LABORATORY STUDIES OF A BRAZILIAN STRAIN OF *AEDES ALBOPICTUS* AS A POTENTIAL VECTOR OF MAYARO AND OROPOUCHE VIRUSES[1]

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ABSTRACT. The vector efficiency of colonized *Aedes albopictus* from Brazil was assessed for Mayaro (MAY) and Oropouche (ORO) viruses. Female mosquitoes, 3–4 days old, were fed on a MAY-infected hamster with a viremia level of 5.3 $\log_{10}$ Vero cell plaque-forming units (PFU) of virus/ml or an ORO-infected hamster circulating 7.3 $\log_{10}$ PFU/ml. Mayaro infection rates among fed mosquitoes were 16, 9 and 11% at 6, 13 and 20 days postfeeding, respectively, and 1/2 and 2/2 infected mosquitoes transmitted virus on days 13 and 20, respectively. Only 13, 5 and 3% of mosquitoes were infected with ORO virus at 6, 13 and 20 days, respectively, and no transmission occurred. Mosquitoes were also fed on 3 dilutions of MAY virus-blood suspensions in membrane feeders. The infection rate among mosquitoes fed the highest concentration (7.7 $\log_{10}$ PFU/ml) was 11/13 (85%), and 5/11 (46%) infected mosquitoes transmitted virus.

INTRODUCTION

Aedes albopictus (Skuse) was first detected in the United States in Houston, TX, in 1985 (Sprenger and Wuithiranyagool 1986) and has since been found in 133 counties in 17 states (Centers for Disease Control 1987). In 1986, the presence of *Ae. albopictus* in Brazil was reported; it currently occurs in several Brazilian states (Forattini 1986, Centers for Disease Control 1987). *Aedes albopictus* from the United States enters diapause in the egg stage, a characteristic of strains from temperate regions. Brazilian strains do not enter diapause and exhibit other biological differences compared with North American strains (Hawley et al. 1989), suggesting a tropical origin for the Brazilian *Ae. albopictus*.

The establishment of *Ae. albopictus* in the U.S. and Brazil is of concern to public health officials because of the ability of this species to serve as a vector of dengue viruses (Jumali et al. 1979). It also is an efficient experimental vector of a number of other viruses of public health importance (Shroyer 1986, Mitchell et al. 1987, Grimstad et al. 1989). A strain of *Ae. albopictus* from Brazil was shown to transmit yellow fever and dengue viruses, although the Brazilian strain appeared to be somewhat less efficient as a vector than its U.S. counterpart (Miller and Ballinger 1988).

We evaluated the vector potential of a Brazilian strain of *Ae. albopictus* for two other Brazilian viruses of public health importance, Mayaro (MAY) and Oropouche (ORO) viruses. Mayaro virus belongs to the genus *Alphavirus*, family Togaviridae. Oropouche virus is in the Simbu group of the genus *Bunyavirus*, family Bunyaviridae. These viruses have caused periodic epidemics, primarily in Para State, Brazil (Causey and Maroja 1957; Pinheiro et al. 1981a, 1981b). Mayaro virus is believed to exist in a sylvatic cycle similar to that of yellow fever virus, with *Haemagogus* species as the primary vectors (Hoch et al. 1981). Previous experimental studies with MAY virus and mosquitoes have shown that the virus replicates readily in *Aedes aegypti* (Linn.) and *Anopheles quadrimaculatus* Say. *Aedes scapularis* (Rondani) was shown to transmit virus to chicks by bite (Aitken and Anderson 1959). Oropouche virus has been isolated from *Coquillettidia venezuelensis* (Theobald), *Aedes serratus* (Theobald), *Culex quinquefasciatus* Say and *Culicoides paraensis* (Goeldi) (Karabatsos 1985). Oropouche virus is thought to be transmitted by *C. paraensis* and possibly *Cx. quinquefasciatus* (Roberts et al. 1981). Monkeys and marmosets are believed to be the principal vertebrate hosts for both viruses (Pinheiro and LeDuc 1988), although antibody to MAY virus has been associated with avian hosts (Hoch et al. 1981).

