Transmission potential of Mayaro virus by *Aedes albopictus*, and *Anopheles quadrimaculatus* from United States

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Short report

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Abstract

Background Mayaro virus (MAYV; *Alphavirus, Togaviridae*) is an emerging pathogen endemic in South American countries. The increase in intercontinental travel and tourism-based forest excursions has resulted in an increase in MAYV spread, with imported cases observed in Europe and North America. Intriguingly, no local transmission of MAYV has been reported outside South America, despite the presence of potential vectors.

Methods We assessed the vector competence of *Aedes albopictus* from New York and *Anopheles quadrimaculatus* for MAYV.

Results We showed that *Aedes albopictus* from New York and *Anopheles quadrimaculatus* are competent vectors for MAYV. However, *Aedes albopictus* was more susceptible to infection. Transmission rates increased with time for both species with rates of 37.21% and 64.44% for *Aedes albopictus*, and 31.11% and 46.34% for *Anopheles quadrimaculatus*, respectively, at 7 and 14 days-post-infection.

Conclusions Our results suggest there is a risk of further MAYV spread throughout the Americas and autochthonous transmission in the United States. Preventive measures such as mosquito surveillance of MAYV will be essential for early detection.

Background

Mayaro virus (MAYV; *Togaviridae, Alphavirus*) is an emerging virus first isolated in Trinidad in 1954 from the serum of febrile patients. *Mayaro virus* strains are grouped into three distinct genotypes: L (limited), N (new), and D (widely dispersed) [1–4]. Like other medically important alphaviruses, MAYV is a mosquito-borne arbovirus that causes fever, headache, myalgia, rash and arthralgia of large joints and, occasionally arthritis in humans [5]. New World primates of the families Cebidae and Callithricidae are considered as potential natural reservoirs for the virus [6,7]. Furthermore, the virus has been found in a migrating bird, equids, anteaters, armadillos, opossums, and rodents [8,9].

Endemic in South America countries, Mayaro cases have increased in number in recent years and imported cases have been detected in previously unaffected areas such as Europe and the United States [3]. Further expansion of MAYV range could be facilitated by global climate change, rapid urbanization and higher mobility of the population, lack of effective vector control, and spreading of vector populations to new geographic regions [5,10,11]. Different mosquito species have been found infected with the virus, including *Mansonia venezuelensis*, *Haemagogus janthinomys*, *Sabethes* spp., and *Culex* spp. [7,10]. Moreover, *Aedes albopictus*, *Aedes aegypti, Anopheles gambiae, Anopheles stephensi, Anopheles quadrimaculatus* and *Culex quinquefasciatus* are known to be competent vectors [12–14].

Many travelers from MAYV endemic areas visit New York each year and no information about the potential of local mosquitoes to transmit MAYV is known. To evaluate risk, we infected *Aedes albopictus* (temperate strain) and *Anopheles quadrimaculatus* with MAYV and evaluated their capacity to transmit the virus. Our results show that both mosquito species are competent vectors of MAYV, with *Aedes albopictus* being the more efficient vector.

Methods

Mosquitoes

A colony of unknown generations of *An. quadrimaculatus* (Orlando strain) was obtained from BEI (MRA-139) and were maintained at 27 °C under standard rearing [15]. *Ae. albopictus* colony (Spring Valley, New York, kindly provided by Laura Harrington, Cornell University) was newly established in 2019 from field collected eggs. *Aedes albopictus* were hatched in distilled water, reared and maintained similarly to the *Anopheles* described above. F4 females were used for the MAYV challenge experiments.

Mosquito Vector Competence For Mayaro Virus

Mayaro virus strain TRVL-4675 (isolated from the serum of an infected human in Trinidad in 1954 and belonging to the D genotype) was freshly propagated in Vero (African Green Monkey kidney) cell cultures maintained at 37 °C 5% CO2. At 48 hours following infection (multiplicity of infection ≈ 1.0), the supernatant was harvested and diluted 1:1 with defibrinated sheep blood plus a final concentration of 2.5% sucrose. Female *An. quadrimaculatus* mosquitoes (3–5 days old) deprived of sugar for 1–2 hours and female *Ae. albopictus* (5–7 days old) deprived of sugar for 24 hours were allowed to feed on MAYV-blood suspension for 45 minutes via a Hemotek membrane feeding system (Discovery Workshops, Acrington, UK) with porcine sausage casing membrane at 37 °C [15]. Following feeding, females were anesthetized with CO_2 and fully engorged mosquitoes were transferred to 0.6-L cardboard containers and maintained with 10% sucrose at 27 °C until harvested for testing. 1 ml aliquot of each blood meal pre-feeding was frozen at -80 °C to determine MAYV titer by plaque assay on Vero cells.

