

Mayaro Virus Disease: An Emerging Mosquito-Borne Zoonosis in Tropical South America

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This report describes the clinical, laboratory, and epidemiological findings on 27 cases of Mayaro virus (MV) disease, an emerging mosquito-borne viral illness that is endemic in rural areas of tropical South America. MV disease is a nonfatal, dengue-like illness characterized by fever, chills, headache, eye pain, generalized myalgia, arthralgia, diarrhea, vomiting, and rash of 3–5 days' duration. Severe joint pain is a prominent feature of this illness; the arthralgia sometimes persists for months and can be quite incapacitating. Cases of two visitors from the United States, who developed MV disease during visits to eastern Peru, are reported. MV disease and dengue are difficult to differentiate clinically.

Mayaro virus (MV) disease is an acute febrile illness of 3–5 days' duration that is characterized by headache, retro-orbital pain, arthralgia, myalgia, vomiting, diarrhea, and rash [1]. MV is a member of the genus *Alphavirus*, family *Togaviridae*. Like most of the other alphaviruses, MV is transmitted to humans by the bite of infected mosquitoes. MV is related to chikungunya, o'nyong-nyong, Ross River, Barmah Forest, and Sindbis viruses, five other alphaviruses that produce a similar clinical illness in infected people [2–7].

MV was first isolated from sick forest workers in Trinidad in 1954 [6]. Subsequently, isolates were recovered from humans, wild vertebrates, and mosquitoes in Colombia, Brazil, Suriname, Guyana, French Guiana, Peru, and Bolivia [1, 6–9]. Serological studies [1, 7–10] indicate that MV infection is relatively common in human populations living in rural areas of northern South America and the Amazon Basin. Although

several small outbreaks of MV disease have been described in residents of rural communities in northern Brazil [11, 12] and in eastern Bolivia [13], human cases of the disease are generally sporadic and occur most commonly in persons in recent contact with humid, tropical forests [1]. This finding is probably due to the fact that mosquitoes of the Neotropical genus *Haemagogus* are the presumed principal vectors [1, 7, 14] and that most members of this genus are forest-dwelling [15].

MV disease is a zoonotic illness. Although MV, its mosquito vectors, and its wild vertebrate hosts have undoubtedly coexisted in Neotropical forests for a long time, recent demographic and land use changes in tropical South America appear to be altering the frequency of this endemic disease in people. As the human population in the region increases and as more people enter forested areas for work and for recreation, the number of persons at risk of MV infection is increasing. Furthermore, with the reappearance of dengue in much of South America and the initiation of a hemisphere-wide measles eradication program, surveillance of febrile exanthems in the region has intensified. Consequently, more cases of MV disease are probably being recognized and diagnosed than in the past.

To illustrate these points, we review our experience with MV disease in Peru and report two representative cases that recently occurred in United States citizens who visited Peru. These cases also illustrate the difficulty of differentiating MV disease from classical dengue fever and other febrile exanthematous diseases and the importance of laboratory confirmation.

Methods

Study Sites and Populations

The cases reported herein are from a variety of sources. The principal study site and source of samples was Iquitos, a city

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The research protocols for use with human subjects in this study were approved by the U.S. Naval Medical Research Institute's Committee for the Protection of Human Subjects, the Peruvian Navy Surgeon General's Ethical Committee, and the University of Texas Medical Branch's Institutional Review Board on the Use of Human Subjects.

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with ~300,000 people that is located in the northeastern Amazon Region of Peru in Loreto Department (figure 1). Twelve of the 27 patients with MV disease were seen in clinics or hospitals in Iquitos. Since 1993, the staff of the U.S. Naval Medical Research Unit Institute Detachment (Lima, Peru), the University of Texas Medical Branch (Galveston, TX), and the U.S. Army Medical Research Institute of Infectious Diseases (Frederick, MD) have been involved in epidemiological studies on the etiology of acute febrile illnesses in civilians and military personnel in the Iquitos area, in collaboration with the Peruvian Ministries of Health and Defense. The study site in Iquitos and the surveillance program have been described previously [16, 17].

Briefly, a sample of persons attending local health facilities and schools in the Iquitos area who had symptoms of a nonspecific febrile illness (temperature, $>38^{\circ}\text{C}$) of ≤ 5 days' duration were selected for study. An acute-phase blood sample was obtained, and all patients enrolled in the study completed an epidemiological questionnaire. Approximately 14 to 21 days later, most of the patients were visited at home by a public health nurse, and a convalescent-phase blood sample was obtained.

