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

doi:10.1016/j.ijid.2010.02.1600

24.016

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

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

Methods: The analytical sensitivity of the assay was validated with a panel of blood donor plasma samples spiked with 10-fold serial dilutions of CHIKV, previously quantified by TCID50 assay, with viral titers ranging from 105 to 1 TCID50/mL. 10 replicates for each viral concentration were analyzed. Following RNA extraction, all samples were amplified by RTNASBA and for each virus concentration, the detection rate (n.positives/n.total) was evaluated. Finally the analytical sensitivity of the NASBA assay has been compared with realtime PCR based methods (RT-PCR).

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

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

doi:10.1016/j.ijid.2010.02.1601

24.017

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

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

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

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