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MAYARO VIRUS: IMPORTED CASES OF HUMAN INFECTION IN SÃO PAULO STATE, BRAZIL

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SUMMARY

Mayaro virus (MAYV) is an arbovirus (*Togaviridae*: *Alphavirus*) enzootic in tropical South America and maintained in a sylvan cycle involving wild vertebrates and *Haemagogus* mosquitoes. MAYV cases occur sporadically in persons with a history of recent activities inside or around forests. This paper reports three cases of MAYV fever detected in men infected in Camapuã, MS, Brazil. Serum samples collected at four days and two months after the onset of the symptoms and examined by hemagglutination inhibition test, revealed monotypic seroconversion to MAYV. Isolation of the virus was obtained from one of the samples by inoculation of the first blood samples into newborn mice. A suspension of the infected mouse brain was inoculated into C6/36 cells culture and the virus was identified by indirect immunofluorescent assay with alphavirus polyclonal antibodies. RT-PCR, performed with RNA extracted from the supernatant of C6/36 infected cells in the presence of alphavirus generic primers as well as specific MAYV primers, confirmed these results. The reported cases illustrate the importance of laboratory confirmation in establishing a correct diagnosis. Clinical symptoms are not always indicative of a disease caused by an arbovirus. Also MAYV causes febrile illness, which may be mistaken for dengue.

KEYWORDS: *Mayaro, Alphavirus*; Virus isolation; Human infection.

INTRODUCTION

Mayaro virus (MAYV) is an arbovirus member of the genus *Alphavirus*, family *Togaviridae.* MAYV is enzootic to tropical South America and endemic to rural areas. It is maintained in a sylvan cycle involving wild vertebrates, including nonhuman primates and *Haemagogus* mosquitoes[12]. Birds can act as secondary hosts, being important for the dissemination of the virus[2,20]. Most of MAYV infections are sporadic and occur in persons with a history of recent activities inside or around forests; but several small outbreaks have been reported in the Amazon Region, usually limited to rural areas near or inside forests, where the vector is found[4,10].

Mayaro fever is a non-fatal, typically dengue-like, acute febrile illness, characterized by frontal headaches, epigastric pain, myalgias, incapacitating arthralgias, maculopapular rash, chills, nausea, photophobia and vertigo. The joint pain may persist for several months[11].

The first isolations of MAYV were made from blood of five febrile forest workers in Trinidad in 1954[1]. The virus has been responsible for epidemics in South America and has been recovered from humans, wild vertebrates and mosquitoes in Brazil, Bolivia, Colombia, French Guiana, Guyana, Peru and Surinam[16,18]. Cases of MAYV were also described among member of the same family in a semirural forested area of Venezuela[19]. In addition, antibodies to MAYV have been found in Costa Rica, Guatemala and Panamá[12,13].

In Brazil the virus is endemic to the Amazon Region, where at least four epidemics have been reported in Pará State; in a community of quarry workers on the Guamá river, in 1955, in Belterra, a rural village of rubber plantations in 1978, in Conceição do Araguaia in 1981 and in Benevides in 1991[4,10,20].

Two other outbreaks were registered in Itaruma, Goiás State, in 1987 and in Peixe, Tocantins State, in 1991[20]. Specific antibodies against MAYV was also found in Xavante indians of Mato Grosso State and in inhabitants from rural areas of Goiás State in Central Brazil[8,13].

Although high antibodies rates are found in some rural communities of the Amazon basin of Brazil[11], it is difficult to isolate MAYV, because of the relatively short period of viremia[20].

This report describes three cases of MAYV infection in São Paulo, detected in patients infected while fishing in Camapuã, MS, from 11 to 19 March, 2000.

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MATERIAL AND METHODS

Case reports

Patient 1: SPH 191220, a 36 year-old male, resident of Flórida Paulista, SP. Onset of the symptoms: 18 March 2000, high fever for four days, arthralgias, joint pains in hand, knees and ankles

Patient 2: SPH 191221, a 59 year-old male, resident of Oswaldo Cruz, SP. Onset of the symptoms: 20 March 2000, high fever, arthralgias, joint pains in hands, knees and ankles for two days. He was hospitalized from 21 to 23 March. The arthralgias lasted for a month.

Patient 3: SPH 191223, a 74 year-old male, resident of Oswaldo Cruz, SP. Onset of the symptoms: 18 March 2000, high fever for two days. The artrhalgias lasted for a month with joints edema.

All patients showed a good course with full recovery.

Laboratorial assays: Blood samples were collected four days after the onset of the symptoms (acute) and two months later (convalescent).