One additional patient from Iquitos was an American visitor who became ill during her plane trip back to the United States. She subsequently visited a hospital in Ohio, where acute- and convalescent-phase serum samples were obtained and forwarded to the Division of Vector-Borne Infectious Diseases Laboratory, Centers for Disease Control and Prevention (CDC; Fort Collins, CO), for diagnostic studies.



Figure 1. Map of Peru and adjacent countries that shows the approximate locality where Mayaro virus infections occurred. The number of cases detected at each geographic locality is given in parentheses.

The 14 other patients with MV disease were seen at health facilities elsewhere in Peru (figure 1). Blood samples from patients from Huanuco, Tocache, and Quillabamba were obtained as part of epidemiological investigations of febrile illness during outbreaks of jungle yellow fever that occurred in eastern Peru in 1995 and 1998. Blood samples from febrile patients in Pucallpa, Yurimaguas, and Tumbes were obtained in 1995, 1997, and 1998 during investigations of suspected dengue outbreaks. From these 14 cases, limited epidemiological information was available, and no convalescent-phase serum samples were obtained. However, MV was isolated from acute-phase serum samples from each patient. All cases reported herein occurred during a 3-year period from April 1995 to April 1998.

Virus Isolation and Identification

Acute-phase serum samples were transported on dry ice to the U.S. Naval Medical Research Unit Institute Detachment's laboratory in Lima, where they were stored in a low-temperature (-70°C) mechanical freezer until processed for virus isolation. At the time of processing, serum samples were thawed and diluted 1:5 in minimum essential medium containing 2% heat-inactivated fetal bovine serum and antibiotics. Single 25-cm² flasks of Vero and/or mosquito (C6/36) cell cultures were each inoculated with 200 μL of the diluted serum. Ten days after inoculation, or sooner if a viral cytopathic effect was observed, cells were scraped from the flask and placed on 12-well glass spot-slides for examination by an indirect fluorescent antibody technique (IFAT) [18] with use of the following mouse immune reference reagents: *Flavivirus* genus-specific monoclonal antibody 4G2 [19]; dengue virus 1- and dengue virus 2-specific monoclonal antibodies 15F3 and 3H5, respectively [19]; yellow fever virus-specific monoclonal antibody 2D12 [20]; and polyclonal hyperimmune ascitic fluids specific to MV and Oropouche, eastern equine encephalitis, and Venezuelan equine encephalitis (VEE) viruses. Immune reagents were obtained from the University of Texas Medical Branch, CDC, or U.S. Army Medical Research Institute of Infectious Diseases reference collections.

The immune ascitic fluids specific to eastern equine encephalitis virus, VEE virus, and MV were used in the IFAT at a 1:50 dilution. Preliminary studies indicated that MV could be differentiated from the other two alphaviruses by IFAT at this dilution. Six of the MV isolates, including isolates from patients 1 and 2 (see case reports), were confirmed by the plaque reduction neutralization test (PRNT) [21]. The isolate from patient 2 was also sequenced (see below).

Sequence Analysis

The nucleotide sequence of the MV isolate from patient 2 was determined and compared with the available sequences of the prototype MV strain (TRVL 4675) and of several other related alphaviruses. Virus was concentrated from an infected BHK-21 cell culture supernatant by precipitation with polyethylene glycol

and RNA extracted from the viral pellet with use of Trizol (Life Technologies, Grand Island, NY) as previously described [22]. The copy DNA was synthesized with use of a poly T oligonucleotide primer (T₂₅V-Mlu; 5'TTACGAATTCACGCGCT₂₅V-3') designed to anneal to the viral polyadenylation sequence. PCR amplification was performed with use of the T₂₅V-Mlu primer with a forward primer (α 10247A; 5'-TACCCNTTYATGTGGGG-3') to generate a fragment containing most of the E1 gene and the complete 3' noncoding region as described previously [22]. The resulting PCR product was cloned into the PCR2.1 vector (Invitrogen, Carlsbad, CA) and was sequenced with use of the plasmid-specific T7 promoter and m13 reverse primers (Gibco BRL, Gaitersburg, MD). The nucleotide sequence was aligned with alphavirus sequences determined previously and obtained from GenBank (Bethesda, MD) with use of the GAP and PILEUP programs in the GCG package [23].

