Isolation and Characterization of Mayaro Virus from a Human in Acre, Brazil


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Abstract. Mayaro virus (MAYV) is widely distributed throughout South America and is the etiologic agent of Mayaro fever, an acute febrile illness often presenting with arthralgic manifestations. The true incidence of MAYV infection is likely grossly underestimated because the symptomatic presentation is very similar to that of dengue fever and other acute febrile tropical diseases. We report the complete genome sequence of a MAYV isolate detected from an Acrelândia patient presenting with fever, chills, and sweating, but with no arthralgia. Results show that this isolate belongs to genotype D and is closely related to Bolivian strains. Our results suggest that the Acre/Mayaro strain is closely related to the progenitor of these Bolivian strains that were isolated between 2002 and 2006.

Mayaro virus (MAYV) is a member of the genus Alphavirus, family Togaviridae. It is widely distributed in Brazil, having been detected in the Amazonia, Central and Northeastern regions.1–5 Outbreaks of arthralgic disease caused by MAYV are usually limited to rural areas within/near rainforests where the mosquito vector Haemagogus janthinomys is abundant.6 The virus is endemic in many parts of the Amazon region of Brazil, with high seropositivity levels among the local populations (typically 5–60%).7–9 However, human viremia is short-lived (around 3 days), thereby making virus isolation from serum samples very difficult.9 Non-human primates and possibly migratory birds are important for enzootic virus circulation.8 The MAYV has the potential to be transmitted in urban settings by the Aedes aegypti mosquito,10 which is widely distributed in Brazilian cities.11

Human infections caused by MAYV have been described in several countries, including Trinidad,12 Bolivia,13,14 French Guiana,15 Peru,14,16 Venezuela,17,18 and Brazil.7,18,19 In 1978, during an epidemic in Belterra, Pará State, Brazil, the virus was isolated from 43 patients.7 In 2000, in São Paulo state (South-east Brazil), MAYV was isolated from a patient who had been fishing in Mató Grosso do Sul State.5 During 2007–2008, surveillance for acute febrile illness cases was performed in Manaus, Amazonia State, and 5.2% of all patients presented with anti-MAYV immunoglobulin M (IgM) antibodies.19

Phylogenetic studies of MAYV based on the E1/E2 envelope glycoprotein genes have recognized two major lineages: Genotype D includes isolates from 1954 to 2003, including samples from Trinidad, Brazil, French Guiana, Surinam, Peru and Bolivia, and Genotype L contains six isolates from the north-central Brazil, isolated between 1955 and 1991.20 Herein, we describe a case of MAYV infection that occurred in Acre, Amazon Basin, Brazil. We also compare the complete genomic sequence of our isolate with those currently available in GenBank to identify its phylogenetic relatedness to other MAYV strains.

The patient was identified in June, 2004, during an ongoing epidemiological survey, in Acrelândia, Acre State, Brazil, which is located in the Western Amazon Basin, bordering with Peru, Bolivia, and the Brazilian states of Amazonas and Rondônia. The blood sample was collected from a 27-year-old malaria-negative female patient presenting with fever, chills, and sweating. The patient was recruited at a health post during a population-based survey of acute febrile illnesses and examined for signs and symptoms of arboviral infections at the time of recruitment. The study was approved by the Ethirical Review Board of the Institute of Biomedical Sciences of the University of São Paulo, Brazil (protocol 538/2004) and additional epidemiological features of this survey were described previously by Silva-Nunes and others, 2008.21

The patient had a non-complicated disease and completely recovered. No signs or symptoms of joint pain, swelling, or arthralgia were recorded at the time of the recruitment, but no follow-up was performed, therefore it is unclear if the characteristic arthralgic symptoms of MAYV infection arose after initial examination. The RNA was extracted from the patient’s serum and multiplex-nested reverse transcription-polymerase chain reactions (RT-PCRs) performed for several flaviviruses and alphaviruses as described by Bronzoni and others, 2005.22 The sample was RT-PCR-positive for MAYV, and the virus was subsequently isolated in C6/36 mosquito cells and the multiplex-nested RT-PCR was used to confirm MAYV infection in the culture supernatant. Viral RNA was obtained using the QIAamp Viral RNA Kit (Qiagen, Germany), double strand complementary DNA (cDNA) was prepared according to Green and Sambrook (2012),23 and Nextera-XT DNA (Illumina, San Diego, CA) was used to prepare the library that was sequenced with the MiSeq v3 Reagent Kit, 150 cycles (Illumina) in the MiSeq System (Illumina). Geneious R6 (Biomatters, Auckland, New Zealand) was used to assemble the sequence using a Brazilian MAYV complete genome (accession NC_003417) as a reference. De novo assembly was also performed with the reads based on the assembled contig to confirm the sequence. The sequence has been submitted to GenBank under accession no. KM400591.