Serological tests with acute and convalescent sera samples were performed by hemagglutination inhibition (HI) method with acetone-extracted serum samples[15], employing four units of the following antigens produced in suckling mice brain: *Alphavirus* (*Eastern, Venezuelan* and *Western equine encephalitis viruses*, and *Mayaro virus*), *Flavivirus* (*Dengue* 1, 2 and 3, Iguape, *Ilheus*, Rocio, *St. Louis encephalitis* and *Yellow fever viruses*) and *Orthobunyavirus* (*Caraparu virus*). The antigens were produced in Adolfo Lutz Institute.

All sera samples were also processed for *Dengue virus* (DENV) IgM by MAC-ELISA[7].

The acute serum samples were inoculated intracerebrally into newborn mice, which were observed for 21 days. A suspension of infected mouse brain from the sick or dead mice was inoculated onto C6/36 mosquito cells, incubated at 28 ^{0}C for nine days, and then examined by indirect immunofluorescent assay (IFA)[17] with alphavirus polyclonal antibodies.

Molecular assay was performed with RNA extracted from a supernatant of infected C6/36 cells using the QIAmp ViralRNA Extraction Kit (Quiagen, Valencia, CA, USA) according to manufacture's instructions. The SuperScript™ One Step RT-PCR System with Platinium Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA) was used for RT-PCR, in a final volume of 25 µL, containing 5 µL of viral RNA and 10 pMol of the M2W/cM3W genus-specific alphavirus primers[9] as well as the MayF1/MayB2 specific MAYV primers[16] (Table 1). Reverse transcription was performed at 50 °C for 30 min. After a 2-min denaturation step at 94 °C, the samples were thermocycled using the following program: denaturation at 94 °C for 30 sec, primer annealing at 55 °C for one min, extension at 72 °C for two min for 35 cycles, and a final extension at 72 °C for seven min. The amplification products were analyzed by electrophoresis in a 1.5% agarose gel, and the separated fragments were visualized by ethidium bromide staining.

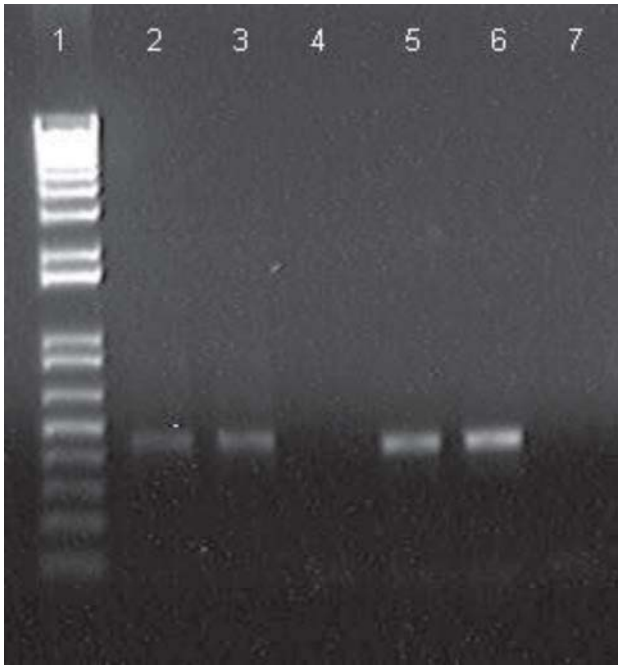

Fig. 1 - Ethidium bromide-stained agarose gel showing the products resulting from RT-PCR amplification performed with Alphavirus genus-specific primers, MAYV specific primers, RNA extracted from the patient (lanes 2 and 5) and MAYV positive control (lanes 3 and 6), respectively. Negative controls are shown in lanes 4 and 7 and molecular markers are shown in lane 1.

Table 1
Alphavirus genus-specific and MAYV specific primers used in RT-PCR

Primer	Sequence	Nucleotide position	Orientation	PCR product (bp)***
M2W*	(CT)AGAGC(AGT)TTTTCGCA(CT)(GC)T(AG)GC(ACT)(AT)	164-186	Forward	434
cM3W	ACAT(AG)AAN(GT)GNGTNGT(AG)TC(AG)ANCC(AGT)A(CT)CC	568-597	Reverse	
MayF1**	CTTCCCATGTTTCCAACCGAG	8235-8255	Forward	462
MayB2	GCCAGGATAAAGTGTCCCATTGTG	8696-8673	Reverse	

*Alphavirus genus-specific primers[9], encompassing the 5' end of the nsP1 gene of the Venezuelan equine encephalitis virus genome reported by KINNEY *et al.* (1992). **MAYV specific primers[16], encompassing the envelope E3 and E2 portion of the MAYV virus genome. *** base pair.