Serological Assays

All acute- and convalescent-phase serum samples were screened initially at a dilution of 1:100 for IgM antibodies to MV and VEE virus by using an IgM antibody capture ELISA (MACEIA) [24]. Reactive samples were subsequently retested at serial dilutions of 1:200 through 1:102,400 to determine end point titers. Several of the serum samples were also titrated by EIA for IgG antibodies to MV and VEE virus.

Serum samples from patients 1 and 2 were also examined for neutralizing antibodies to MV and VEE, chikungunya, Ross River, and Getah viruses by PRNT [21] at twofold dilutions from 1:10 to 1:640. The highest serum dilution at which $\geq 80\%$ plaque reduction occurred was considered the end point.

Diagnostic Criteria

A confirmed case of MV disease was defined as an acute dengue-like illness and isolation of MV. A presumptive diagnosis of MV disease was made if the patient had a compatible clinical illness, no arbovirus was isolated, and titers of IgM antibodies to MV and VEE virus were $>1:400$ and $<1:100$, respectively, in a single serum sample.

Case Reports

Case 1

A 48-year-old American female nurse, who had spent the previous 2 weeks working at a mission clinic in a rural community near Iquitos, developed fever, chills, frontal headache, and generalized myalgia during her plane flight home to the United States in February 1996. These symptoms as well as weakness and joint pains (especially in the knees and ankles) continued for the next 2 days. On the third day of her illness, she was seen by a local physician and admitted to a hospital in Ohio with a presumptive diagnosis of dengue fever because of continued fever and dehydration. Before visiting Peru, the patient

had received yellow fever, hepatitis A and B, rabies, and typhoid vaccines. During her visit, she had taken mefloquine as prophylaxis for malaria.

Physical examination at the time of admission revealed a thin female in some distress because of headache, but no other physical abnormalities were found. Multiple insect bites were noted on the feet, lower legs, and thighs. Initial blood studies showed a hemoglobin level of 13.4 g/dL, hematocrit of 38.2%, platelet count of 124,000/mm³, and leukocyte count of 3,200/mm³, with 65% neutrophils and 4% band cells. Urinalysis was unremarkable, as was a chest roentgenogram. Examination of a blood smear for malaria and a serological test for *Salmonella* were both negative. A blood sample was obtained at the time of admission; a serum sample was frozen and submitted to the CDC for diagnostic testing for dengue.

At the time of admission to the hospital, the patient was given intravenous fluids for her dehydration and propoxyphene napsylate and acetaminophen tablets for pain. The following day, the patient was afebrile, and her headache and joint pains had diminished; she was discharged at her request. On the sixth day after the onset of symptoms, a diffuse papular rash developed on her arms, legs, and buttocks. MV was subsequently isolated from the acute-phase serum specimen submitted to the CDC. MV-specific IgM as well as neutralizing antibodies were absent from the first serum sample, but both antibodies were present in a second (convalescent-phase) serum sample obtained 20 days after the onset of the illness (table 1). The patient had a complete recovery, although joint pain and stiffness persisted for several months in her hands and knees; effusions were also noted in her knees and joints of the hands.

Case 2

A 29-year-old American female graduate student, who was trapping small mammals in a forest reserve near Iquitos, devel-

Table 1. Results of plaque reduction neutralization tests of serum samples from patients with two confirmed cases of MV disease.

Patient, date specimen collected	Titer of antibody to indicated virus				
	VEE virus	MV	CHIK virus	RR virus	GET virus
Patient 1					
13 February 1996	<10	<10	NT	NT	NT
1 March 1996	<10	80	NT	NT	NT
Patient 2					
6 February 1996	<10*	<10	<10	<10	<10
27 May 1997	160	<10	<10	<10	<10
18 August 1997	80	<10	<10	<10	<10
26 August 1997	640	40	<10	<10	<10
15 September 1997	≥ 640	80	<10	10	10
16 October 1997	≥ 640	40	<10	10	<10

NOTE. CHIK = chikungunya; GET = Getah; MV = Mayaro virus; NT = not tested; RR = Ross River; VEE = Venezuelan equine encephalitis.

