Anopheles mosquitoes may drive invasion and transmission of Mayaro virus across geographically diverse regions

Marco Brustolin*, Sujit Pujhari*, Cory A. Henderson, Jason L. Rasgon☯

Department of Entomology, The Pennsylvania State University, University Park, PA, United States of America

☯ These authors contributed equally to this work.
* jlr54@psu.edu

Abstract

The Togavirus (Alphavirus) Mayaro virus (MAYV) was initially described in 1954 from Mayaro County (Trinidad) and has been responsible for outbreaks in South America and the Caribbean. Imported MAYV cases are on the rise, leading to invasion concerns similar to Chikungunya and Zika viruses. Little is known about the range of mosquito species that are competent MAYV vectors. We tested vector competence of 2 MAYV genotypes in laboratory strains of six mosquito species (Aedes aegypti, Anopheles freeborni, An. gambiae, An. quadrimaculatus, An. stephensi, Culex quinquefasciatus). Ae. aegypti and Cx. quinquefasciatus were poor MAYV vectors, and had either poor or null infection and transmission rates at the tested viral challenge titers. In contrast, all Anopheles species were able to transmit MAYV, and 3 of the 4 species transmitted both genotypes. The Anopheles species tested are divergent and native to widely separated geographic regions (Africa, Asia, North America), suggesting that Anopheles may be important in the invasion and spread of MAYV across diverse regions of the world.

Author summary

Mayaro virus (MAYV) is a mosquito-borne Alphavirus responsible for outbreaks in South America and the Caribbean. In this study we infected different species of mosquito (belonging to the genera Aedes, Anopheles and Culex) with MAYV and tested their capacity to transmit the virus at different time points. Results show that Anopheles mosquitoes were competent vectors for 2 genotypes of MAYV, while Aedes and Culex were poor vectors. The capacity of Anopheles mosquitoes to transmit MAYV highlights their importance as neglected vectors of arboviruses. These data suggest that Anopheles mosquitoes have the potential to sustain transmission cycles of neglected pathogens in naïve regions, including the United States.
Introduction

Mayaro virus (MAYV) is a member of the genus *Alphavirus* (family Togaviridae) which was first isolated from the blood of five febrile workers in Mayaro County, Trinidad, in 1954 [1]. MAYV has a single-stranded positive-sense RNA genome of approximately 11.7 kb and is classified into three genotypes: D, L, and N [2,3]. Genotype D (dispersed) includes strains isolated in several South American countries, whereas genotype L (limited) includes strains isolated only in Brazil. In 2010, a minor genotype called N (new), was isolated in Peru, but it is limited to one known sequence. Since its first isolation, MAYV has caused sporadic outbreaks and small epidemics in several countries in South and Central America (reviewed in [4]). In 2015, an 8-year-old child from Haiti was reported as co-infected with MAYV and dengue virus (DENV), suggested that MAYV may be actively circulating in the Caribbean [5]. Several imported cases recently reported in the Netherlands [6], Germany [7], France [8], and Switzerland [9] highlight the need to survey naive regions, including the United States, for possible introductions of this neglected arthropod-borne virus (arbovirus).

The symptoms of MAYV infection include rash, fever, myalgia, retro-orbital pain, headache, diarrhea, and arthralgia, which may persist for months or even years [10], and are similar to those caused by other arboviruses such DENV or chikungunya virus (CHIKV). Due to the absence of routine differential diagnostics, reported cases of MAYV likely underestimate the real prevalence, and the circulation of the virus can pass undetected in areas with ongoing DENV or CHIKV outbreaks [4,11].

MAYV is thought to be principally transmitted by the bite of diurnal canopy-dwelling mosquitoes of the genus *Haemagogus* [4]. These mosquitoes are responsible for maintaining the sylvatic cycle involving nonhuman primates and birds as primary and secondary hosts, respectively. Human infections are sporadic, likely because *Haemagogus* spp. rarely display anthropophilic behaviors, and they possess a preference for rural areas with proximity to forests [12]. Vector competence (VC) studies demonstrated that anthropophilic and urban-adapted species, such as *Aedes aegypti* (Linnaeus, 1762) and *Ae. albopictus* (Skuse, 1895), are competent vectors for MAYV in laboratory conditions [13–15]. *Culex quinquefasciatus* mosquitoes positive for MAYV have also been identified from field collections during a DENV outbreak in Mato Groso County, Brazil [16]; however, their capacity to transmit MAYV has not been demonstrated.