RESULTS

Virus isolation was obtained from only one of the acute blood samples (SPH 191221). All of the convalescent sera samples revealed monotypic seroconversion to MAYV by HI, with titer of 1:80 in the 2nd sera samples. Although this titer can be considered low, it is compatible with the period between the first and second samples. MAC-ELISA was not reactive to DENV. IFA done on the C6/36 cells was positive for alphavirus. This result was confirmed by the RT-PCR assay, done with the viral RNA and genus-specific alphavirus primers (Table 1). A final identification of MAYV virus was obtained in a further RT-PCR assay, performed with specific MAYV primers, designed to amplify a 462 base-pair product from the envelope E3 and E2 portion of the genome[16] (Table 1).

DISCUSSION

Arboviruses infections cannot usually be diagnosed only based on clinical symptoms; MAYV for example, causes a febrile illness that may be mistaken for DENV or other exanthematous diseases[5]. For this reason, many cases may be misdiagnosed. The surveillance system to diagnose acute undifferentiated febrile syndromes in the western Amazonian region of Brazil, analyzes serum samples obtained from patients with clinical suspicion of dengue and IgM antibodies to MAYV have been detected in a number of cases[5]. In addition, during two related epidemics of MAYV in the Amazonia, occurred concurrently with yellow fever[12]; and virological and serological studies made possible the differentiation of both diseases. Thus, the related cases illustrate, once more, the importance of laboratory confirmation in establishing a correct diagnosis.

None of the three patients presented rash which is reportably observed in about two thirds of MAYV infections, being more frequent in children[12]. The brief viremia, that occurs during MAYV infections may be responsible by the fact that the virus was isolated in only one out of the three cases.

The detection of MAYV in Goiás and Mato Grosso[8,13,20] and the present cases in Mato Grosso do Sul indicates that the virus also occurs out of the Amazonian Region.

It is noteworthy to consider the possibility of urbanization of the disease. Although mosquitoes of the genus *Haemagogus* are presumed to be the principal vector of the virus, experimental studies indicate that MAYV can also infect and be transmitted by *Aedes aegypti*[1]. So potentially, it might be transmitted in urban areas under the appropriate conditions.

Despite the fact that there are no fatal cases due to MAYV infection, the disease may cause a significant morbidity among persons who live in rural areas[12]. Intense arthralgia causes temporary incapacitation to work and some severely affected patients require hospitalization. Consequently, epidemics might have an important social and economic impact.

Infections by MAYV virus are sporadic; and as pointed out in this study, affect susceptible persons who are exposed to mosquitoes in forested areas. Human surveillance is difficult, since the infections are sometimes asymptomatic and they occur in rural areas, near or inside forests. Hence measures to control the vectors or vertebrates reservoirs would be impractical. At present, avoiding mosquito bites remains the only effective preventive control at present.

RESUMO

Vírus Mayaro: casos importados de infecção humana no Estado de São Paulo, Brasil

O vírus *Mayaro* (MAYV) é um arbovírus do gênero *Alphavirus,* família *Togaviridae,* enzoótico na América do Sul, sendo mantido em ciclo silvestre envolvendo vertebrados e mosquitos *Haemagogus.* Casos de MAYV são esporádicos e ocorrem em pessoas com história de recentes atividades dentro ou próximo a florestas. Este artigo relata infecção por MAYV detectada em três pacientes, infectados em Camapuã, MS, Brasil. Amostras de sangue, coletadas no 4° dia e no 2° mês após o início dos sintomas, foram usadas para teste de inibição da hemaglutinação, que revelou soroconversão monotípica para MAYV. O isolamento do vírus foi obtido somente de uma das amostras, por inoculação em camundongos lactentes. Suspensão de cérebro de camundongo infectado foi inoculada em cultura de células C6/36 e o vírus foi identificado por imunofluorescência indireta com anticorpos policlonais para alphavirus. RT-PCR realizado com RNA extraído do sobrenadante de células C6/36 infectadas, na presença de "primers" genéricos para alphavirus assim como "primers" para MAYV, confirmou os resultados. Os casos relatados ilustram a importância da confirmação laboratorial em estabelecer um diagnóstico correto. Os sintomas clínicos não são sempre indicativos de uma doença causada por arbovírus. MAYV causa doença febril, que pode ser confundida com dengue.

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