Mayaro Fever in the City of Manaus, Brazil, 2007–2008

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Abstract

Mayaro *Alphavirus* is an arbovirus that causes outbreaks of acute febrile illness in the Amazon region of South America. We show here the cases of Mayaro fever that occurred in 2007–2008, in Manaus, a large city and capital of the Amazonas State, in Western Brazilian Amazon. IgM antibodies to Mayaro virus (MAYV) were detected by an enzyme immunoassay using infected cell cultures as antigen in the sera of 33 patients from both genera and 6–65 years old. MAYV genome was also detected by RT-PCR in the blood of 1/33 of these patients. The patients presented mainly with headache, arthralgia, myalgia, ocular pain, and rash. These cases of Mayaro fever are likely to represent the tip of an iceberg, and probably a much greater number of cases occurred in Manaus in the study period.

Key Words: *Alphavirus*—Arbovirus—Brazilian Amazon—Mayaro fever.

Introduction

ARTHROPOD-BORNE VIRUSES (arboviruses) are important causative agents of many infectious diseases that, in tropical countries, can occur endemically or epidemically, emerge producing large outbreaks, and become global public health problems. *Alphavirus* genus of the *Togaviridae* RNA virus family includes many zoonotic arboviruses distributed worldwide, infecting domestic animals and man, and producing acute febrile illness, arthralgia, and encephalitis (Griffin 2007). Mayaro virus (MAYV) is one of the 28 species of *Alphavirus* (Powers et al. 2001). These are 70 nm enveloped viruses that have an icosahedral capsid and a genome consisting of a linear, positive-sense, single-stranded RNA molecule of ~11.8 kb including eight genes. The virus genome encodes the nonstructural proteins, required for viral replication, in the 5' two thirds of the genome; and the structural genes are collinear with the 3' one third. The structural proteins are produced by translation of an mRNA that is generated from an internal, subgenomic promoter immediately downstream of the nonstructural open reading frame. The 5' end of the genome has a 7-methylguanosine cap, whereas the 3' end is polyadenylated. The structural gene products after

generation are included in a polyprotein that is processed to produce a capsid protein, two major envelope surface glycoproteins (E1 and E2), and two small peptides, E3 and 6K (Powers et al. 2001, Griffin 2007, Powers and Logue 2007).

In the phylogenetic tree of *Alphavirus* species based on the E1 virus envelope glycoprotein gene, there are mainly three different clades: the Semliki Forest clade, the Sandbis-Equine encephalitis clade, and the Aquatic virus clades. MAYV is grouped in Semliki Forest clade (also including Una, Bebaru, Semliki Forest, Getah, and Ross River viruses) (Griffin 2007). The complete genome analysis confirms this classification on the Semliki Complex clade, together with Chikungunya, Igbo-Ora, O'nyong nyong, Semliki forest, Ross River, and Sagiyama viruses (Luers et al. 2005).

MAYV was first isolated in 1954 from patients of Trinidad having acute febrile illness (Anderson et al. 1957). MAYV has been reported as causing outbreaks of a dengue-like disease in South American rural communities including those from Brazil, Peru, Bolivia, Venezuela, and French Guyana (LeDuc et al. 1981, Talarmin et al. 1998, Tesh et al. 1999, Torres et al. 2004). Mayaro fever is an acute disease that includes fever, headache, myalgia, cutaneous rash, and arthralgia which is classically described as severe in many cases where

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predominantly large joints are affected (Taylor et al. 2005). The acute febrile phase of the disease usually takes 3–5 days and finishes with the appearance of the rash. After this phase, convalescence can take a couple of weeks, with the patient feeling weakness and arthralgia (Pinheiro et al. 1981).

MAYV is maintained in nature as a zoonosis of primates that become infected after the virus transmission by bites of *Haemagogus* mosquitoes living in forest canopy (Hoch et al. 1981). This maintenance cycle is similar to the sylvatic cycle of yellow fever virus (YFV) (Figueiredo 2007).

The first outbreak of Mayaro fever in Brazil was reported in 1957, close to Guama River, in Para State, striking about 100 individuals. In this outbreak, MAYV was isolated from six patients. Serologic surveys performed in 17 localities of the Amazon region have shown a MAYV seroprevalence of 2%–40.9% (Causey et al. 1957). However, much of our understanding of the clinical and epidemiological studies on Mayaro fever were done in 1978, during an outbreak that occurred in Belterra County, Para State, when 55 patients were confirmed as having the disease. These patients presented an acute febrile exanthematic disease that also struck large joints (Pinheiro et al. 1981).

Since then, MAYV infections have been reported in many localities of Amazon and Central Plateau regions of Brazil (Vasconcelos et al. 2001, Tavares-Neto et al. 2004, Silva-Nunes et al. 2006, Coimbra et al. 2007, Figueiredo 2007).