Overall, little data is available about the VC of mosquitoes for MAYV [14,15,17,18] and, there have been no studies about the VC of vector species in the United States (with the exception of two field populations of *Ae. aegypti* and *Ae. albopictus* from Florida [15]). To address this knowledge gap, we evaluated the ability for *Ae. aegypti* and *Anopheles freeborni* (Aitken, 1939), *An. gambiae* (Giles, 1902), *An. quadrimaculatus* (Say, 1824), *An. stephensi* (Liston, 1901) and *Cx. quinquefasciatus* (Say, 1823) to become infected with and transmit MAYV after feeding on a viremic blood meal. Our results demonstrate that while the laboratory strains of *Ae. aegypti* and *Cx. quinquefasciatus* tested are poor vectors for MAYV, all tested *Anopheles* species were competent laboratory vectors for MAYV, including species that they have the potential to support the transmission cycle if the virus is introduced into the United States. Additionally, the results of our study provide useful information to improve entomologic surveillance programs and prevent future outbreaks of this emerging neglected pathogen.

Material and methods

Mosquitoes

Six mosquito species were used in this experimental study. The *An. gambiae* (NIH strain) were originally obtained from The National Institutes of Health (Bethesda, MD, USA). *An. stephensi*
(Liston strain) were provided by Johns Hopkins University (Baltimore, MD, USA). Cx. quinquefasciatus (Benzon strain) were provided by the Wadsworth Center (Slingerlands, NY, USA) and was initially derived from a colony maintained by Benzon Research (Carlisle, PA, USA). An. quadriramulatus (Orlando strain, MRA-139) and An. freeborni (F1 strain, MRA-130) were provided by BEI Resources (Manassas, VA, USA). Ae. aegypti (Rockefeller strain) were provided by Johns Hopkins University. Anopheles species were selected to cover different geographical macroregions: North America (An. freeborni and An. quadriramulatus), Africa (An. gambiae) and Southeast Asia (An. stephensi).

Mosquito colonies were reared and maintained at the Millennium Sciences Complex insectary (The Pennsylvania State University, University Park, PA, USA) at 27˚C ± 1˚C, 12:12 h light:dark diurnal cycle at 80% relative humidity in 30×30×30-cm cages. Ground fish flakes (TetraMin, Melle, Germany) were used to feed Anopheles spp. and Aedes sp. larvae. A 1:1:1 mixture of bovine liver powder (MP Biomedicals, Solon, OH, USA), koi pellets (TetraPond Koi Vibrance; TetraPond, Prestons, Australia), and rabbit pellets (Kaytee, Chilton, WI, USA) was used for Culex sp. larvae. Adult mosquitoes were provided with 10% sucrose solution ad libitum for maintenance. For reproduction and virus infection purposes, adults were fed with expired anonymous human blood (Biological Specialty Corporation, Colmar, PA, USA).

**Virus**

Two strains of MAYV were used for the experimental infections: BeAr 505411 (BEI Resources, Manassas, VA, USA), a genotype L strain isolated from Haemagogus janthinomys mosquitoes in Para, Brazil, in March 1991, and BeAn 343102 (BEI Resources, Manassas, VA, USA), a genotype D strain originally isolated from a monkey in Para, Brazil, in May 1978. Both viruses were passed once in African green monkey kidney (Vero) cells. Virus-infected supernatant was aliquoted and stored at −70˚C until used for mosquito infections. Viral stock titers were obtained by the focus forming unit (FFU) technique, as described below.

**Vector competence assays**

Five- to seven-day-old females that had not previously blood-fed were used for experiments. Mosquitoes were allowed to feed on human blood spiked with virus via a glass feeder jacketed with 37˚C distilled water for 1 h. Aliquots of the infectious bloodmeals were collected and titers of MAYV were determined by FFU (Table 1). After blood feeding, mosquitoes were anesthetized and fully engorged females were selected and placed in cardboard cages. Infection rate (IR), dissemination rate (DIR), transmission rate (TR), and transmission efficiency (TE) were assessed at 7 and 14 days post-infection (dpi). IR was measured as the rate of mosquitoes with infected bodies among the total number of analyzed mosquitoes. DIR was measured as the rate of mosquitoes with infected legs among the mosquitoes with positive bodies. TR was measured as the rate of mosquitoes with infectious saliva among the mosquitoes with positives legs, and TE measured as the rate of mosquitoes with infectious saliva among the total number of analyzed mosquitoes [19].