Detection of Mayaro virus

Infection, dissemination, and transmission were determined on days 7 and 15 post infectious blood meal as previously described [15]. Mosquito bodies, legs, and salivary secretions were assayed for infection by plaque assay on Vero cells [16]. Dissemination rate is the proportion of mosquitoes with infected legs among the mosquitoes with infected bodies. Transmission rate is the proportion of mosquitoes with infectious saliva collected by capillary transmission method [15] among mosquitoes with disseminated infection. Dissemination efficiencies and transmission efficiencies refer to the proportion of mosquitoes with infectious virus in the legs or in the saliva, respectively, among all mosquitoes that fed.

Statistical analysis

A Fisher's exact test was used to compare combined infection rates, dissemination rates, dissemination efficiencies, transmission rates and transmission efficiencies between or within mosquito species and between time points. All statistical analyses were carried out at a significance level of $p < 0.05$. OpenEpi,

Version 3, open source calculator–TwobyTwo (http://www.openepi.com/TwobyTwo/TwobyTwo.htm) was used for all statistical analysis.

Results

A total of 180 *An. quadrimaculatus*, and 180 *Ae. albopictus* were analyzed in this study.

Oral challenge with MAYV led to the establishment of high infection rates in both mosquito species. Infection rates of *Ae. albopictus* and *An. quadrimaculatus* were significantly different at both time points (100.00% vs. 82.22%, $p < 0.0001$ and 100.00 vs 74.4, $p < 0.0001$, at 7 dpi and 14 dpi, respectively; Table 1); but no significant difference between time points within mosquito species was observed.

Table 1

Aedes albopictus and *Anopheles quadrimaculatus* infection rates, dissemination rates, and transmission rates after exposure to *Mayaro*

Mosquito species	Replicate	Blood meal titer (log10 PFU/ml)	7DPI						14DPI			
			N tested	IR N (%)	DR N (%)	D.E N (%)	TR N (%)	T.E N (%)	N tested	IR N (%)	DR N (%)	D.E N (%
Ae. albopictus	1	8.23	30	30 (100.00)	29 (96.67)	29 (96.67)	13 (44.82)	13 (43.33)	30	30 (100.00)	30 (100.00)	30 (10
	2	8.14	30	30 (100.00)	30 (100.00)	30 (100.00)	10 (33.33)	10 (33.33)	30	30 (100.00)	30 (100.00)	30 (10
	3	7.97	30	30 (100.00)	27 (90.00)	27 (90.00)	9 (33.33)	9 (30.00)	30	30 (100.00)	30 (100.00)	30 (10
	Total	**NA**	**90**	**90 (100.00)**	**86 (95.55)**	**86 (95.55)**	**32 (37.21)**	**32 (35.55)**	**90**	**90 (100.00)**	**90 (100.00)**	**90 (10**
An. quadrimaculatus	1	8.06	30	25 (83.33)	19 (76,00)	19 (63.33)	8 (42.11)	8 (26.66)	30	21 (70.00)	16 (76.19)	16 (53
	2	7.87	30	25 (83.33)	8 (32,00)	8 (26.67)	0 (0.00)	0 (0.00)	30	19 (63.33)	7 (36.84)	7 (23
	3	8.39	30	24 (80.00)	18 (75,00)	18 (60.00)	6 (33.33)	6 (20.00)	30	27 (90.00)	18 (66.67)	18 (60
	Total	**NA**	**90**	**74 (82.22)**	**45 (60.81)**	**45 (50.00)**	**14 (31.11)**	**14 (15.55)**	**90**	**67 (74.44)**	**41 (61.19)**	**41 (45**

DPI: Days post-infection N: Number; IR: Infection rate; DR: Dissemination rate; DE: Dissemination efficiency; TR: Transmission rates; TE: Transmission efficien

We used the infection rates, dissemination rates and transmission rates for comparison within mosquito species, and significant difference was only observe transmission ($p < 0.001$). Significant differences were observed between *Ae. albopictus* and *An. quadrimaculatus* infection rates and dissemination efficienci 0.0001). Between species the transmission efficiencies were significantly different at both time points with ($p < 0.001$ at 7 dpi and $p < 0.0001$ at 14 dpi).

Within mosquito species similar dissemination rates were observed for *Ae. albopictus* and *An. quadrimaculatus* for both time points (95.55%, 7dpi vs 100%, 14 dpi and 60.81% vs 61.19, at 7 dpi and 14 dpi, respectively; Table 1). Dissemination efficiencies at 7 and 14 dpi showed a significant difference between mosquito species ($p < 0.0001$; Table 1). Detection of infectious viral particules in mosquitoes collected at 7 and 14 dpi indicated that *Ae. albopictus* and *An. quadrimaculatus* are highly susceptible to MAYV through oral challenge and subsequently supports viral replication.

