MRSA occurred in infants < 1 year, 13 (18.6%) in children aged 1-4 years and 8 (11.4%) in those ≥ 5 years. Seventy-seven (58.8%) of 131 children with known HIV result were HIV-infected. CA *S. aureus* isolates were more likely to be MRSA in HIV-infected (49.4%) compared with HIV-uninfected children (29.6%; $P = 0.02$). There was no difference in mortality between CA-MRSA (12.3%) and CA-MSSA isolates (12.8%; $P = 0.93$). However, mortality was lower for CA-MRSA than for HA-MRSA (50.0%; $P < 0.001$). Clindamycin resistance was present in 75.4% of CA-MRSA (70.3% in HA-MRSA; $P = 0.80$), ciprofloxacin resistance in 32.6% of CA-MRSA (48.4% in HA-MRSA; $P = 0.17$), and cotrimoxazole resistance in 96.9% of CA-MRSA (97.7% in HA-MRSA; $P = 0.80$). In HIV-infected subjects, all CA-MRSA isolates were cotrimoxazole resistant.

Conclusion: Although MRSA frequently had its onset in the community, especially among HIV-infected children, antibiotic resistance patterns of CA-MRSA in South African children suggest that most of these infections are probably due to community spread of hospital strains. Molecular analysis is required to further investigate the epidemiology of MRSA in this community.

Final abstract number: 57.009

Session: Hot Topics (Oral Presentation)

Date/time: Sunday, 22 June, 2008, 10:15 - 12:15 hrs

Room: 301

Retrospective Investigation of a Dengue-Like Syndrome in a Rural Area in Western Cameroon

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Background In the last ten years, reports of arbovirus circulation have increased in Central Africa. In 2006, Chikungunya virus (CHIKV) was isolated after a simultaneous outbreak of febrile syndrome in the two main cities of Cameroon (Douala and Yaounde). Evidence of co-circulation of CHIKV and Dengue virus (DENV) was also established during these outbreaks. Following a dengue-like syndrome outbreak (sudden high fever combined with prolonged and severe arthralgia) in three villages of Kumbo-East Health District (western Cameroon) in 2006, a cross sectional study was carried out one year later to characterize this epidemic and to determine its aetiology.

Methods: Volunteers among those who suffered of dengue-like syndrome one year before the investigation period were included. A total of 106 people (sex ratio 0.3) were sampled for blood collection. Most of the people examined were farmers (85.8%), and the mean age was 50.5±17.5 years (minimum 8, maximum 81). An entomological investigation was also carried out.

All the 106 sera were tested for CHIKV specific IgM and IgG antibodies and for DENV specific IgM and IgG antibodies by capture enzyme-linked immunosorbent assay (ELISA).

Results: Antibodies to CHIKV were detected in 63 (59.4%) sera; IgM antibodies were found in 5 (4.7%) specimens and IgG antibodies were detected in 59 (55.6%) specimens including two sera from persons still complaining of arthralgia. No DENV antibodies were detected.

The entomological survey revealed the presence of *Aedes aegypti* breeding sites in the raffia plantations situated in the valleys of villages where people fetch water and palm wine.

Conclusion: The findings suggest that the dengue-like syndrome outbreak was due to an Alphavirus, probably CHIKV. Added to previous findings (Yaounde and Douala outbreaks), these results provide further evidence of the circulation of arboviruses in Cameroon.