At 7 and 14 dpi, mosquitoes were anesthetized with triethylamine (Sigma, St. Louis, MO, USA). Legs were detached from each body and placed in 2-mL tubes filled with 1 mL of mosquito diluent (20% heat-inactivated fetal bovine serum [FBS] in Dulbecco’s phosphate-buffered saline [PBS], 50 μg/mL penicillin/streptomycin, 50 μg/mL gentamicin, and 2.5 μg/mL fungizone) and a single zinc-plated, steel, 4.5-mm bead (Daisy, Rogers, AR, USA), and tubes immediately placed on ice. Saliva was collected by forced salivation into a capillary tube as previously described [20], expelled into in a 2-mL tube filled with 100 μL of mosquito diluent, and immediately placed on ice. Body and leg samples were homogenized at 30 Hz for 2 min using...
a TissueLyser II (QIAGEN GmbH, Hilden, Germany) and centrifuged for 30 sec at 11,000 rpm. All samples were stored at −70˚C until tested.

**FFU assay**

The presence of infectious MAYV particles in the body, legs, and saliva samples was tested by FFU assay in Vero cells. Vero cells were grown to a confluent monolayer in 96-well plates at 37˚C with 5% CO₂ in complete media (Dulbecco’s modified-essential media [DMEM], 100 units/mL penicillin/streptomycin, and 10% FBS). The next day, wells were washed with DMEM without FBS and incubated with a 30-μL of 10-fold serial dilutions (10⁻¹ to 10⁻⁴) of each homogenized tissue sample for 2 h at 37˚C. Saliva samples were not diluted. After the incubation step, the 30-μL aliquot was removed from the cell monolayer and any unattached viral particles removed with a DMEM wash. A total of 100 μL of overlay medium (1% methyl cellulose in complete growth medium) was dispensed into each well, and plates incubated at 37˚C with 5% CO₂. Cells were fixed at 24 h (bodies and legs samples) or 48 h (saliva samples) post-infection with 4% paraformaldehyde (Sigma, St. Louis, MO, USA). Fixed cells were blocked and permeabilized for 30 min with blocking solution containing detergent (3% bovine serum albumin and 0.05% Tween 20 in PBS) and washed with cold PBS. Viral antigens in infected cells were labeled using the monoclonal anti-chikungunya virus E2 envelope glycoprotein clone CHK-48 (which reacts with Alphaviruses) (BEI Resources, Manassas, VA, USA) diluted 1:500 in blocking solution. Subsequently, cells were washed 4 times with cold PBS to remove unbound primary antibodies. The primary antibody was labeled with the Alexa-488 goat anti-mouse IgG secondary antibody (Invitrogen, Life Science, Eugene OR, USA) at a dilution of 1:500, and green fluorescent foci observed and enumerated with an Olympus BX41 microscope equipped with an UPlanFI 4× objective and a FITC filter. Fluorescent foci were counted by eye (the dilution with less than 100 foci was selected for each sample) and virus titers calculated and expressed as FFU/mL.

![Table 1. Infection, dissemination and transmission rates for mosquitoes orally exposed to Mayaro virus.](https://doi.org/10.1371/journal.pntd.0006895.t001)
Statistical analysis

Data were analyzed using GraphPad Prism version 7.04. Differences in the IR, DIR, TR, and TE of mosquitoes challenged with BeAr 505411 and BeAn 343102 were analyzed by Fisher’s exact test. The Mann–Whitney U test was used to compare the body, legs, and saliva viral titers of mosquitoes exposed to BeAr 505411 or BeAn 343102.

Results

A total of 115 Ae. aegypti, 19 An. freeborni, 31 An. gambiae, 29 An. quadrimaculatus, 132 An. stephensi and 60 Cx. quinquefasciatus were analyzed in this study. Details of analyzed mosquitoes and the IR, DIR, TR, and TE are in Table 1.

