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Detection of Mayaro virus infections during a dengue outbreak in Mato Grosso, Brazil

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ABSTRACT

Arboviruses are common agents of human febrile illness worldwide. In dengue-endemic areas illness due to other arboviruses have been misdiagnosed as dengue based only on clinical–epidemiological data. In this study we investigated the presence of Brazilian arboviruses in sera of 200 patients presenting acute febrile illness, during a dengue outbreak in Sinop, MT, Brazil. The results showed that 38 samples were positive to Dengue virus (DENV) type 1, two samples to DENV type 4, and six to Mayaro virus. These results indicate that arboviruses others than DENV are circulating in Sinop and the surrounding region, which are going undiagnosed. In addition, molecular and evolutionary analyses indicate that two MAYV genotypes are co-circulating in Mato Grosso, Brazil. Thus, a strong surveillance program must be implemented to evaluate and monitor the distribution and the true importance of non-dengue arboviruses in the etiology of acute febrile illnesses.

1. Introduction

Arboviruses are a large group of zoonotic RNA viruses maintained in nature in cycles involving hematophagous arthropod vectors and a broad range of vertebrate hosts (Calisher, 1994). Most of these arboviruses belong to the families *Togaviridae*, *Flaviviridae*, and *Bunyaviridae*. Mayaro virus (MAYV), dengue virus (DENV), yellow fever virus (YFV), Rocio virus (ROCV), Saint Louis encephalitis virus (SLEV), and Oropouche virus (OROV) are responsible for more than 95% of the human arbovirus diseases cases described in Brazil (Mondini et al., 2007; Terzian et al., 2009; Vasconcelos et al., 1998). Although many arboviral infections in humans are asymptomatic or result in a mild to moderate febrile illness, some arboviruses can cause arthritis, hepatitis, hemorrhagic disease, encephalitis, and death. In most cases, a differential diagnosis between arboviruses based on clinical grounds can be very difficult, especially during the acute phase of illness, where symptoms are nonspecific (Calisher, 1994).

Dengue is the most prevalent arboviral disease in Brazil. Over the last 31 years, successive epidemics involving all four dengue serotypes (DENV-1 to 4) have been occurring in the country (Figueiredo, 2012). There is growing evidences indicating that other arboviruses relevant to public health such as YFV (Moreno et al., 2011), SLEV (Terzian et al., 2011), MAYV (Azevedo et al., 2007; Mourão et al., 2012; Zuchi et al., 2014), and OROV (Azevedo et al., 2007; Mourão et al., 2009; Terzian et al., 2009) are present and causing outbreaks in different regions.

In DENV-endemic areas illness due to co-circulating arboviruses have often been misclassified, and frequently are diagnosed as dengue solely based on clinical–epidemiological data (clinical signs compatible with dengue and occurrence of an outbreak, according to the Brazilian Ministry of Health). Therefore, it has been postulated that many febrile cases due to others arbovirus than DENV are misdiagnosed and the incidences of these infections are much higher than reported (Figueiredo, 2012; Terzian et al., 2011).

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Brazil is a tropical country with more than 50% of the region covered by rainforests or other natural ecosystems, which provide an ideal environment for the maintenance of zoonotic arboviruses with the potential for emergence as significant human pathogens (Figueiredo, 2007). Both natural and manmade ecological changes can have a significant impact on public health due to the emergence and reemergence of arboviruses, however, little is known about the geographic range, relative impact, and epidemiologic characteristics associated with most arbovirus infections in this region.

In this study we have investigated the presence of arboviruses in sera of patients presenting with acute febrile illness, during a dengue outbreak in Sinop, in the state of Mato Grosso, Brazil. We have used molecular methods based on duplex-RT-PCR to detect *Alphavirus* and *Flavivirus* genuses followed by multiplex-nested-PCR methods to identify the species from each genus. We have detected MAYV in six patients clinically diagnosed with DENV and demonstrated the co-circulation of two MAYV genotypes in Mato Grosso.

2. Materials and methods

2.1. Study site

The study was conducted in Sinop (11°50′53″S and 55°38′57″W), located 503 km from the capital of Cuiabá, and is considered the main city of the northern state of Mato Grosso, Brazil. The city was founded in 1974 and today has a population of 120,000 inhabitants with approximately 17% of them living in rural areas. Principle economic activities include agriculture, animal husbandry, logging, and human services, such as healthcare and education. It is located in a geographical transition zone between savannah and rainforest. The climate is tropical with average temperature of 28 °C and high precipitation levels (1900 mm/year), particularly from January to March.

2.2. Clinical samples

Patients seen by the Sinop Municipal Emergency Services, between January 2011 and February 2012, presenting with a clinical diagnosis of dengue, were selected for this study. Two hundred serum samples were collected from patients with acute febrile illness, clinical symptoms of headache, diffuse body pain, arthritis and retro-orbital pain. Samples were transported on dry ice to the Laboratory of Immunology and Molecular Biology of the Universidade Federal de Mato Grosso (UFMT) *campus* of Sinop and stored at −80 °C. Viral RNA was isolated from 140 μL of serum using the QIAamp Viral RNA Mini kit (QIAGEN, USA), as per the manufacturer's instructions. The Ethics Committee from Júlio Müller University Hospital—UFMT approved this study (128/CEP-HUJM/2011).

