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

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

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

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

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

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24.003

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

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

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

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

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

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24.004

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

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

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

Results: Sequence analysis revealed unique amino acid substitutions D67G, E122G or D277A in the envelope glycoprotein. Each mutation resulted in an increase of net charge and hydrophobicity on the virion surface. When introduced individually into the TBEV infectious clone (IC), each substitution inhibited haemagglutination and reduced mouse neuroinvasiveness from 65% to 15-30%. Antibody production in infected mice was 1.5-3 times lower for IC-E122G and IC-D277A suggesting lower levels of viraemia and/or deficient immune stimulation induced by these viruses. All mutants demonstrated delayed growth in PS cells during the first 24hpi; however, mutant IC-D67G exhibited significantly

better growth characteristics than IC-E122G and IC-D277A. The reproduction of IC-E122G and IC-D277A in fasting ticks was similar to that of control HA positive virus whereas the titres of IC-D67G were significantly lower (2.5-3 vs. 1-2 log10PFU/ml, respectively). In feeding ticks, the titre of IC-E122G increased approximately 1000-fold and IC-D277A and IC-D67G - approximately 300-fold, whereas for control virus the increase was about 10-fold. Non-viraemic transmission efficiency from infected to uninfected ticks was increased by each individual substitution in nymphal *I. ricinus* (Figure 1).

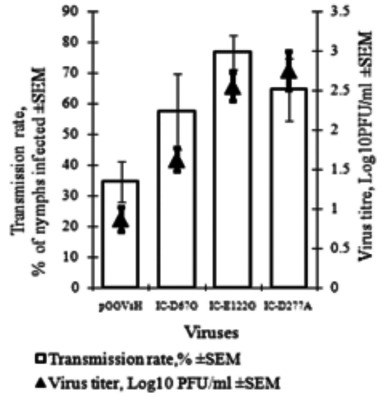

Figure 1 Tick-to-tick transmission rate (clear bars) is expressed as the proportion of infected I.ricinus nymphs. Black triangles show the average virus titres in individually infected recipient nymphs as determined by plaque assay.

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

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24.005

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

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

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

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

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

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24.006

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

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

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

Results: Plaque reduction neutralization test demonstrated that MAb 2A10G6 was active to neutralize dengue 1-4, yellow fever and West Nile viruses. *In vivo* protection