All six mosquito species were susceptible to infection with MAYV to some degree, although there were MAYV strain–specific differences. IRs for Ae. aegypti exposed to strain BeAr 505411 were significantly higher compared to strain BeAn 343102 (p<0.0001) at 7 dpi, and IRs for strain BeAr 505411 at 7 dpi were significantly higher than 14 dpi (p<0.0001). Moreover, no Ae. aegypti exposed to strain BeAn 343102 became infected at 14 dpi despite the presence of positive mosquitoes at 7 dpi. IRs for An. gambiae and An. stephensi were similar across MAYV strains, and IRs increased over time in An. gambiae. An. freeborni and An. quadrimaculatus were susceptible to infection with both strains of MAYV, and Cx. quinquefasciatus was susceptible only to a low-frequency infection with strain BeAr 505411 and refractory to BeAn 343102 infection.

Once infected, all tested mosquito species developed a disseminated infection. Disseminated infection was generally detected as early as 7 dpi, with the exception of An. freeborni exposed to the BeAr 505411 strain. DIRs were similar for both virus strains in An. gambiae and An. stephensi at both timepoints and for Ae. aegypti at 7 dpi. There was a trend toward higher DIRs for strain BeAn 343102 compared to strain BeAr 505411 in An. quadrimaculatus and An. freeborni at day 7. There was also a trend toward a higher DIR at 14 dpi than at 7 dpi for strain BeAr 505411 in Ae. aegypti, both strains in An. gambiae, and strain BeAr 505411 in An. freeborni.

Transmission was detected in all Anopheles species and Ae. aegypti (albeit very poorly), but not in Cx. quinquefasciatus. An. gambiae, An. quadrimaculatus and An. stephensi were able to transmit both MAYV strains tested. For Ae. aegypti only a single transmission event was detected for virus strain BeAr 505411. Only virus strain BeAn 343102 was transmitted by An. freeborni. Both virus strains could be transmitted by An. gambiae, quadrimaculatus and stephensi.

MAYV titers for all samples were calculated and expressed as FFU/mL. Ae. aegypti exposed to strain BeAr 505411 had significantly greater titers in the bodies (7 and 14 dpi) and legs (7 and 14 dpi) compared to strain BeAn 343102 (p<0.0001) (Fig 1). Conversely, An. stephensi exposed to strain BeAn 343102 had significantly greater titers in the bodies (7 dpi, p<0.05; 14 dpi, p<0.001) and legs (7 dpi, p<0.001) compared to strain BeAr 505411 (Fig 1). There were no significant differences in body, legs, or saliva titers between the MAYV strains in An. freeborni, An. gambiae, An. quadrimaculatus and Cx. quinquefasciatus.

Discussion

These results demonstrate that An. freeborni, An. gambiae, An. quadrimaculatus, and An. stephensi are competent laboratory vectors for MAYV. The two viral strains tested present significant differences in their ability to infect and disseminate in Ae. aegypti and An. stephensi. In An. stephensi, the strain BeAn 343102 had a statistically higher titer in body and leg samples than BeAr 505411. Conversely, strain BeAn 343102 has a statistically lower body titer in Ae.
Fig 1. Viral titer in body, legs, and saliva of six mosquito species mosquitoes exposed to Mayaro virus. Each dot corresponds to a single mosquito sample. Viral titers were statistically compared between strains by Mann-Whitney U test.

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Ae. aegypti and was not detected in legs, likely indicating the presence of a midgut escape barrier. Strain BeAn 343102 failed to infect Cx. quinquefasciatus, likely due to the presence of a midgut infection barrier. The VC differences between the strains may be explained by the theory of the host genotype and pathogen genotype (G × G) interaction [21]. G × G interactions have been found in many systems, including DENV [22]. For example, Lambrechts et al. [22] showed that DENV vector competence varied greatly depending on the specific Ae. aegypti population and DENV genotype combination. This provides evidence that G × G interactions may be responsible for the adaptation of a lineage/strain to a specific population.

Ae. aegypti, An. freeborni, An. quadrinaculatus and An. stephensi were able to transmit the virus at 7 dpi but we did not detect transmission at 14 dpi. The short extrinsic incubation period (EIP) of MAYV for these species might represent a notable increase in their vectorial capacity [23] and must be considered when establishing a future surveillance plan. In An. stephensi, the absence of transmission at 14 dpi corresponds with a decrease of the viral titer in the legs between 7 dpi and 14 dpi. These data suggest that in An. stephensi, MAYV infection may not persist, and may be progressively eliminated or limited by the vector. Similar results were recently published for Ae. aegypti infected with DENV [24], where a progressive decrease in transmission began at 14 dpi and continued until 25 dpi, at which point no viral transmission was recorded. To test this hypothesis and to better understand the kinetics of MAYV infection, a study with a longer EIP and more intermediate timepoints should be performed.