The Acre/Mayaro sequence was manually aligned with MAYV sequences available in the GenBank database using Se-Al (http://tree.bio.ed.ac.uk/software/seal/). A set of 61 taxa representing all available partial E2-E1 envelope glycoprotein sequences was prepared and used for phylogenetic analysis. A Bayesian phylogeny was inferred in Mr. Bayes24 using the
General Time Reversible (GTR+I+Γ₄) nucleotide substitution model. The analysis was performed for 1 million steps sampling every 1,000 and discarding the first 10% as burn-in.

Silva-Nunes and collaborators (2006) previously reported strong evidence of MAYV circulation and human infection in Acre state. Approximately half of the individuals sampled who were seropositive for MAYV were native Acreans, but the virus was never isolated there. Our study further supports their suggestion of endemicity with the isolation of MAYV from a serum sample collected in 2004.

Our Bayesian phylogeny supports the MAYV genetic structure previously described by Powers and others, with both genotypes being clearly delineated. The Acre/Mayaro strain grouped most closely with Bolivian strains isolated between 2002 and 2006 within genotype D (Figure 1). This result was not unexpected given the proximity of Acre state to Bolivia. In our phylogeny, the Acre/Mayaro strain was positioned basal to the clade containing the Bolivian strains, suggesting that it is closely related to the progenitor for these Bolivian strains. However, the Acre/Mayaro isolate was phylogenetically distinct from the Bolivian strains with which it clustered, and from all previously published Brazilian sequences. This relationship was supported by the high posterior probability value (>0.99) at the node supporting the Acre/Mayaro branch. According to its position in the phylogeny and its genetic divergence from other Brazilian strains isolated between 1978 and 1991 (92.1–98.8% nt sequence identity), relative to Bolivian strains isolated between 1955 and 2006 (98.8–99.3% nt sequence identity), and Peruvian strains isolated between 1995 and 2003 (98.6–99.4% nt sequence identity), Acre/Mayaro may represent another circulating phylogenetically distinct lineage within Brazil. However, there are very limited sequence data available for Brazilian MAYV strains after 1991, and these would be required to confirm this finding.

Human MAYV infections are strongly associated with occupational or recreational exposure in rainforest environments. However, increasing intercontinental travel and tourism-based forest excursions have increased the chance of acquiring these infections and possibly spreading the virus internationally. Therefore, infectious disease specialists need to be aware of

![Figure 1. Midpoint-rooted Bayesian Markov chain Monte Carlo (MCMC) phylogeny based on Mayaro virus (MAYV) partial E2-E1 sequences. Numbers at nodes indicate posterior probabilities ≥ 0.9. Taxon/tip labels include years of isolation, accession numbers, strain names, and countries where the virus was isolated. Scale bar shows percent nucleotide sequence divergence. Acre/Mayaro sequence is highlighted in gray and indicated with an arrow.](image-url)
febrile illnesses followed by persistent arthralgia, as patients can be easily misdiagnosed because Mayaro disease is not well known outside endemic regions.25–27

The threat of Mayaro and other arbovirus outbreaks (Dengue virus [DENV], Yellow fever virus [YFV], St. Louis encephalitis virus [SLEV], Chikungunya virus [CHIKV], Oropouche virus [OROV], equine encephalitis viruses, etc.) is extremely important to public health in the Americas and warrants further surveillance as part of an effective control program for humans and domestic animals. However, the epidemiology of the diseases caused by them is poorly explored in Brazil and other South America countries,1,2 because laboratory diagnostics for suspected cases are generally unavailable and clinical diagnosis based on signs and symptoms is difficult.1

This study reports the symptomatic profile of a patient presenting with Mayaro fever in the absence of acute arthralgia. The sequence reported here is the first to be described from Brazil in ~15 years and represents a genetically distinct Brazilian MAYV lineage. Surveillance of healthy at risk populations and those presenting with acute undifferentiated febrile illnesses will be the best approach to understanding the epidemiology and clinical presentation of MAY disease among infected individuals.

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