Key words: Dengue-like syndrome, Chikungunya, *Aedes aegypti*, IgM and IgG antibodies

Final abstract number: 7.001

Session: Epidemiology and Public Health (Oral Presentation)

Date/time: Friday, 20 June, 2008, 10:15:15 hrs

Room: 301

Activities of Artesunate and Amodiaquine against Intestinal Helminth in Children with *Plasmodium falciparum* Malaria in Endemic Area

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The activities of the antimalarial drugs- artesunate and amodiaquine against intestinal helminth were evaluated in 109 of 242 Nigerian children with uncomplicated *Plasmodium falciparum* malaria who had helminth before, during and after treatment with the two therapies. The children were randomized to the standard dose regimens of the drugs. Clinical recovery from malaria occurred in all children. Helminth was detected in 102 patients (93.6 %) before treatment and in another 7 patients (6.4%) after treatment. The children treated with artesunate had similar pretreatment helminth ova load, significantly shorter malaria parasite clearance times (1.3 + 0.5 versus 2.8+1.8 d, P = 0.0001), high total parasite reduction potential in 24 h (TPRP24h) (P= 0.0001) and lower geometric mean helminth ova load by day 14 (P= 0.012) following treatment than those treated with amodiaquine. However, helminth parasite excretion time was significantly shorter in those treated with amodiaquine (P= 0.003). Kaplan-Meier survival curve of cumulative probability of remaining helminth ova carrier showed that by day 14 of follow-up, children treated with artesunate had significant propensity to still carry helminth ova than in amodiaquine treated children (Log rank statistics 4.67, df= 1, P =. 0.03). These results suggest that both artesunate and amodiaquine not only clear malaria parasite, but also offer considerable anti-helminth activity that is more pronounced with amodiaquine-an additional advantage for combination therapy.

Final abstract number: 7.002

Session: Epidemiology and Public Health (Oral Presentation)

Date/time: Friday, 20 June, 2008, 10:15:15 hrs

Room: 301

Transmission Dynamics of MDR-TB and XDR-TB in Areas of Varying HIV Prevalence

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Background and aim: There have been many reports on co-infection with HIV and Tuberculosis, however, the disease dynamics of drug-resistant *Mycobacterium tuberculosis* strains in areas of high HIV prevalence remains largely unknown. This study aimed to investigate the population structure of drug-resistant strains in settings of high HIV prevalence in South Africa.

Methods: A molecular epidemiological approach (IS6110-RFLP, spoligotyping, MIRU-typing, drug-resistance genotyping, phylogenetic tree analysis) was used to study the dynamics of drug-resistant strains in 3 settings with different HIV prevalence's (Western Cape, Eastern Cape and a mine in the Northern Province).

Results: Cluster analysis showed that more than 60% of drug-resistant TB was due to the transmission of resistant strains in the 3 study settings. A hyper transmissible drug-resistant clone with the Beijing genotype was responsible for the significant increase in the number of drug-resistant cases in the Western Cape setting over a 5 year period. Phylogenetic analysis, of isolates collected in the mine setting, coupled with contact tracing, demonstrated how MDR-TB acquired additional resistance markers (pyrazinamide, ethambutol and ofloxacin) in a stepwise manner despite an excellent TB control program. This stepwise evolution culminated in the emergence of XDR-TB. In the Eastern Cape setting the molecular epidemiological data showed that the atypical Beijing genotype (attenuated phenotype) can become virulent and spread in patients infected with HIV despite the acquisition of resistance markers which have a fitness cost.

Conclusion: Our results raise concern for the spread of all drug-resistant strains in vulnerable populations. Greater vigilance is required to contain the drug-resistant TB epidemic in high HIV prevalence settings. This can be achieved by the development and implementation of rapid diagnostics, ensuring treatment adherence and intensified screening of contacts. However, in order for diagnosis and treatment to be effective it is essential that communities are educated to improve health seeking behavior.

Final abstract number: 7.003

Session: Epidemiology and Public Health (Oral Presentation)

Date/time: Friday, 20 June, 2008, 10:15:15 hrs

Room: 301

The Emergence of Nipah Virus in Malaysia: The Role of Pteropus Bats as Hosts and Agricultural Expansion as a Key Factor for Zoonotic Spillover