An. gambiae also is a competent laboratory vector for MAYV but the longer EIP (14 dpi) required for the transmission of the virus might limit the role of this species in the transmission cycle.

With the Ae. aegypti strain tested here, we obtained similar IR and DIR results compared to those previously described with a different strain [14,15]. However, the MAYV TR in this study is considerably lower than that described by Long et al. using a field population from Peru [14] (6.7% vs. 88%) or Wiggins et al. [15] with a field population from Florida (6.7% vs. ~25%). This discrepancy could be due to the genetic differences in the mosquito population (salivary gland infection barrier in the strain tested) or in the viral strain used for the experiment. Is important to highlight that results presented by Wiggins et al. are obtained by RT-PCR, which allows identification of viral RNA but does not ensure the presence of infectious viral particles in the saliva producing in some cases an overestimation of the TR.

The global expansion of CHIKV demonstrates how rapid virus adaptation may alter the transmission potential of alternate arthropod vectors [25]. The adaptation of MAYV to Aedes has been analyzed [26], and the emergence of hybrid genotypes D and L suggests that Aedes mosquitoes can play an important role in the urban diffusion of MAYV. Ae. aegypti and Ae. albopictus are well adapted to urban and peri-urban habitats, and, contrary to Haemagogus mosquitoes, consistently have anthropophilic feeding behavior. Our results confirm that Ae. aegypti is a possible (if potentially inefficient) vector for MAYV, but more studies are needed to understand the differences in the VC for the genotype D and genotype L strains.

We found that Cx. quinquefasciatus can be infected with MAYV strain BeAr 505411, but is not able to transmit the virus. Conversely, another study found MAYV-positive Cx. quinquefasciatus during an outbreak of DENV in Mato Grosso, Brazil, and suggested that this species could sustain the transmission cycle [16]. The MIRs obtained for the pools of Cx. quinquefasciatus collected in two of four study areas seems to indicate a quite low prevalence of infected mosquitoes (MIRs = 1.39 and 16). These results highlight the important point that merely detecting virus in a mosquito does not necessarily implicate it as a vector.

Previously, only two alphaviruses were known to be transmitted by Anopheles mosquitoes: O’nyong-nyong virus [27] and a single record for CHIKV [28]. However, in the original paper describing the isolation of MAYV, the authors present an anecdote (no data) stating that when
inoculated into *An. quadrimaculatus* from Trinidad, MAYV was able to replicate (although neither oral infection nor transmission was investigated) [1]. The capacity of *An. quadrimaculatus* and *An. freeborni* to transmit MAYV is particularly relevant to the United States, because the estimated geographic distribution of these species covers the entirety of the country [29,30]. The sylvatic cycle of MAYV involves principally non-human primates and birds, however there is serological evidence of MAYV circulation in other hosts including rodents and marsupials [31]. Nothing is known about the capacity of North American mammal species to act as vertebrate reservoirs. However, MAYV could be possibly maintained in a human-mosquito-human urban cycle similarly to what is observed with CHIKV. According to Tesh et al. [32] human viremia during the acute-phase is comparable to that reached in experimentally infected nonhuman primates. *An freeborni* and *An. quadrimaculatus* are opportunistic feeders and have a wide range of hosts including humans. Depending on ecological and epidemiological factors, these two mosquito species might have the capacity to sustain the transmission cycle and spread the MAYV in the United States in synergy with other competent species like *Ae. aegypti* and *Ae. albopictus* [15]. An interesting and important aspect of *Anopheles* vector biology is their tendency to have multiple feeding events during a single gonotrophic cycle [33]. The bite frequency of *Anopheles* mosquitoes increases their vectorial capacity [23]. For these reasons, we highlight the need for more studies on the possible role of *Anopheles* mosquitoes in spreading arboviruses in the United States.

