Mayaro Virus in Child with Acute Febrile Illness, Haiti, 2015

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Mayaro virus has been associated with small outbreaks in northern South America. We isolated this virus from a child with acute febrile illness in rural Haiti, confirming its role as a cause of mosquitoborne illness in the Caribbean region. The clinical presentation can mimic that of chikungunya, dengue, and Zika virus infections.

Mayaro virus (MAYV; genus *Alphavirus*, family *Togaviridae*) is a single-stranded positive RNA virus that was first isolated in Trinidad in 1954 (*1*) and is one of the viruses that comprise the Semliki Forest virus complex (*2*). Its transmission cycle is thought to occur mainly through mosquito vectors, especially those of genus *Haemagogus* (*3*), but *Aedes* spp. mosquitoes may also be competent vectors (*4*,*5*). The natural reservoirs of MAYV have been reported to be sylvatic vertebrates, mainly nonhuman primates but also birds and reptiles (*3*).

Since May 2014, when chikungunya virus (CHIKV) swept across the island of Hispaniola, researchers at the University of Florida (Gainesville, FL, USA) have studied alphavirus and flavivirus transmission in Haiti in collaboration with the Christianville Foundation. This foundation operates 4 schools in the Gressier/Leogane region of Haiti (≈20 miles west of Port-au-Prince) that serve ≈1,250 students from prekindergarten to grade 12 (*6*). The University of Florida has protocols in place for the collection of diagnostic blood samples from children seen at the school clinic with acute undifferentiated febrile illness (i.e., febrile illness with no localizing signs, such as would be expected with pneumonia, urinary tract infections, etc.).

From May 2014 through February 2015, blood samples were obtained from 177 children who met the criteria for acute undifferentiated febrile illness. The protocol for sample collection was approved by the University of Florida Institutional Review Board and the Haitian National Institutional Review Board. Written parental informed consent was obtained from parents or guardians of all study participants. Plasma samples were screened by reverse transcription PCR (RT-PCR) for CHIKV and dengue virus (DENV); samples that were negative for CHIKV were cultured by using cell lines and conditions as previously described (*7*). Zika virus and enterovirus D68 have been previously isolated from members of this school cohort (*7*,*8*). We report detection of MAYV in a child as part of this screening process.

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The Case

On January 8, 2015, an 8-year-old boy was examined at the school clinic because of fever and abdominal pain. His temperature was 100.4°F, lung sounds were clear, and his abdomen was soft and not tender. He had no rash and no conjunctivitis. On the basis of this clinical presentation, the clinic physician empirically diagnosed typhoid and administered co-trimoxazole.

A blood sample was collected, and RNA was extracted from plasma by using RT-PCR primers and the procedure described by Santiago et al. (*9*). The sample was negative for CHIKV but positive for DENV-1 (cycle threshold 26). In Vero E6 cells, which had been inoculated with the specimen, diffuse cytopathic effects typical for DENV-1 developed but at a much later time than for DENV-1–positive plasma specimens from other patients; this finding raised the possibility that DENV-1 had either mutated to reduced replication fitness or that the cells were co-infected with ≥2 incompatible viruses that were interfering with the replication of each other. DENV-1 viral RNA was detected by RT-PCR in the spent cell media of the plasma-inoculated cells but not in the spent media from noninoculated cells (negative control; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/11/16-1015-Techapp1.pdf). Furthermore, no CHIKV- or Zika virus–specific amplicons were amplified from the spent media. However, apart from DENV-1, an alphavirus amplicon corresponding in size to that expected for MAYV was detected in viral RNA extracted from infected Vero cells. Sequencing confirmed that the amplicon corresponded to MAYV (GenBank accession no. KX496990).

The MAYV genome from Haiti was aligned with all MAYV strains available in GenBank, and a neighbor-joining

tree was inferred from pairwise genetic distances estimated with the best fitting nucleotide substitution model (general time reversible plus gamma), as previously described (*7*). The phylogeny clearly shows 2 major and well-supported (bootstrap >90%) clades (Figure). The first clade includes strains sampled over the past 60 years from several South American countries (Peru, Bolivia, Venezuela, Trinidad and Tobago, and French Guiana); the second clade clusters the new Haiti strain with isolates from Brazil sampled during 1955–2014.

