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 20 mg/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.

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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.

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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
viruses do not protect against this virus.

**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.

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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 intra-muscular (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.

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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 ± 0.02 μA. 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 then 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.

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