* Reciprocal of highest serum dilution at which $\geq 80\%$ plaque reduction occurred (no antibody, $<1:10$).

oped fever, headache, generalized myalgia, weakness, and diarrhea on 14 August 1997. These symptoms continued for the next 2 days. The patient did not consult a physician and continued her field work; however, she noted that it became increasingly difficult to walk because of severe myalgia and arthralgia. On the fourth day of her illness she reported that she "could hardly move" because of severe joint pains and spent the entire day in bed. On the fifth day (18 August 1997), she felt better and was visited by a public health nurse who noted a maculopapular rash on her neck and arms, made a presumptive diagnosis of dengue fever, and obtained a blood sample for diagnostic studies.

MV was subsequently isolated from that sample at the U.S. Naval Medical Research Unit Institute Detachment's laboratory in Lima. MV-specific IgM, IgG, and neutralizing antibodies also were demonstrated in convalescent-phase serum samples obtained at various intervals after her illness (tables 1 and 2). The patient's symptoms gradually abated; she resumed work on 23 August 1997, although stiffness, joint pains (wrists and ankles), and fatigability persisted for about 2 months.

The patient was taking mefloquine as prophylaxis for malaria at the time of her illness in Peru. Before her departure from the United States to Peru in June 1997, the patient had received hepatitis A and B, yellow fever, and VEE (TC-83 live-attenuated) vaccines [25]. Several days after receiving the VEE vaccine in early May 1997, she had symptoms of low-grade fever, chills, headache, and myalgia, which lasted 24–36 hours. Analysis of a postvaccination blood sample obtained on 27 May 1997 indicated that she had seroconverted; a titer of neutralizing antibody to VEE virus of 1:160 was detected in her serum at that time (table 1). The latter result was confirmed at the U.S. Army Medical Research Institute of Infectious Diseases.

Results and Discussion

During the period from April 1995 to April 1998, a total of 27 cases of MV disease in persons living or working in Peru

Table 2. Results of IgM and IgG antibody capture ELISA of serial serum samples from a patient with MV disease (case 2).

Date serum collected	Titers of antibody to indicated antigen			
	VEE virus*		MV	
	IgM	IgG	IgM	IgG
18 August 1997	3,200 [†]	6,400	<100	<100
26 August 1997	6,400	≥102,400	12,800	1,600
15 September 1997	1,600	102,400	12,800	800
29 September 1997	800	12,800	3,200	3,200
16 October 1997	3,200	25,600	3,200	12,800

NOTE. MV = Mayaro virus; VEE = Venezuelan equine encephalitis.

* The patient had received VEE (TC-83) vaccine about 3 months before MV infection.

[†] Reciprocal of highest serum dilution at which a positive result occurred.

were diagnosed. MV was isolated from acute-phase serum samples from 21 (77.8%) of these patients (confirmed cases); the remaining six cases (presumptive) were diagnosed by serology. Of the 24 patients for whom demographic data were collected, 13 were females, and 11 were males. The ages of these patients ranged from 9 to 65 years (figure 2). Occupational information was available for 17 of the patients and was as follows: general laborer, 4 patients; agricultural worker, 3; housekeeper, 5; student, 2; soldier, 1; biologist, 1; and missionary, 1.

The cases of MV disease were sporadic (figure 3) and occurred over a wide geographic area of the country (figure 1). Twelve of the 27 cases were detected during prospective surveillance of patients with febrile illness in Iquitos. The remaining 15 cases were detected fortuitously by the isolation of MV from serum samples from patients with suspected cases of dengue or yellow fever that were submitted to our laboratory for confirmation. To our knowledge, this is the first report of MV disease in Peru. However, MV isolations from people in other South American countries have been reported previously [1, 6, 11–13] under similar circumstances; sporadic cases were detected coincidentally during the investigation of other disease outbreaks. Collectively, these results and the relatively high prevalence of antibodies to MV that was observed among persons living in rural areas of tropical South America [1, 7, 9, 10] suggest that MV disease is much more common than is generally reported.

The prevalence of presenting symptoms and signs in 22 of the patients with MV disease who were included in our study is shown in table 3. The symptomatology was similar to that reported in previous clinical descriptions of the disease [1, 6, 11, 12]. The relatively low prevalence of rash (31.9%) in our series can probably be explained by the fact that most of the patients were seen in the early phase of their illness, while they still had viremia. Pinheiro et al. [12] reported that the rash associated with MV disease usually appears about the fifth day of illness, at the time that the viremia ends and humoral antibodies first appear. Patients 1 and 2 in our study were observed over a longer period, and their rashes appeared on the sixth and fifth day of the illness, respectively.