We tested 4 *Anopheles* species (2 from North America, one from Africa, and one from Southeast Asia) for MAYV VC, and all were able to transmit the virus. Our results illustrate the knowledge gaps that remain about this important emerging virus. *Anopheles* mosquitoes in general are currently neglected as potential vectors of arboviral pathogens, however they are widely dispersed worldwide (reviewed in [34]). Our data suggest that *Anopheles* spp. may be important vectors driving the emergence and invasion of MAYV (and potentially other arboviruses) across geographically diverse regions of the globe, and their epidemiological role in virus invasions should be further studied.

**Caveats of this study**

It should be noted that all results were conducted with laboratory mosquito strains. Results with other laboratory strains or wild mosquitoes that differ in their nuclear genotype or microbiome may differ from those we obtained. All mosquitoes in our experiments were infected with approximately 7 logs of virus; for mosquitoes that did not readily become infected it is possible that higher viral titers might result in higher infection rates. In our experiments we only tested two timepoints post-infection (seven and fourteen days). It is possible that longer incubation times may result in different infection and/or transmission rates.

**Supporting information**

S1 Table. Manuscript raw data.

(XLSX)

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**Author Contributions**

Conceptualization: Marco Brustolin, Sujit Pujhari, Jason L. Rasgon.
Data curation: Marco Brustolin, Sujit Pujhari.

Formal analysis: Marco Brustolin, Sujit Pujhari, Jason L. Rasgon.

Funding acquisition: Jason L. Rasgon.

Investigation: Marco Brustolin, Sujit Pujhari, Cory A. Henderson.

Methodology: Marco Brustolin, Sujit Pujhari.

Project administration: Jason L. Rasgon.

Resources: Jason L. Rasgon.

Supervision: Jason L. Rasgon.

Validation: Sujit Pujhari, Jason L. Rasgon.

Writing – original draft: Marco Brustolin, Sujit Pujhari, Jason L. Rasgon.

Writing – review & editing: Marco Brustolin, Sujit Pujhari, Jason L. Rasgon.

References

1. Anderson CR, Downs WG, Wattley GH, Ahin NW, Reese AA. Mayaro virus A new human disease agent II. Isolation from bood of patients in Trinidad, B.W.I. Am J Trop Med Hyg. 1957; 6: 1012–1016. Available: http://www.ajtmh.org/docservlet/fulltext/1476164S/6/6/TM0606061012.pdf?expires=1507823028&id=id&accname=12234&checksum=C860A78D2092EFEFD4A5D8080FFE89D6 PMID: 13487973

2. Powers AM, Aguilar P V, Chandler LJ, Brault AC, Meakins TA, Watts D, et al. Genetic relationships among Mayaro and Una viruses suggest distinct patterns of transmission. Am J Trop Med Hyg. 2006; 75: 461–469. doi: 75/3/461 [pii] PMID: 16968922

3. Auguste AJ, Liria J, Forrester NL, Giambalvo D, Moncada M, Long KC, et al. Evolutionary and Ecological Characterization of Mayaro Virus Strains Isolated during an Outbreak, Venezuela, 2010. Emerg Infect Dis. 2015; 21: 1742–1750. https://doi.org/10.3201/eid2110.141660 PMID: 26401714

4. Mota MT de O, Ribeiro MR, Vedovello D, Nogueira ML. Mayaro virus: a neglected arbovirus of the Americas. Future Virol. 2015; 10: 1109–1122. https://doi.org/10.2217/fvl.15.76

5. Lednický J, Beau De Rochars VM, Elbadry M, Loeb J, Telisma T, Chavannes S, et al. Mayaro virus in child with acute febrile illness, Haiti, 2015. Emerg Infect Dis. 2016; 22: 2000–2002. https://doi.org/10.3201/eid2211.161015 PMID: 27767924

6. Hassing R-J, Leparc-Goffart I, Blank SN, Thevarayan S, Tolou H, van Doornum G, et al. Imported Mayaro virus infection in the Netherlands. J Infect. 2010; 61: 343–345. https://doi.org/10.1016/j.jinf.2010.06.009 PMID: 20603000

7. Liagonne-Barets M, Icard V, Leparc-Goffart I, Prat C, Perpoint T, André P, et al. A case of Mayaro virus infection imported from French Guiana. J Clin Virol. 2016; 77: 66–68. https://doi.org/10.1016/j.jcv.2016.02.013 PMID: 26921736