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Nipah virus (NiV) emerged in Malaysia in 1998 as a respiratory and neurologic disease in pigs and caused a severe febrile encephalitis in humans, carrying a 40% mortality rate (n=265). Bats of the genus *Pteropus* are considered a natural reservoir for Nipah virus and other related henipaviruses. We proposed two hypotheses for NiV emergence: 1) Nipah virus is endemic and circulating in pteropid bats throughout Malaysia and these bats normally occurred in the area of the index farm where Nipah virus emerged; and 2) the intensification of pig farms in Malaysia enabled sustained NiV epidemics to occur in pigs, facilitating NiV emergence in humans. We performed cross-sectional serological surveys of *Pteropus vampyrus* and *P. hypomelanus* from spatially disparate colonies across Peninsular Malaysia. A longitudinal sero-survey of *P. hypomelanus* from a single population on Tioman Island was conducted between October 2003 and November 2006. Bat population counts and satellite telemetry were used to assess abundance and long-range movements of *P. vampyrus*. We also analyzed livestock production data from the index farm and modeled within-farm infection dynamics.

Serological studies showed a widespread distribution of Nipah virus antibodies in both species of *Pteropus* bats in Peninsular Malaysia and provided evidence for continued viral circulation in bats. Results from the pig farm analyses suggest that repeated introduction of NiV from the wildlife reservoir into this intensively managed pig population led to changes in infection dynamics in the pigs. Long-term within-farm persistence permitted regional spread of the virus, ultimately producing widespread human infection. Thus, while pteropid bats have likely been the reservoir for Nipah virus for a long time, the cause of emergence of NiV can be essentially characterized as due to agricultural intensification. Targeted surveillance of these farms in areas where flying fox distributions overlap commercial pig farms is therefore important to detect spillover events early-on and prevent widespread infection.

Final abstract number: 7.004

Session: Epidemiology and Public Health (Oral Presentation)

Date/time: Friday, 20 June, 2008, 10:15:15 hrs

Room: 301

Increased Risk of Death in HIV-Infected Patients with Pneumococcal Meningitis, South Africa, 2003-2005

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Background: Pneumococcal disease is an important cause of mortality in developing countries. We aimed to determine whether HIV-infection was associated with increased risk of death amongst invasive pneumococcal disease (IPD) cases.

Methods: Cases with IPD presenting to enhanced surveillance sites as part of national laboratory-based surveillance between January 2003 and December 2005 were reviewed. Surveillance officers collected epidemiologic data on cases and offered all cases HIV ELISA testing. Meningitis was defined as pneumococcal growth on cerebrospinal fluid specimen culture (with or without growth from another site) and other IPD as pneumococcal growth from other normally sterile site specimens. Risk factors for death in patients with meningitis and other IPD were evaluated using multivariable logistic regression.

Results: Of 11,116 reported IPD cases, 4890 (44%) presented to enhanced surveillance sites and had available outcome data; 1154 (24%) cases of meningitis and 3736 (76%) cases of other IPD. Of cases with available age, the age distribution was: <5 years, 1770/4882 (36%); 5-24 years, 774/4882 (16%); 25-44 years, 1693/4882 (35%); ≥45, 645/4882 (13%). The overall case fatality rate was 28% (1360/4890); 45% (520/1154) in meningitis and 22% (840/3736) in other IPD cases ($p < 0.001$). Of patients tested for HIV, HIV-seroprevalence was 512/664 (77%) amongst meningitis cases and 2062/2346 (88%) amongst other IPD ($p < 0.001$). On multivariable analysis of meningitis cases, HIV-coinfection was associated with increased odds of death when controlling for age group, severity of illness [Pitt bacteraemia score], prior antibiotic use and province (odds ratio 2.2, 95% confidence interval 1.3-3.6). HIV-coinfection was not an independent risk factor for death in other IPD cases.

Conclusions: Pneumococcal meningitis has a high mortality in South Africa, and HIV-infected patients are at increased risk of death. Access to antiretroviral therapy for HIV-positive patients and introduction of the pneumococcal conjugate vaccine for routine immunization should be prioritized.

