Abstracts

Session 4
Arbovirus and Other Viruses

Plenary paper

S4.1 The control and eradication of zoonotic diseases in livestock in Malaysia

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National programmes for the control and eradication of zoonotic diseases in farmed animals has been implemented in the country since the 1980's. The Department of Veterinary Services Malaysia, has identified and made compulsory, notification of 8 zoonotic diseases in the country in its efforts to control and/or eradicate these diseases. The 8 diseases are Bovine Brucellosis, *B. melitensis* infection in goats, Rabies, Johne's Disease, melioidosis and more recently, Nipah Virus infection, highly pathogenic Avian Influenza (HPAI) infections, and BSE. Effective surveillance, monitoring and control systems supported by currently approved international laboratory methods and testings have been well established in the country for each of the disease. Surveillance programs principles and aims vary for each of this disease although the general task of detecting outbreaks as early as possible remains the main objective. Malaysia wants to maintain the status claimed as free of BSE, Nipah virus and AI, and has undertaken surveillance programmes of laboratory testing of brain samples of cattle from abattoir and fallen stock to substantiate claims of freedom of disease/infection for BSE and testing of swine sera from abbatoirs for Nipah virus infection. HPAI threats, however, are less predictable and surveillance programmes are formulated for rapid detection of outbreaks. For all these diseases, surveillance has been geared towards eradication and control of the disease. Research towards the development of sensitive assays for detecting low level antibody and molecular-based assays to detect small quantities of the virus/bacteria in live animals are the major and critical issues.

S4.2 Confirmation of 2006 chikungunya outbreak in Sri Lanka using RT-PCR

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Chikungunya, a mosquito-borne viral infection caused by a single-stranded RNA virus of the family *Togaviridae*, is considered as a rare, non-fatal disease. During February to October 2006, an epidemic of over 1.3 million suspected cases was reported in India and neighbouring countries causing a significant economic loss due to crippling manifestations of this infection. With the outbreak of many viral fevers including dengue and dengue haemorrhagic fever, in October–November 2006, patients with manifestations suggestive of chikungunya such as high fever, headache, arthralgia and arthiritis (particularly, in ankle, knee and small joints of hands) were reported in many parts of Sri Lanka. As no chikungunya cases had been officially reported in the island since 1969, laboratory investigations for the presence of chikungunya virus was a prime requirement for confirmation of the outbreak. A total of 60 venous blood samples collected from suspected patients from different geographical regions of Sri Lanka were analysed using a reverse transcriptase-polymerase chain reaction (RT-PCR) technique to confirm the presence of chikungunya virus. Viral RNA was extracted from samples collected within 1-4 days of fever by using a Qiagen RNA extraction kit. RT-PCR was performed using chikungunya specific oligonucleotides. Both positive and negative controls were included in each set of reactions. The amplified products (354 bp) were visualized by running in a 1.5% agarose gel followed by ethidium bromide staining. Of the 60 samples, 33 (55%) were positive for chikungunya. They were distributed among almost all the geographical regions, highlighting the presence of a wide-spread epidemic in the country.

S4.3 Determination of dual infection with dengue and chikungunya virus in *Aedes aegypti*

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Dengue and chikungunya viruses are members of Arboviruses that cause diseases in humans. Chikungunya virus co-circulates in regions where dengue virus is also endemic. It has been postulated that many cases of dengue virus infection are misdiagnosed. Dual infection of dengue and chikungunya viruses have been reported in human sera but no report of dual infection in mosquito vector yet. Artificial membrane feeding technique was used to orally feed the mosquitoes with dengue and chikungunya viruses. Virus detection was carried out by reverse-transcriptase polymerase chain reaction (RT-PCR). Both chikungunya and dengue viruses cannot multiply in the same mosquito simultaneously. The study did not detect dual infection of *Aedes aegypti* with dengue and chikungunya from the same pools of mosquito. Out of ten pools of mosquitoes fed with both viruses, six pools showed positive with chikungunya only while four pools showed positive with dengue virus only. Oral receptivity of *Aedes aegypti* to chikungunya virus was higher than that of dengue.

S4.4 Silent transmission of the dengue fever in Gampaha District, Sri Lanka

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Dengue fever is a major infectious disease in Sri Lanka. Silent transmission of dengue virus *has been suggested as a possible risk factor for the increasing* incidence of dengue. The present study was carried out in the District of Gampaha using cluster investigation method. A cluster consisted of a minimum of 20 volunteers (family members and immediate neighbours) of a hospitalized serologically/molecular biologically confirmed dengue patient. Serum samples were collected from 148 volunteers in 7 clusters. Samples were tested for anti-dengue antibodies using Dengue Duo IgM and IgG Rapid Strip Test. Of these, positives were further tested for anti-dengue IgG antibody by Haemagglutination Inhibition (HAI) assay, the gold standard test for serological diagnosis of virus infection. Of the 148, 41 had evidence of exposure to dengue virus by Dengue Duo IgM and IgG Rapid Strip Test [positive for IgM: 28(68.4%), IgM & IgG: 7(17%) and IgG: 6(14.6%)]. Of that 41, paired sera were collected from 36 volunteers and tested by HAI assay which confirmed dengue virus infection in 4(11.1%) [confirmed secondary-4(100%)]. Additional 32(88.9%) were diagnosed as recent dengue infections [probable secondary-17(53.1%), probable dengue- 15(46.9%)]. Out of 36 volunteers, 12(33.3%) were symptomatic [confirmed secondary-1(8.3%), probable secondary-10(83.4%), probable dengue-1(8.3%)] and 24(66.7%) were asymptomatic [confirmed secondary-3(12.5%), probable secondary-7(29.2%), probable dengue-14(58.3%)]. Presence of dengue vectors, *Aedes aegypti* and/or *Aedes albopictus* were detected around all 7 clusters. The present study serologically confirms the persistence of silent transmission of dengue virus with a trend towards clustering around cases. Presence of vector species in the area further supports this phenomenon.