Conclusions

Although MAYV was originally isolated in Trinidad in 1954, subsequent reports of illness associated with this virus have tended to be associated with small, occasional outbreaks (30–100 cases) in northern South America (*10*,*11*), within and close to the Amazon forest. Signs and symptoms reported in association with MAYV infection include arthralgias, eye pain, fever, headache, myalgias, rash, and occasionally nausea and vomiting, photophobia, abdominal pain, cough, diarrhea, sore throat, and bleeding gums (*12*). A fatal infection associated with hemorrhagic fever has been reported (*13*). MAYV infections are probably underdiagnosed because of confusion with other mosquitoborne virus infections, especially dengue fever, which is endemic to the same areas. The emergence of CHIKV has further added to this confusion, especially because prolonged arthralgia is reportedly associated with CHIKV and MAYV infections (*3*).

Our findings suggest that MAYV is actively circulating in the Caribbean region and that there may be a link between the strain circulating in Haiti and the strains that have been circulating in Brazil since isolation of the virus in the 1950s. The patient from whom we isolated the organism had fever and abdominal pain but no rash or arthralgia. However, given that the patient was co-infected with DENV-1, it is difficult to separate out symptoms that are specific for MAYV infection. Of note, the clinic physician empirically diagnosed typhoid and treated the patient accordingly. The patient was from a rural/semi-rural area of Haiti, reflecting an ecologic setting that differs greatly from sylvan Amazon regions where many of the other reported MAYV infections have occurred. Little is known about vectors for MAYV in Haiti; potential animal reservoirs, if any, remain to be identified.

The recent emergence of Zika virus infection in the Caribbean region, and its identification as a major cause of birth defects, has brought a great deal of attention to arboviruses. Our findings highlight the multiplicity of arbovirus species in Haiti and the evolutionary relatedness among the viruses in Haiti and those circulating in Brazil, in keeping with prior work on Zika virus (*7*). Findings also underscore the complexity of the interactions among different species and the apparent proclivity for Zika virus/DENV (*7*) and MAYV/DENV co-infections. Although a better understanding of Zika virus infection is clearly needed, careful studies of other arboviruses

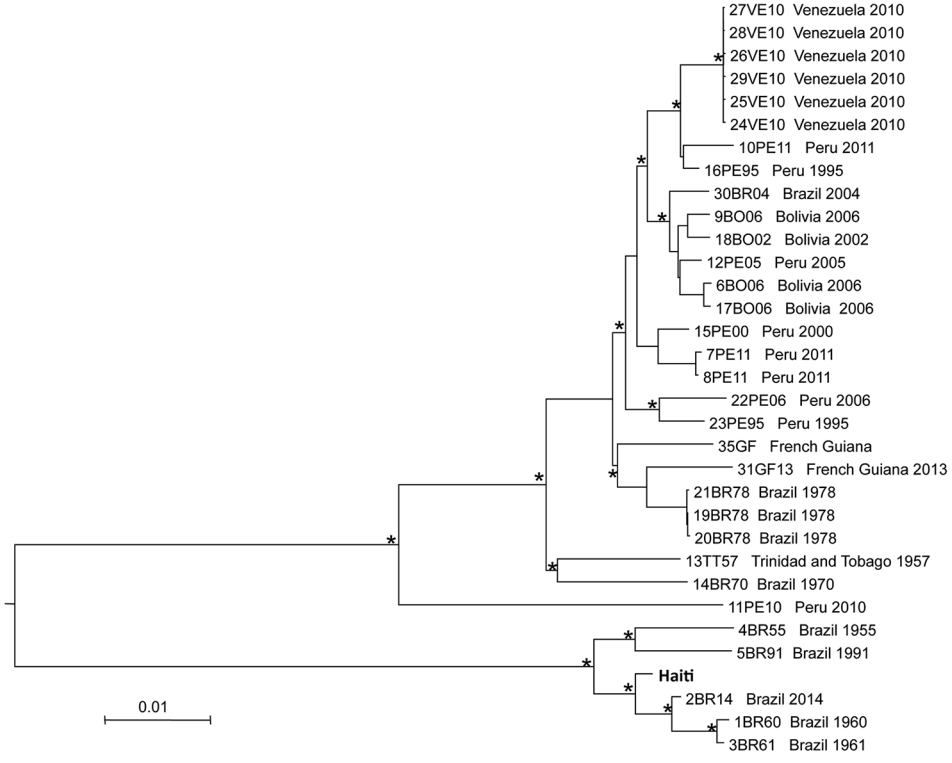

Figure. Neighbor-joining tree of full-genome Mayaro virus sequences. The tree was inferred from pairwise distances estimated with the best fitting nucleotide substitution model (general time reversible plus gamma). The tree includes the isolate from Haiti identified in this study (in boldface) and all full-genome sequences with known country of origin and sampling date downloaded from GenBank. Branches are drawn according to the scale bar at the bottom, which indicates nucleotide substitutions per site. An asterisk along a branch indicates bootstrap support >90% for the subtending clade.