Final abstract number: 7.005

Session: Epidemiology and Public Health (Oral Presentation)

Date/time: Friday, 20 June, 2008, 10:15:15 hrs

Room: 301

Intradermal Influenza Vaccine Elicits Superior Immunogenicity in Adults Aged ≥ 60 Years: A Randomized Controlled Phase 3 Trial

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Background: Annual trivalent inactivated vaccines (TIV) provide protection against influenza and its complications for hundreds of millions of individuals. Yet among adults aged ≥ 60 years, vaccine efficacy is lower than in younger adults. Elderly adults are also the most at risk, with the highest influenza morbidity and mortality. An influenza vaccine, injected using a novel intradermal microinjection system, has been developed with the aim of offering improved protection for this vulnerable population.

Methods: A multicenter, randomized controlled phase 3 trial was conducted to assess whether TIV given via ID microinjection induces a superior immune response in adults ≥ 60 years, compared with an intramuscular (IM) control vaccine (Vaxigrip®). Each vaccine dose contained 15 μ g hemagglutinin/strain. Strain-specific hemagglutination inhibition titers were assessed on D0 and 21 using a standard assay.

Results: 3701 subjects aged 60-94 years (mean: 70.8 \pm 6.8) were enrolled and vaccinated ID (n=2612) or IM (n=1089). 54.4% were female. Seroprotection rates were significantly higher in the ID group (p=0.0003 for H1N1 and B, p<0.0001 for H3N2) with a difference of 5.5-6.6 percentage for each strain. Mean titer increases after ID vaccination were: H1N1 3.97, H3N2 8.19 and B 3.61, which were 24.5%, 53.1% and 18.8% higher (p<0.0001) than the corresponding values in the IM group. Seroconversion rates among those with a prevaccination titer <10 were significantly higher with ID: H1N1 64.3% vs 55.6% p=0.0127, H3N2 80.9% vs 69.3% p=0.0003 and B 41.3% vs 35.5% p=0.0282.

Conclusion: In a large phase 3 population of adults aged 60-94, the immunogenicity of a new ID influenza vaccine was superior to that of a conventional IM control vaccine. Increased serum antibody responses should provide improved protection against influenza for this vulnerable population.

Final abstract number: 7.006

Session: Epidemiology and Public Health (Oral Presentation)

Date/time: Friday, 20 June, 2008, 10:1512:15 hrs

Room: 301

Comparison of Fluorescence Microscopy with Ziehl-Neelsen Technique in the Examination of Sputum for Acid-Fast Bacilli Using Bleach Centrifugation Method

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Background: The reliability of direct smear microscopy for diagnosis of tuberculosis (TB) using Ziehl-Neelsen (ZN) technique has frequently been questioned due to low sensitivity. Fluorescent microscopy (FM) is more sensitive than ZN but its sensitivity is less than culture. Treatment of sputum with bleach has been used to increase sensitivity in many settings. However, no study has compared use FM and ZN methods for detection of acid-fast bacilli (AFB) using bleach method.

Objectives: Comparison of results with fluorescence and bright-field microscopy for AFB using bleach centrifugation method.

Methods: Three hundred and seventy sputum specimens were collected from new TB suspects attending Mbagathi District Hospital and processed for direct microscopy using both ZN and FM. Culture on Lowenstein Jensen egg media was used as the gold standard. FM and ZN smear negative specimens were treated with 3.5% bleach and left to stand for 30 minutes before centrifugation. Smears were prepared from each bleach treated specimen, processed and examined using either ZN or FM staining methods.

Results: Of the 370 specimens, 200(54%) were culture positive. The number of smear positive by direct ZN was 138 (37.2%) which increased to 171 (46.2%) and direct FM positive was 165 (44.6%) which increased to 180 (48.6%), after treatment of direct ZN and FM smear negative specimens with 3.5% bleach, respectively. There was a significant increase in sensitivity from 66% to 81.1% ($p < 0.05$) using ZN technique and 75.5% to 83% ($p < 0.05$).

Conclusion: Bleach centrifugation method significantly increases the sensitivity of smear negative specimens irrespective of the staining method used. However, FM appears to be more sensitive than ZN.