Infectious viral particules were detected in saliva of individuals with disseminated infections for 37.21% and 64.44% *Ae. albopictus* and, 31.11% and 46.34% *An. quadrimaculatus*, at 7 and 14 dpi, respectively (Table 1). In both mosquito species, the transmission rates increased with time; however, a significant difference was only observed for *Ae. albopictus* ($p < 0.0001$; Table 1). Furthermore, transmission efficiencies were significantly different between mosquito species at both time points ($p < 0.001$ and $p < 0.0001$, at 7 dpi and 14 dpi, respectively; Table 1).

Discussion

To our knowledge, this is the first study to examine the vector competence of a temperate population of *Ae. albopictus* from the Northeast U.S., and second for *An. quadrimaculatus* for MAYV. As mosquitoes and their viruses continue to expand their geographic range and emerge in unpredictable ways, the U.S. could face an increased threat from MAYV in the future. Our data demonstrates that NY *Ae. albopictus* and *An. quadrimaculatus* are highly competent vectors of MAYV.

When multiple mosquito species are involved in the transmission of an arbovirus, the effort needed to prevent human exposure increases. Determining the role of each species is important [17]. Dissemination and transmission rates were lower for *An. quadrimaculatus* than for *Ae. albopictus*. In locations where *Ae. albopictus* is prevalent, it might play a role in the epidemiology of MAYV considering its high vector competence, were it to be introduced.

Aedes albopictus, is a highly invasive species that has been introduced into the U.S. and became permanently established in at least 27 states, including New York [18, 19]. It is predicted that this mosquito species will continue to spread globally over the coming decades, increasing the risk to human health [20]. In the U.S., *Ae. albopictus* may be infected with a number of arboviruses including *Eastern Equine Encephalitis virus*, *Dengue virus*, *St. Louis encephalitis virus*, and *West Nile virus* [19]. In addition, its role as a vector is recognized for *Chikungunya virus* and *Zika virus*, both introduced recently into the U.S.[19, 20]. Using a temperate population of *Ae. albopictus* from New York, we confirmed earlier studies that demonstrated the potential of *Ae. albopictus* to transmit MAYV [13, 14, 21].

The high infection rates (85–100%) obtained in our results were similar to others [13, 14]. Moreover, the high dissemination and transmission rates observed in our study corroborate findings of Diop *et al* 2019. However, Wiggins et *al* 2018, using the same MAYV strain that we used, found lower transmission rates compare to our studies and Pereira et *al* 2020 studies. These differences could be due to the genetic background (geographical origin) of the vector and /or the difference of mosquito incubation temperature as has been shown for *Chikungunya virus* [22, 23].

Anopheles mosquitoes are persistently exposed in nature to diverse arboviruses, but in general assessment of their potential to transmit arboviral pathogens has been neglected. In addition to MAYV, vector competence of *Anopheles* mosquitoes for *O'nyong nyong* (ONNV) *virus*, *Rift Valley fever phlebovirus*, *Eastern equine encephalitis virus*, and *Cache Valley orthobunyavirus* have been reported [12, 24–26]. However, only ONNV is known to rely on *Anopheles spp* as primary vectors [27, 28]. *An. quadrimaculatus* are primarily mammalophagic mosquitoes. In the Northeast U. S., white-tailed deer are the predominately identified vertebrate host [29]. However, this may be an artefact of human accessibility rather than an indication of preference. *An. quadrimaculatus* mosquitoes are historically important vectors of human malaria parasites (*Plasmodium vivax*) [30], suggesting that they have a high level of anthropophily. Furthermore, white-tailed deer overabundance and availability throughout the region may explain mosquitoes feeding behavior [17, 31]. It is suggested that *An. quadrimaculatus* and *An. punctipennis* may contribute to transmission of Eastern equine encephalitis, Jamestown Canyon, Cache Valley viruses in the Northeast U.S. [29]. Recently, the capacity of *An. quadrimaculatus* to transmit MAYV at 7 dpi but not at 14 dpi was demonstrated [12]. In our study, *An. quadrimaculatus* mosquitoes were able to transmit the virus at both time points, suggesting this species may be an overlooked vector for MAYV emergence and invasion in the U. S.

Conclusion

Information on the competence of mosquito vectors is essential for controlling and preventing viruses transmitted by arthropods. While it is not possible to accurately predict the emergence of a disease, in light of our results, MAYV presents a threat to the U.S. and local authorities should reinforce epidemiological and entomological surveillance to detect the introduction of this viral pathogen.

List Of Abbreviations

MAYV: Mayaro virus

ONNV: O'nyong nyong virus

DPI: Days post-infection

Ae: Aedes

An: Anopheles

N: Number

IR: Infection rate

DR: Dissemination rate

DE: Dissemination efficiency

TR : Transmission rates

TE: Transmission efficiency

NA : Not applicable

U.S: United States

NY: New York

Declarations

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Authors' contributions

Designed research : CD, ATC and LDK. Performed research : CD. Analyzed data : CD, ATC and LDK. Wrote the paper : CD, ATC and LDK. All authors read and approved the final manuscript.

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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