(and their vectors and possible reservoirs) in these same geographic regions are correspondingly needed. We do not know if MAYV has epidemic potential; however, in light of recent observations with CHIKV, DENV, and Zika virus and the potential for transmission of MAYV by *Aedes* and *Haemagogus* spp. mosquitoes, inclusion of MAYV in studies of arbovirus transmission seems to be indicated.

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Dr. Lednicky is an associate professor of Environmental and Global Health in the College of Public Health and Health Professions at the University of Florida. His research interests are virus isolation and identification from human and environmental sources.

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Supplemental Material

Viral Isolation/Sequencing

Virus DNA (vDNA) and RNA (vRNA) were extracted from spent cell growth media and, separately, from the virus-infected cells. Spent cell growth media was centrifuged at low speed to pellet cellular debris, and the clarified spent media treated with cyanase nuclease to degrade nucleic acids external to that packaged (and thus protected) in virions using a Nucleic Acid Removal Kit (RiboSolutions, Inc., Cedar Creek, Texas), and vDNA and vRNA co-extracted from the treated material using a QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA) (Lednicky et al). In contrast, for the virus-infected cells, total RNA was extracted by an RNeasy kit (Qiagen), following the manufacturer's instructions.

PCR of vDNA purified from spent cell media: PCR for DNA viruses was performed using primers specific for the detection of Epstein Barr virus and cytomegalovirus, and polyomaviruses (information provided upon request). PCR was performed with One Taq DNA polymerase (New England Biolabs, Ipswich, MA, USA)

RT-PCR of vRNA purified from spent cell media: Real-time RT-PCR (rtRT-PCR) was performed for CHIKV, DENV, and ZIKV using the primers systems discussed in Lednicky et al (*1*). Standard RT-PCR was performed using virus group-specific primers that included universal α- and flavivirus primers (*2*) and universal primers for other RNA viruses (arena-, bunya-, corona-, paramyxo-, picorna-, and rubella viruses, information provided upon request). Briefly, a High-Capacity cDNA Reverse Transcription Kit (Applied Biosytems, ThermoFisher Scientific, Wyman, MA, USA) primed with non-ribosomal hexamers (0.6 mM) in the presence of SUPERase-In RNase inhibitor (Ambion, Austin, TX) was used for cDNA synthesis. Noteworthy, as an additional precaution, non-ribosomal hexamers were used was used to favor the reverse transcription of viral genomes over rRNA (*3*), even though the vRNA had already been pre-

treated using the RiboSolutions kit components, which is designed to remove contaminating rRNA. PCR was performed with One Taq DNA polymerase (New England Biolabs). Amplicons from standard RT-PCR reactions were analyzed on a 2% agarose gel stained with ethidium bromide (EtBr).

RT-PCR of RNA purified from virus-infected cells: A High-Capacity cDNA Reverse Transcription Kit (Applied Biosytems) was used with non-ribosomal hexamers (as described above) in the presence of SUPERase-In RNase inhibitor, and rtRT-PCR or RT-PCR performed using the CHIKV, DENV, and ZIKV described in Lednicky et al [1], the α- and flavivirus primer systems of de Morais et al (*2*), and RT-PCR systems for arena-, bunya-, corona-, paramyxo-, picorna-, and rubella viruses, as mentioned above. No MAYV strains/isolates of nucleic acid were present in the laboratory at the of the study; MAYV genomic sequences have never been detected in any work in our laboratory until the present study, and the cell lines and mock infected cells do not have Mayaro virus sequences.