8. Receveur MC, Grandadam M, Pistone T, Malvy D. Infection with Mayaro virus in a French traveller returning from the Amazon region, Brazil, January, 2010. Eurosurveillance. 2010; 15: 1–3. https://doi.org/10.2807/ese.15.18.19563-en

9. Neumayr A, Gabriel M, Fritz J, Günther S, Hatz C, Schmidt-Chanasit J, et al. Mayaro Virus Infection in Traveler Returning from Amazon Basin, Northern Peru. Emerg Infect Dis. 2012; 18: 695–696. https://doi.org/10.3201/eid1804.111717 PMID: 22469145

10. Esposito DLA, Fonseca BAL da. Will Mayaro virus be responsible for the next outbreak of an arthropod-borne virus in Brazil? Brazillian J Infect Dis. 2017; 2: 540–544. https://doi.org/10.1590/1981-43752017.06002

11. Zuchi N, Borges L, Heinen DS, Mendes MA, Santos D, Pereira FC, et al. Molecular detection of Mayaro virus during a dengue outbreak in the state of Mato Grosso, Central-West Brazil. Mem Inst Oswaldo Cruz Rio Janeiro. 2014; 109: 820–823. https://doi.org/10.1590/0074-0276140108 PMID: 25141284

12. Abad-Franch F, Grimmcr GH, de Paula VS, Figueiredo LTM, Braga WSM, Luz SLB. Mayaro Virus Infection in Amazonia: A Multimodel Inference Approach to Risk Factor Assessment. Weaver SC, editor PLoS Negl Trop Dis. Public Library of Science; 2012; 6: e1846. https://doi.org/10.1371/journal.pntd.0001846 PMID: 23071852
13. Smith GC, Francy DB. Laboratory studies of a Brazilian strain of Aedes albopictus as a potential vector of Mayaro and Oropouche viruses. J Am Mosq Control Assoc. 1991; 7: 89–93. Available: http://www.ncbi.nlm.nih.gov/pubmed/1646286 PMID: 1646286

14. Long KC, Ziegler SA, Thangamani S, Hausser NL, Kochel TJ, Higgs S, et al. Experimental transmission of Mayaro virus by Aedes aegypti. The American Society of Tropical Medicine and Hygiene; 2011; 85: 750–757. https://doi.org/10.4269/ajtmh.2011-0359 PMID: 21976583

15. Wiggins K, Eastmond B, Alto BW. Transmission potential of Mayaro virus in Florida Aedes aegypti and Aedes albopictus mosquitoes. Med Vet Entomol. 2018; https://doi.org/10.1111/mve.12322 PMID: 30006976

16. Pereira Serra O, Cardoso BF, Lúcia A, Ribeiro M, Leal FA, Santos D, et al. Mayaro virus and dengue virus 1 and 4 natural infection in culicids from Cuiabá, state of Mato Grosso, Brazil. Mem Inst Oswaldo Cruz Rio Janeiro. 2016; 111: 20–29. https://doi.org/10.1590/0074-02760150270 PMID: 26784582

17. Smith GC, Francy DB. Laboratory studies of a Brazilian strain of Aedes albopictus as a potential vector of Mayaro and Oropouche viruses. J Am Mosq Control Assoc. 1991; 7: 89–93. Available: http://www.ncbi.nlm.nih.gov/pubmed/1646286 PMID: 1646286

18. Galindo P, Srihongse S. Transmission of arboviruses to hamsters by the bite of naturally infected Culex (Melanocionion) mosquitoes. Am J Trop Med Hyg. 1967; 16: 525–530. https://doi.org/10.4269/ajtmh.1967.16.525 PMID: 4952151

19. Chouin-Carneiro T, Vega-Rua A, Vazeille M, Yebakima A, Giordi R, Goindin D, et al. Differential Susceptibilities of Aedes aegypti and Aedes albopictus from the Americas to Zika Virus. PLoS Negl Trop Dis. Public Library of Science; 2016; 10: e0004543. https://doi.org/10.1371/journal.pntd.0004543 PMID: 26938668

20. Dodson BL, Rasgon JL. Vector competence of Anopheles and Culex mosquitoes for Zika virus. PeerJ. PeerJ, Inc; 2017; 5: e3096. https://doi.org/10.7717/peerj.3096 PMID: 28316896

