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Detection and identification of Rift Valley fever virus in mosquito vectors by quantitative real-time PCR.

Mwaengo D, Lorenzo G, Iglesias J, Warigia M, Sang R, Bishop RP, Brun A.

Abstract

Diagnostic methods allowing for rapid identification of pathogens are crucial for controlling and preventing dissemination after disease outbreaks as well as for use in surveillance programs. For arboviruses, detection of the presence of virus in their arthropod hosts is important for monitoring of viral activity and quantitative information is useful for modeling of transmission dynamics. In this study, molecular detection of Rift Valley fever virus (RVFV) in mosquito samples from the 2006 to 2007 East African outbreaks was performed using quantitative real-time PCR assay (qRT-PCR). Specific RVFV sequence-based primer/fluorogenic (TaqMan) probe sets were derived from the L and S RNA segments of the virus. Both primer-probe L and S segment-based combinations detected genomic RVFV sequences, with generally comparable levels of sensitivity. Viral loads from three mosquito species, Aedes mcintoshi, Aedes ochraceus and Mansonia uniformis were estimated and significant differences of between 5- and 1000-fold were detected between Ae. mcintoshi and M. uniformis using both the L and S primer-probe-based assays. The genetic relationships of the viral sequences in mosquito samples were established by partial M segment sequencing and assigned to the two previously described viral lineages defined by analysis of livestock isolates obtained during the 2006-2007 outbreak, confirming that similar viruses were present in both the vector and mammalian host. The data confirms the utility of qRT-PCR for identification and initial quantification of virus in mosquito samples during RVFV outbreaks.