Dengue was initially suspected in a number of the patients with MV disease in our study. As illustrated by cases 1 and 2, MV disease and classical dengue fever cannot be easily differentiated clinically. In addition, the hematologic studies in case 1 showed leukopenia and thrombocytopenia, findings which are also common in dengue virus infections [26]. This study illustrates the value and importance of laboratory confirmation in establishing a correct diagnosis. During the past 2 decades, dengue has reappeared in most cities and towns in tropical America, including the Amazon Basin [27]. Since most of these communities lack virus diagnostic capabilities, dengue in patients with febrile exanthems, regardless of the etiology, is commonly diagnosed. This is a serious problem in the current dengue reporting system for the Americas.

All of the MV isolates in this study were initially recovered in Vero and/or C6/36 cell cultures. Most of the samples were

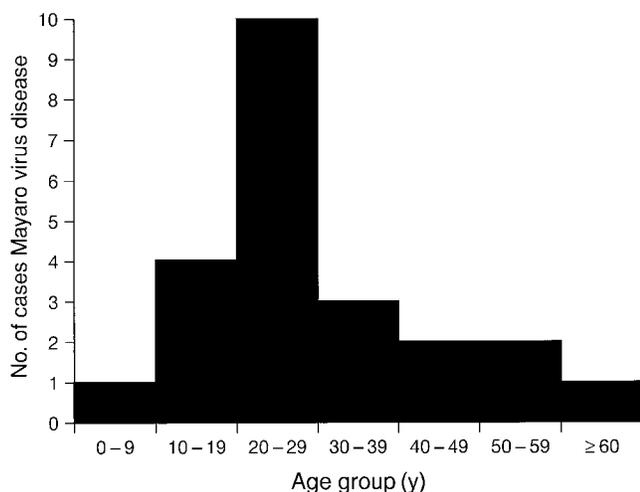


Figure 2. Dendrogram showing the age distribution for 23 patients with Mayaro virus disease in Peru.

inoculated only into mosquito cells. The number of infected serum samples inoculated into both cell lines was small; therefore, it was not possible to accurately compare the relative sensitivity of the two culture systems. MV produced a marked cytopathic effect in Vero cells, thus revealing viral infection. In contrast, infection in the mosquito cells was often inapparent, although occasionally syncytium formation was observed [28].

Six of the MV isolates, including the strains recovered from patients 1 and 2, were confirmed by PRNT. In addition, the MV isolate from patient 2 (designated as IQT-4235) was sequenced. The PCR product, encompassing the E1 gene and the 3' noncoding region, was analyzed by comparison of the nucleotide sequence with those of other known alphaviruses. Pairwise comparisons and phylogenetic analysis by using parsimony showed that IQT-4235 was most closely related to viruses within the antigenic complex of the Semliki Forest virus. The isolate IQT-4235 had a 95% nucleotide identity with the

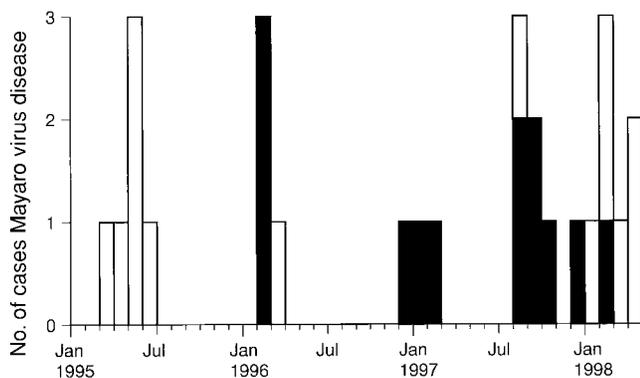


Figure 3. Monthly frequency of cases of Mayaro virus disease that were detected in Peru during the period April 1995 to April 1998. Black bars = cases from Iquitos; white bars = cases from six other localities.

Table 3. Prevalence of presenting symptoms and signs in 22 patients with Mayaro virus disease in Peru.