Infections by MAYV are very common in the Brazilian State of Amazonas, an arbovirus serologic survey performed in inhabitants of the Rio Negro region having 335 participants showed that 41.2% had IgG antibodies to MAYV (unpublished data). The first eight cases of Mayaro fever in the State of Amazonas were reported in 1999, as a part of a surveillance program on acute febrile illness performed at Fundação de Medicina Tropical Dr. Heitor Dourado (FMT-HVD), a tertiary care center on tropical and infectious diseases located in the city of Manaus (Figueiredo et al. 2004). This surveillance program, which had been started in 1998, also made possible the local identification of the first confirmed cases of human parvovirus infections (Thatcher et al. 2000), dengue hemorrhagic fever (Mourão et al. 2001), and Hantavirus pulmonary syndrome (Santos et al. 2006), as well as the reappearance of dengue serotype 4 producing disease in Brazil after 25 years (Figueiredo et al. 2008). The present study was done in 2007–2008 as a part of this surveillance program on acute febrile illness.

Materials and Methods

From January 2007 to December 2008, patients attended in the FMT-HVD and having more than 5 days of acute febrile illness were tested by the blood smear for malaria and by the MAC-ELISA serology for dengue. Those patients having negative results in both tests were selected for the study.

The diagnosis of MAYV infection was done by specific IgM detection in an enzyme immunoassay using infected cell culture as antigen (EIA-ICC) (Figueiredo 1989, 1990). MAYV infection was also confirmed in 1 case by RT-PCR followed by nucleotide sequencing of amplicon.

EIA-ICC

For EIA-ICC, monolayers of C6/36 cells, originally from *Aedes albopictus*, were grown in 96-well microplates (TRP) in Leibovitz L15 medium with 10% fetal calf serum. Wells of

alternate columns were then infected with MAYV TRVL4675 strain, and the plates were kept at 28°C. After 3 days, the cells were fixed overnight in the wells with 7% formalin buffered at pH 7.0 and at 4°C. After that, 5% skim milk was used as a blocking solution, for 2 h, at 37°C, followed by three washes of the wells with PBS containing 1:2000 Tween 20 (PBS-Tween). Then, 100 mL of serum diluted 1:400 in PBS with 0.5% bovine serum albumin was added into infected and uninfected wells. The plates were incubated for 1 h at 37°C, washed thrice with PBS-Tween, and 100 µL of peroxidase-conjugated goat anti-human IgM 1:2000 in PBS (KPL) was added to the wells. The plates were incubated for 1 h at 37°C and after five washes with PBS-Tween, 100 µL of the ABTS substrate (KPL) was added into the wells, the plates were incubated for 15 min at 37°C, and read on a spectrophotometer (Asys) at 405 nm. The cutoff of the test was determined as the mean of the optical densities read in all wells containing uninfected cells plus 3 standard deviations of the mean (Figueiredo et al. 1989).

RT-PCR

RNA was extracted from the sera of all 33 patients showing IgM to MAYV, by using the Qiaamp viral RNA Mini Kit (Qiagen), following the manufacturer's specifications. The reverse transcription was conducted in 5 µL of the RNA extracts with random primers and RevertAid™ H Minus Reverse Transcriptase (Fermentas), by incubating the mixture 1 h at 45°C. PCR was performed with primers MAYV_FNS (sense CCTTTTATGTGGGGAGGTGC 10090–10109nt) and MAYV_FNAS (reverse CATGGTCACCGTTCACATACG 10291–10271nt, nucleotides positions are related to MAYV reference sequence NC_003417), which were designed with the aid of Primer-BLAST software, targeting two conserved regions of envelope glycoprotein E1 for specific amplification of MAYV genome. This 202 bp region was chosen based on an alignment of all MAYV sequences available at GenBank. Briefly, a reaction mixture was prepared including 1 U of Taq DNA polymerase (Invitrogen), 0.2 µM of both FN primers, 1.5 µM of MgCl₂, 1.0 µM of dNTPs, 5.0 µL of the RT mixture, and DEPC water to complete a 50 µL volume. This mixture was submitted to 40 thermal cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 7 min. MAYV amplicons were visualized under UV light in an agarose gel stained with ethidium bromide.

Nucleotide sequencing and amplicon analysis

Amplicons obtained in the RT-PCR were directly sequenced after purification. Aliquots of 40 µL of each amplicon were purified with the Qiaamp PCR purification kit (Qiagen) and sequenced in both directions by using the BigDye Terminator Cycle Sequence Kit v3.1 in an ABI3130xl automated sequencer (Applied Biosystems) using MAYV_FN primers.

The obtained nucleotide sequence was subjected to the basic local alignment search tool (BLAST) (www.ncbi.nlm.nih.gov/blast) analysis using the megablast algorithm for highly similar sequences (Altschul et al. 1990) and deposited in GenBank.

Results

IgM antibodies to MAYV were detected in 33 sera of 631 tested patients (5.2% positivity). MAYV cases were seen in

FIG. 1. Seropositivity to Mayaro virus (MAYV) during the study period (months) of 2007–2008 in Manaus, Brazil.

both genera and included patients 6–65 years old (23 ± 13 years). Most of these patients with Mayaro fever lived in Central–West (33.3%), South (27.8%), and North (22.2%) regions of Manaus. Mayaro fever occurred all year long in Manaus, but the cases were more frequent from November through March, thus coinciding with the rainy months of the year (Fig. 1).