21. Lambrechts L, Fellous S, Koella JC. Coevolutionary interactions between host and parasite genotypes. Trends Parasitol. Elsevier Current Trends; 2006; 22: 12–16. https://doi.org/10.1016/j.pt.2005.11.008 PMID: 16310412

22. Lambrechts L, Chevillon C, Albright RG, Thaisomboonsuk B, Richardson JH, Jarman RG, et al. Genetic specificity and potential for local adaptation between dengue viruses and mosquito vectors. BMC Evol Biol. BioMed Central; 2009; 9: 160. https://doi.org/10.1186/1471-2148-9-160 PMID: 19589156

23. Reisen WK. Estimation of vectorial capacity, Palm Springs CA, November 16, 1988. Bull Soc Vector Ecol. 1989; 14: 39–70.

24. Ye YH, Chenoweth SF, Carrasco AM, Allen SL, Frentiu FD, van den Hurk AF, et al. Evolutionary potential of the extrinsic incubation period of dengue virus in Aedes aegypti. Evolution (N Y). 2016; 70: 2459–2469. https://doi.org/10.1111/evo.13039 PMID: 27530960

25. Tsatsarkin KA, Vanlandingham DL, McGee CE, Higgs S. A single mutation in chikungunya virus affects vector specificity and epidemic potential. PLoS Pathog. 2007/12/ 12. 2007; 3: e201. https://doi.org/10.1371/journal.ppat.0030201 PMID: 18069894

26. Mavian C, Rife BD, Dollar JJ, Cella E, Ciccozzi M, Prosperi MCF, et al. Emergence of recombinant Mayaro virus strains from the Amazon basin. Sci Rep. Nature Publishing Group; 2017; 7: 8718. https://doi.org/10.1038/s41598-017-07152-5 PMID: 28821712

27. Rezza G, Chen R, Weaver SC. O’nyong-nyong fever: a neglected mosquito-borne viral disease. Pathog Glob Health. Taylor & Francis; 2017; 111: 271–275. https://doi.org/10.1080/20477724.2017.1355431 PMID: 28829253

28. Yadav P, Gokhale MD, Barde P V, Singh DK, Mishra AC, Mourya DT. Experimental transmission of Chikungunya virus by Anopheles stephensi mosquitoes. Acta Virol. 2003; 47: 45–7. Available: http://www.ncbi.nlm.nih.gov/pubmed/12828344 PMID: 12828344

29. Sinka ME, Rubio-Palis Y, Manguin S, Patil AP, Temperley WH, Gething PW, et al. The dominant Anopheles vectors of human malaria in the Americas: occurrence data, distribution maps and bionomic précis. Parasit Vectors. BioMed Central; 2010; 3: 72. https://doi.org/10.1186/1756-3305-3-72 PMID: 20712879

30. Levine RS, Peterson AT, Benedict MQ. Distribution of Members of Anopheles quadrivulatus Say (Diptera: Culicidae) and Implications for Their Roles in Malaria Transmission in the United States. J Med Entomol. Oxford University Press; 2004; 41: 607–613. https://doi.org/10.1603/0022-2585-41.4.607 PMID: 15311451

31. de Thoisy B, Gardon J, Salas RA, Morvan J, Kazanjii M. Mayaro Virus in Wild Mammals, French Guiana. Emerg Infect Dis. 2003; 9: 1326–1329. https://doi.org/10.3201/eid0910.030316 PMID: 14609474
32. Tesh RB, Watts DM, Russell KL, Damodaran C, Calampa C, Cabezas C, et al. Mayaro Virus Disease: An Emerging Mosquito-Borne Zoonosis in Tropical South America the Division of [Internet]. Available: https://academic.oup.com/cid/article-abstract/28/1/67/302503

33. Klowden MJ, Briegel H. Mosquito Gonotrophic Cycle and Multiple Feeding Potential: Contrasts Between Anopheles and Aedes (Diptera: Culicidae). J Med Entomol. 1994; 31: 618–622. Available: https://pdfs.semanticscholar.org/fba8/dacb33ee0a3af70074b4231230b8bae8f44.pdf PMID: 7932610

34. Sinka ME, Bangs MJ, Manguin S, Rubio-Palis Y, Chareonviriyaphap T, Coetzee M, et al. A global map of dominant malaria vectors. Parasit Vectors. 2012; 5: 69. https://doi.org/10.1186/1756-3305-5-69 PMID: 22475528