2.3. RT-PCR assays

Initially, duplex-RT-PCR (D-RT-PCR) for *Flavivirus* and *Alphavirus* genus detection was performed using specific primers targeting the NS5 and nsP1 regions, respectively, followed by multiplex-nested-PCR (M-N-PCR) performed using inner primers to DENV types 1 to 4. DENV negative samples were tested by conventional Nested-PCRs (N-PCR) for YFV, SLEV, ROCV and ILHV identification; a separate M-N-PCR was performed to identify MAYV, Venezuelan equine encephalitis virus (VEEV), Eastern equine encephalitis virus (EEEV), Western equine encephalitis virus (WEEV), and Aura virus (AURAV) from *Flavivirus* negative samples. These RT-PCR methods were performed as previously described (Bronzoni et al., 2005, 2004). Finally, the *Alphavirus* and *Flavivirus* negative samples were tested by RT-PCR methods for the presence of OROV using two different set of primers to amplify the S segment (Saeed et al., 2000; Moreli et al., 2002) and one set to amplify the M segment (Vasconcelos et al., 2011).

2.4. Nucleotide sequencing and analyses

Amplicons obtained in the D-RT-PCR were directly sequenced using BigDye v3.1 Terminator, and an ABI377 automatic sequencer (Applied Biosystens, Foster City, CA, USA) using the genus primers. The nucleotide sequences were subjected to the basic local alignment search tool (BLAST) (www.ncbi.nlm.nih.gov/blast) and submitted to GenBank.

In order to get some insights into the MAYV genotypes circulating in Sinop-MT, Brazil, nsP1 sequences from MAYV, Una virus (UNAV), Semliki Forest virus (SFV), Getah virus (GV) and Ross River virus (RRV) were retrieved from Genbank and aligned using Clustal W, implemented in MEGA 6 (Tamura et al., 2013). The nucleotide substitution model that best fit the data was chosen using jModelTest2 (Darriba et al., 2012). Thus, K80 + G (Kimura, 1980) was used to reconstruct phylogenetic trees, by Maximum Likelihood method, with 1000 bootstrap sampling. Sequences from UNAV, SFV, GV and RRV were used as the outgroups. Evolutionary divergence between and within groups of MAYV strains were estimated by sequence comparisons, using K80 + G model (Kimura, 1980), implemented in MEGA 6 (Tamura et al., 2013). Bayesian analyses were also performed using software from BEAST package v.1.8 (Drummond et al., 2002–2014). One hundred million chains were run, eliminating the first 10 million chains. Data and trees were sampled every 5000 steps. The convergence of parameters was verified with the Tracer v1.6.0 (Rambaut et al., 2003–2013) and the maximum clade credibility tree was displayed in FigTree v1.4.2 (Rambaut, 2006–2014).

3. Results and discussion

From the 200 clinical samples analyzed, arboviruses genomes were detected in 46 samples (23%). The remaining 154 samples are febrile illnesses, which are not related to the arboviruses that were tested herein. DENV-1 sequences were detected in 38 samples, DENV-4 sequences were detected in two samples, and MAYV was detected in six samples. DENV-1, 4 and MAYV each showed greater than 98% sequence identity with DENV or MAYV sequences obtained from GenBank. The NS5 gene partial sequences of DENV-1 and DENV-4, and nsP1 gene partial sequence of MAYV were submitted to GenBank, and the accession numbers are KF305670, KF305671, and KF305672, respectively. Dengue is considered to be one of the most important and widespread reemerging infectious diseases in tropical countries. DENV (serotypes 1–4) is endemic in many regions of Brazil with 700,000 cases per occurring over the last decade (Figueiredo, 2012). The *Aedes aegypti* mosquito, with a range of dissemination across most of Brazil including Mato Grosso, is the primary vector for DENV transmission. The recent years, Mato Grosso has suffered successive epidemics of DENV1–3 including: 34,851 cases with 53 deaths in 2010, 4004 cases with 4 deaths in 2011, and 26,451 cases with 13 deaths in 2012 (Ministério da Saúde, 2013). DENV-4 was re-introduced into Brazil in 2008 in Manaus, the capital of Amazonas state (de Figueiredo et al., 2008). From there the DENV-4 has spread to other geographic regions, including the North (Roraima, Amazonas and Para states), Northeast (Bahia, Pernambuco and Piaui states), and Southeast (Rio de Janeiro and São Paulo states) of Brazil. In February 2012, DENV-4 was reported for the first time in Mato Grosso. Interestingly in this study, we have detected DENV-4 in two patients samples from Sinop city collected in February of 2011. It has been shown that an introduction of a new dengue serotype into a susceptible population can cause an increase in disease severity and/or a large

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