The patients with Mayaro fever presented mainly with headache, arthralgia, myalgia, and ocular pain, as shown in Table 1. A cutaneous rash was observed in 8 patients (24.2%) and hemorrhagic phenomena in 4 (12.1%). All patients recovered without sequelae and were not hospitalized.

MAYV genome was detected in the serum of one patient by RT-PCR. The nucleotide sequence of 201 nucleotides from one of these amplicons (AM28725–Gene Bank submission numbers: HQ664947) showed 97% homology when compared with the prototype MAYV sequence (TRVL 15537) assessed by BLAST.

Discussion

Despite known occurrences of several arboviruses in the Amazon region, most cases of arboviral disease remain un-

diagnosed, probably owing to their generally mild and self-limited clinical manifestations, usually leading to complete recovery after a couple of days. However, even more severe cases may remain undiagnosed, especially because of long distances to health-care facilities, difficulties for sample transportation, and lack of laboratory facilities capable of conducting the diagnostic assays. With regard to MAYV infections, diagnosis may be easily confused by other acute febrile illness, including malaria, dengue, and Oropouche fever, all highly endemic in Manaus.

IgM antibodies to MAYV were detected in the sera of 33 patients by EIA-ICC, which was found suitable for diagnosis of infections by this virus in the acute phase.

Therefore, a combination of a systematic surveillance for acute febrile illnesses and an efficient laboratory diagnosis for MAYV resulted in the discovery of cases that would probably be ignored if occurring in any region, simultaneously with large dengue outbreaks, or in the absence of laboratory diagnosis. A similar scenario occurred in this same study period in Manaus, with retrospective identification of a silent Oropouche fever outbreak in patients with clinical diagnosis of dengue fever (Mourão et al. 2009). Similarly, in 2006 in the city of São José do Rio Preto, state of São Paulo, an outbreak of Saint Louis encephalitis virus would have been missed during a large dengue outbreak if there wasn't an active laboratory surveillance for emerging viruses (Mondini et al. 2007).

The clinical presentation of most cases of MAYV fever found in this study was similar to previously reported disease descriptions (Pinheiro et al. 1981). Remarkably, however, four cases from Manaus had spontaneous hemorrhagic phenomena (petechiae, epistaxis, and gingival bleeding) that had never been described earlier in MAYV fever. These findings are probably due to a closer examination of enrolled patients, considering that many of them had minor bleeding evidences. As long as we are in a dengue endemic area, the tourniquet test is an important clinical tool for searching potential severe cases in febrile patients without spontaneous bleeding and may lead to an increased number of hemorrhagic findings.

TABLE 1. SYMPTOMS OBSERVED IN PATIENTS WITH IgM AGAINST MAYARO VIRUS IN MANAUS, BRAZIL (2007–2008)

Symptoms	n	%
Fever	33	100.0
Headache	19	57.6
Arthralgia	18	54.5
Myalgia	16	48.5
Ocular pain	13	39.4
Rash	8	24.2
Bleeding	4	12.1
Vomiting	3	9.1
Photophobia	1	3.0
Jaundice	1	3.0

The cases of MAYV fever evaluated here are likely to represent the tip of an iceberg, and probably a much greater number of cases occurred in Manaus in the study period. Cases of MAYV fever occurred all year long, with a higher frequency in the rainy season as occurs with dengue (Figueiredo et al. 2004). The disease affected people of both genders and all ages that are, probably, bitten by sylvatic *Haemagogus* mosquitoes. The transmission of MAYV in Manaus, a state capital and a large city with about 2 million people, is a relevant public health problem, as there is no vaccine to this virus and the control of sylvatic *Haemagogus* mosquito vectors is not feasible. Besides, considering that the transmission of MAYV by *Aedes* mosquitoes has been previously reported (Weaves and Reisen 2010), the occurrence of this virus in Manaus, which is infested by *Aedes aegypti*, could originate urban cycles of Mayaro fever involving man as reservoir (Tesh et al. 1999). Further studies are necessary to study the ecology of vectors and reservoirs of MAYV in Manaus.

The maintenance cycle of MAYV as a zoonosis of primates transmitted by *Haemagogus* mosquitoes is similar to the sylvatic cycle of YFV (LeDuc et al. 1981); and, in fact, on some occasions, YFV outbreaks follow MAYV report of cases (Figueiredo et al. 1989, Van der Stuyft et al. 1999). In the Amazonas state, during the study period, two confirmed cases of yellow fever were reported with 100% of fatality. It is estimated that more than 90% of the population is vaccinated against yellow fever. Thus, the transmission of MAYV in Manaus, the capital of the Amazonas state, is worrisome, because it indicates that local transmission of sylvatic yellow fever could occur anytime and that this is a risk place for urbanization of the disease with occurrence of large outbreaks. Therefore, it is important to give priority for vaccination to yellow fever to maintain a high immunization level of the population of Manaus and to avoid outbreaks of this severe disease.

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Disclosure Statement

All authors declare that no competing financial interests exist.

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