S4.5 A comparative retrospective study of novel Reverse-Transcription Polymerase Chain Reaction-based Liquid Hybridization (RT-PCR-LH) assay with Polymerase Chain Reaction (PCR) amplification, virus isolation and serological techniques for early, definitive laboratory diagnosis of dengue infection

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Dengue is an important vector borne viral infection in South East Asia. Dengue virus is responsible for dengue fever, dengue haemorrhagic fever and dengue shock syndrome. Early diagnosis of infection helps in monitoring the disease, determining when hospital admission is necessary and in reducing case fatalities. The objective of the study was to carry out a comparative retrospective study of a novel Reverse Transcription-Polymerase Chain Reaction-based Liquid Hybridization (RT-PCR-LH) assay with PCR amplification, virus isolation and serological techniques for laboratory diagnosis of dengue infection. Amplified products of Non Structural-3 gene were hybridized with a mixture of the 4 dengue type-specific Deoxyribonucleic Acid (DNA) probes in liquid phase. The assay was validated in a comparative retrospective study using acute serum samples collected from 88 patients with dengue confirmed by Haemagglutination Inhibition (HAI) assay. The assay was highly specific for diagnosis of dengue infection. As an early (≤5 days of fever) laboratory diagnostic method, this assay had 100% sensitivity for detection of dengue patients confirmed by HAI assay. A high analytical sensitivity of 2 fluorescent focus units of dengue virus/reaction was achieved. Novel RT-PCR-LH assay using a single serum specimen offers distinct advantages of specificity and sensitivity over other diagnostic techniques for early definitive laboratory diagnosis of dengue infection at the time during which serological methods can not be used.

S4.6 Dengue Entomological and Virological Surveillance in Singapore

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Singapore's comprehensive vector control programme resulted in over 15 years of low dengue incidence. However dengue has resurged in recent years despite a low vector population, with an unprecedented 14,209 reported cases in 2005. This paper highlights the dengue surveillance effort that has contributed to vector control operations and understanding of disease epidemiology. Entomological surveillance has further revealed *Aedes aegypti* presence in dengue outbreak areas. Singapore is hyper-endemic, with all four dengue serotypes circulating. Through analysis of samples collected from private hospitals and general practitioners, we detected a transition from DEN-2 to DEN-1 as the predominant serotype at end-2003, with a huge increase in cases following in 2004. The epidemic continued through 2005, when DEN-3 emergence was observed at certain foci at the height of the outbreak. Gene sequencing and phylogenetic analysis of the viral envelope gene, revealed that Singaporean DEN-1 and DEN-2 are contained within genotype I and the Cosmopolitan genotypes respectively. Although evidence of importation was noted, our data suggests that the serotype shift was not the result of importation of a novel virus strain, but more likely from neutral processes rather than through specific selection. DEN-3 viruses are contained within genotype III, shared by Sri Lankan (1980-1990s) and Venezuelan (2000-2001) strains. Co-circulation of DEN-1 and DEN-3 could have serious implications, since significant pathogenesis has been associated with DEN-3 (Bangladesh, 2000-2001), DEN-1 (Nicaragua), and both (Thailand, 2000-2002). Our demonstration of rapid changes in dengue serotype highlights the importance of sustained monitoring of dengue viruses in Singapore.

S4.7 Screening of hepatitis C (HCV) antibody reactive donors by RT-PCR in a sample population of blood bank donors in Sri Lanka

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The practice of screening donors for HCV antibodies has substantially lowered the risk of acquiring HCV infection from a transfusion. However, detection of molecular markers in blood is the most reliable means of diagnosing active viral infection. Molecular studies on HCV antibody reactive donors have not been previously performed in Sri Lanka. The present study was carried out to investigate the RNA positive rates in a sample population of anti-HCV antibody reactive blood donors in Sri Lanka, with a view of determining whether RT-PCR testing for HCV RNA should be carried out at the initial donor screening level. Eighty nine (89) HCV antibody reactive donors were tested for the presence of HCV RNA by RT-PCR (sensitivity 200 copies/ml) during the period October 2005 to May 2006. The 89 serology positive donors were initially detected by a third generation ELISA by routine screening of an initial pool of 26,176 blood donors. Of the 89 Anti-HCV antibody positive donors (0.34% of the total donor pool), 6 (0.023% of the total donor pool, and 6.74 % of antibody positive individuals) were positive for HCV RNA. The prevalence of HCV-RNA positivity was low in this cohort of Sri Lankan blood donors. This is in keeping with the low prevalence of HCV infection in the community. Routine individual HCV-RNA screening of donors does not seem cost-effective in our setting. The RNA negative, antibody positive profiles reflect either false positive serology results or donors who have been exposed to HCV previously and subsequently resolved their infections.