Sequencing of MAYV: For RT-PCR, first-strand synthesis was performed using non-ribosomal hexamers and Accuscript High Fidelity 1st strand cDNA kit (Agilent Technologies, Santa Clara, CA, USA in the presence of SUPERase-In RNase inhibitor. PCR was performed with Phusion Polymerase (New England Biolabs) with denaturation steps performed at 98°C. A gene walking approach with overlapping primers was used (Technical Appendix Table), with most of the specific amplicons in the 800 bp range. A 5′ and 3′ system for the Rapid Amplification of cDNA Ends (RACE) was used per the manufacturer's protocols (Life Technologies, Carlsbad, CA, USA) to obtain the 5′ and 3′ ends of the MAYV genome. The reverse primer used for 5′ reaction consisted of MAYV-5R-RACE-JAL (5′-CCTGCTGTGCTTCCACTTCAAATGC-3′), and the 3′ forward primer was: MAYV-3F-RACE-JAL (5′-GGTCACCTATCCGGCAAATCACAACG-3′). Additionally, for 3′ RACE, 5′-T_{25}A-3′ was used as the reverse primer. Virus-specific PCR amplicons were purified, sequenced bidirectionally using Sanger Sequencing, and the sequences assembled with the aid of Sequencher DNA sequence analysis software v2.1 (Gene Codes, Ann Arbor, MI, USA). The GenBank accession number is KX496990.

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Technical Appendix Table. Primers used for sequencing of MAYV Haiti/1236/2015*

Primer	Primer nucleotide sequence, 5′→3′†	Nucleotide position‡
5R-RACE-JAL	CCTGCTGTGCTTCCACTTCAAATGC	182–158
MAYV-1F	CGCCGGACGTCTCTAAGCTCTTCCT	36–61
MAYV-1R	CACTGACGGTAAATGCCAACTC	877–856
MAYV-2F	GCAACCAATTGGGCTGACGAACAG	674–697
MAYV-2R	GACTGCAGAAGAAGCCTAATTCGG	1509–1486
MAYV-3F	GGTCCGCAGGGTTGTCGATAGGAATCAG	1455–1482
MAYV-3R	CACGCCCAAGACCCCGACCAC	2257–2237
MAYV-4F	CGAAGACCGGCGGCCCCGTAC	2207–2227
MAYV-4R	GGTTGCTGTAAAATTCCCTTTTGG	3034–3011
MAYV-5F	GTGATCCCTGGATCAAGACCTTGAG	2979–3003
MAYV-5R	GGTAGTGGTGTAGCCTATACGGTGTG	3779–3754
MAYV-6F	CGCTGGCAGGTACGACCTGGTCTTC	3718–3742
MAYV-6R	CGCGGCAGTAGATAGTGACCCGTGC	4523–4499
MAYV-7F	CAATCACTCTCGCATCTGTTGGC	4457–4459
MAYV-7R	GGGCGAGGCGGCGGTATGGGA	5271–5251
MAYV-8F	CCTACGCCAACGGCAGAACTTGC	5222–5244
MAYV-8R	CGTCTACCATATCCAAGTATGCGTC	6041–6017
MAYV-9F	GGCGTCCTACCAGATTACGGATG	5989–6011
MAYV-9R	CGATAAGGTCCAATAGCTGGTTATC	6794–6770
MAYV-10F	GTTGATGATATTGGAGGACCTG	6742–6763
MAYV-10R	GGGCCTGTTGGTCTGGTCTCTGCATTG	7555–7529
MAYV-11F	CCACCACGCCCTTGGAGACCAC	7498–7519
MAYV-11R	GAATTTACGTTCTCCTCCAGCATCCTCAG	8351–8323
MAYV-12F	GCTGTGCACCGTGCTGCTATGAAAAGG	8282–8308
MAYV-12R	GCCATTTTGTGTGGCTCGTGACGTAAGCCTG	9121–9091
MAYV-13F	CCATTAATAGCTGTACCGTTGAC	9062–9084
MAYV-13R	GTGCTCGTAAGCACTGGCAACG	9870–9849
MAYV-14F	GTCGCAATGAGCATCGGGAGTG	9826–9847
MAYV-14R	CGCCTTGGCGTTAAGCGGTGAGTC	10626–10603
MAYV-15F	CCCTATACCCAGACTCCATCTGGC	10555–10578
MAYV-15R	GTTATTATATGCGCCATTAGAGACG	11374–11350
3F-RACE-JAL	GGTCACCTATCCGGCAAATCACAACG	11016–11041
$T_{25}A$	TTTTTTTTTTTTTTTTTTTTTTTTA	polyA tail

*MAYV, Mayaro virus.
†Mismatches with corresponding nucleotide in MAYV BR/SJRP/LPV01/2015 (GenBank accession no. KT818520.1) indicated in red.
‡Nucleotide position in MAYV BR/SJRP/LPV01/2015 and in MAYV Haiti/1236/2015.