Symptom or sign	Prevalence (% of patients)
Fever	100
Headache	100
Myalgia	77.3
Eye pain	63.6
Chills	59.1
Arthralgia	50.0
Rash	31.9
Nausea	18.2
Cough	18.2
Sore throat	18.2
Vomiting	13.6
Abdominal pain	13.6
Nasal congestion	9.0
Diarrhea	9.0
Photophobia	4.5
Bleeding gums	4.5

prototype strain of MV (TRVL 4675), while the next closest relatives, Bebaru virus (strain MM 2354) and Una virus (strain BeAr13136), had only a 60%–66% nucleotide identity [7].

Results of serological studies of acute- and convalescent-phase serum samples from patients 1 and 2 are shown in tables 1 and 2. Patient 1 seroconverted, and the titer of neutralizing antibody to MV was 1:80 in a second serum sample taken 20 days after the onset of her illness on 1 March 1996 (table 1). IgM antibody capture ELISA of the same sample gave a positive reaction up to a dilution of 1:1,600 (data not shown).

The serological results for patient 2 were more complicated because she previously had received live-attenuated VEE vaccine. In a serum sample obtained in February 1996, before VEE vaccination and MV infection, there were no detectable neutralizing antibodies to any of the five alphaviruses (MV and VEE, chikungunya, Ross River, and Getah viruses) tested (table 1). In a serum sample obtained on 27 May 1997, about 3 weeks after she had received VEE vaccine, the titer of neutralizing antibody to VEE virus was 1:160, but no antibodies to the other four viruses were detected (titer, <1:10). Similar results of PRNT of a third serum sample obtained on 18 August 1997 were found; MV was isolated from this sample. Neutralizing antibodies to MV (titer, 1:40) were detected in a fourth serum sample obtained on 26 August 1997, 13 days after the onset of MV disease. It is interesting that there was an eightfold increase in the titer of neutralizing antibody to VEE virus between serum samples taken on 18 August and 26 August 1997. Neutralizing antibodies to VEE virus and MV also were present in two subsequent serum samples taken on 15 September and 16 October 1997 (~1 and 2 months after the infection), respectively.

Results of IgM and IgG antibody capture ELISAs of serum samples from patient 2 are given in table 2. In the serum sample from which MV was isolated (which was obtained on 18 August 1997), both IgM and IgG antibodies to VEE virus were

present, but no antibodies to MV were detected. However, in the sample obtained on 26 August 1997 and subsequent samples, both IgM and IgG antibodies to MV were present. As PRNT revealed, titers of IgG antibody to VEE virus also increased following MV infection.

The increase in the titer of IgG antibody to VEE virus, observed in case 2 following MV infection (tables 1 and 2), is interesting, but this phenomenon (original antigenic sin) has been described for alphaviruses before [29–31]. Earlier experiments with animals [32, 33] suggested that infection with one alphavirus might protect against or attenuate a subsequent heterologous alphavirus infection. However, in the case of patient 2, previous immunization with live-attenuated VEE vaccine and the presence of heterologous (VEE virus-specific) neutralizing antibodies did not appear to alter her subsequent MV infection.

In view of the current rural and endemic nature of MV disease in tropical South America, it is worth considering the possibility of urbanization of the disease. The available ecological data indicate that MV is maintained in a continuous sylvan cycle involving wild vertebrates (mammals and possibly birds) and mosquitoes [1]. To date, most of the arthropod isolations of the virus have been from mosquitoes of the genus *Haemagogus*; therefore, it is presumed that they are the principal vectors [1, 7, 14]. However, experimental studies [34] indicate that MV can also infect and be transmitted by *Aedes aegypti*.

As noted before [27], this highly anthropophilic urban mosquito has now reinfested most communities in tropical America. Humans infected with MV develop detectable viremia of 3 to 5 days' duration [12]. Acute-phase serum samples from two of our patients with MV disease were titrated in C6/36 cells, and IFAT revealed that the median infective dose was about $10^{5.0}$ tissue culture/mL (data not shown). Comparable levels of viremia were also observed in nonhuman primates with experimental MV infections [14, 32]. Titers of this magnitude should be adequate to infect susceptible mosquitoes. One of the distinguishing features among the alphaviruses is their ability to infect a wide range of mosquito species [1, 35]; therefore, it is conceivable that MV could infect *A. aegypti* and cause urban MV disease.

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