Final abstract number: 7.007

Session: Epidemiology and Public Health (Oral Presentation)

Date/time: Friday, 20 June, 2008, 10:15:15 hrs

Room: 301

Polymerase Chain Reaction and Mosquito Dissection as Tools to Monitor Filarial Infection Levels Following Mass Treatment in Gampaha District, Sri Lanka

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Background: Mass Drug Administration (MDA)-based Global Lymphatic filariasis (Lf) eradication programmes are aimed at stopping transmission of *Wuchereria bancrofti* by its mosquito vector. The study was designed to compare one year post treatment (mass distribution of Diethylcarbamazine-Albendazole) infection rates of *Wuchereria bancrofti* in *Culex quenuifaciatus*, the main vector of Lf in Sri Lanka using Conventional dissection techniques and a Polymerase Chain Reaction (PCR) assay based on parasite specific *Ssp1* repeat which amplifies a fragment of 188 bp.

Methods: Field study was conducted in 45 sites in all Medical Officer of Health (MOH) areas in the Gampaha district, Sri Lanka; identified by the Anti Filariasis Campaign (AFC) as having high-risk for bancroftian filariasis transmission. Indoor-resting mosquitoes were collected by aspiration from 20 houses per each site. Part of the mosquitos were used for dissection and the remainder was used for PCR to detect the filarial parasites in mosquito.

Results: Mosquito dissection data revealed 42.22% (19/45) of the sites were infested with mosquitoes positive for *Wuchereria bancrofti*, indicating 8 transmission active MOH areas (53.33%; 8/15). An infection rate of 5.26% was observed among the mosquitoes caught from households and the larval density was 8.7 per positive mosquito. PCR investigation revealed that 46.67% (21/45) of the sites were positive for *W. bancrofti* DNA, indicating 11 transmission active areas (73.33%; 11/15). The PCR was found to be more sensitive compared to microscopy in detecting the filarial parasite in field collected mosquito samples with respect to the MOH areas. **Conclusion:** The PCR technique employed offers scope for detection of the filarial parasites with higher sensitivity and specificity; is efficient and rapid. This technique applied for the first time in Sri Lanka, can be adopted as a diagnostic tool for the detection of filarial parasites in the vector population in surveillance to enable effective control of filariasis in the country.

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Final abstract number: 7.008

Session: Epidemiology and Public Health (Oral Presentation)

Date/time: Friday, 20 June, 2008, 10:15:15 hrs

Room: 301

Condom Use Varies with Age and Partner Types Among High Risk Heterosexual Men in Hong Kong

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Background: Monitoring the trend of practice of safer sex in high risk population is an important element contributing to assessment of the overall risk for HIV and STI spread in a locality. Hong Kong public STI clinics provide free STI management to local residents. The profile of attendees provides a convenient platform to track the sexual risk pattern.

Methods: An annual survey of all new attendees at the public STI clinics was conducted during November from 2000 to 2005. The nurse-administered survey included questions on demographics and risk behaviours. Only male heterosexual attendees were included. Data on age, ethnicity, frequency of condom use with commercial sex workers and non-commercial sex workers in previous 3-month period were collected for analysis.

Results: There were 6272 heterosexual male attendees from 2000 to 2005. Over 95% were Chinese and median age 40. Ninety-percent reported ever visited a commercial sex worker, 72% visited in previous 1 year and 61% in previous 3 month period. The proportions of always, usually (>50%), sometimes (≤50%), never using a condom during commercial sex in the preceding 3 months were: 44%, 27%, 9% and 20% respectively. Two-thirds reported having regular sex partners in the preceding three months, and only 21% and 18% reported always and often use of condoms with these partners. Proportion of men practiced regular (always or usually) condom use with commercial sex workers was less among those below 20, and the rapidly decreased among men after 40 years of age. A similar trend was observed for condom use with non-commercial sex partners (Graphs 1 and 2).

Conclusion: The younger and older men are less likely to practice safer sex. Only one in four men over 40 regularly used condoms with non-commercial sex partners. Condom use varies significantly with age and partner types among high risk heterosexual men in Hong Kong.

Final abstract number: 7.009

Session: Epidemiology and Public Health (Oral Presentation)

Date/time: Friday, 20 June, 2008, 10:15:15 hrs

Room: 301

Large Outbreak of Orally-Acquired Acute Chagas' Disease, in a Public School of Caracas, Venezuela

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Introduction: In December 2007, a sharp increase of medical consultations and absenteeism was noticed among students and workers of a Municipal School in Caracas. Patients complained of fever lasting more than 7d, abdominal pain, headache, dry cough, and myalgias; and to a lesser degree, diarrhea, facial edema, malaise, arthralgias, dyspnea and tachycardia. After trypomastigotes of *Trypanosoma cruzi* were noticed on stained blood smears of a 9 year-old patient, a thorough epidemiological investigation was carried out.

Methods and Results: A total of 984 exposed individuals were evaluated; 127 of them showed specific anti-*T. cruzi* IgM and/or IgG, by the ELISA test (attack rate 12.49%), 10 cases were also parasitemic. Of those infected, 75% were symptomatic, 16.15% required hospitalization, and 28.34% had clinical and/or EKG findings of acute cardiac involvement. One patient, died due to a severe dilated acute myocarditis at the onset of the outbreak. About 75% of the cases were younger than 18 years. Epidemiological pattern was typical of an orally-transmitted, outbreak. The incriminated source was a contaminated fresh juice prepared under precarious sanitary conditions, at a location where wild infected vectors were collected in the surroundings. One female worker involved in the elaboration of the beverage, was found seropositive for specific IgM and IgG.

Discussion: This represents the largest known outbreak of orally-acquired, acute Chagas' diseases. It is unique in affecting a predominantly young, healthy population. The institution involved is located in an urban area with no current vectorial transmission. The attack rate was significant and virulence high. Evolution of symptomatic cases treated with nifurtimox or benznidazole was favorable. On follow up 60 days later, all treated patients remained asymptomatic, and original EKG abnormalities had subsided. Food-borne transmission of *T. cruzi* may occur more often than currently recognized. In endemic countries, Chagas' diseases must be considered in patients with FUO.

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Final abstract number: 7.010

Session: Epidemiology and Public Health (Oral Presentation)

Date/time: Friday, 20 June, 2008, 10:15:15 hrs

Room: 301

Extensive Transmission of Mycobacterium Tuberculosis from a 9-Year Old Child with Sputum - Smear-Negative Pulmonary Tuberculosis

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Background and Methods: Patients with pulmonary tuberculosis who are sputum smear-negative and particularly young children without cavitating lesions have previously not been considered to be infectious. Following the diagnosis of pulmonary tuberculosis in a 9-year old boy (the index case) with a right upper lobe consolidation and Mycobacterium tuberculosis grown from a smear-negative sputum, we detected a high rate of infection in his family and class contacts. This led to the screening of all pupils (n= 200) and staff (n=108) of a UK junior school for M. tuberculosis infection.

Results: Altogether, 85 (42%) pupils of the junior school had a reactive gamma interferon release assay indicating infection with M. tuberculosis. X-ray screening revealed 18 children with pulmonary changes consistent with tuberculosis. One child had acid-fast bacilli on gastric lavage but there was no increased rate of infection among his family or class contacts. The infection rate in the class of the index case was significantly higher (79%) than the infection rate among the other pupils at the school (35%) (p<0.01). None of the adult contacts screened had pulmonary tuberculosis. Genetic finger printing revealed that the strain of M. tuberculosis of the two pupils was identical and matched a strain of a family contact of the index case (and the most likely source for this school outbreak) encountered six years previously.

Conclusions: Smear-negative sputum and lack of cavitating disease on a chest x-ray do not exclude significant risk of transmission of infection from a patient with pulmonary tuberculosis.