MATERIALS AND METHODS

Mosquitoes: A Brazilian strain of *Ae. albopictus* was colonized in our insectary in December 1987, from eggs provided by SUCEN (Superintendencia de Controle de Endemias), Department of Disease Control, São Paulo State, Brazil. The F_2 through F_5 laboratory generations of the Ft. Collins colony were used during this study. All mosquitoes were reared and held at 27 ± 1°C, 80% RH and a 16:8 L:D photoperiod.

[1] These studies were reviewed and approved by the Institutional Animal Care and Use Committee. Programs and facilities are managed to conform with the U.S. Public Health Service policy on the humane care and use of laboratory animals.

Viruses: A MAY virus strain (TR4675), which had been passed 8 times in mice, and an ORO virus strain (TR9760), which had been passed 14 times in mice, were obtained from the Arbovirus Diseases Branch, Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Ft. Collins, CO.

Both virus strains were originally isolated from humans. Each virus was inoculated intracranially into newborn white mice, and a working stock of each was prepared from dead and moribund baby mice by making a 10% mouse brain suspension in BA-1 (tris-buffered medium-199, pH 7.6, containing 1% bovine albumin and antibiotics).

Hamster viremia study: Viremia profiles in hamsters were determined for each virus. Individual hamsters (3–4 wk old) were inoculated subcutaneously with approximately 4.2 $\log_{10}$ Vero cell plaque-forming units (PFU) of MAY virus (3 hamsters) and approximately 3.9 $\log_{10}$ PFU of ORO virus (2 hamsters). At 24, 48, 72 and 96 h postinoculation, each of the 5 hamsters was anesthetized and a blood sample was taken via cardiac puncture. All samples were diluted 1:10 using BA-1 diluent and frozen at −70°C until assayed in Vero cell culture.

Mosquito infection: Female mosquitoes, 3–4 days old, were starved overnight and allowed to feed approximately 1 h on a MAY- or ORO-infected hamster during periods of anticipated peak viremia. A second group of mosquitoes were fed through a pig-gut membrane on artificial feeders (Ogston and Yanovski 1982). Three 10-fold dilutions of MAY virus, suspended in defibrinated human blood, were used to feed the mosquitoes. Prefeeding and postfeeding blood samples were taken from all infective blood meal sources. Mosquitoes that had engorged on the membrane feeder were sorted into lots of 25 and held in pint cartons. Mosquitoes feeding to repletion on infected hamsters were placed individually in half pint-cartons covered with fine mesh netting. Each carton contained a small amount of water and a red velour paper oviposition strip. All cartons were provided with a saturated cotton pad containing 5% sugar water and a pad saturated with deionized water. Mosquitoes that had been fed were incubated under the insectary conditions described above.

Virus transmission: Transmission of each virus was attempted at 6, 13 and 20 days postfeeding. Mosquitoes that had oviposited were selected to refeed individually on newborn white mice restrained on top of the carton netting. After 1 h of exposure, each baby mouse was removed, marked for later identification and returned to its mother, along with other exposed litter mates. Each litter was checked daily for 14 days. Dead and moribund baby mice were removed and frozen at −70°C until they were tested for virus. All mosquitoes were stored at −70°C. The refeeding status of each mosquito was verified at the time it was ground for testing.

Studies of MAY virus dissemination were conducted at 6 and 13 days postinfection, using mosquitoes that had fed on a virus-blood suspension in the artificial feeder. Each mosquito was dissected for salivary glands and midgut tissue in a drop of BA-1 diluent. The tissues were rinsed twice in a drop of diluent before being transferred, individually, to 1 ml of diluent. Also, the drop containing the remnants of the dissected mosquito was transferred to 1 ml of diluent. All specimens were stored at −70°C until assayed in Vero cell culture.

Mosquitoes fed on the virus-blood suspension with the highest titer in the membrane feeders were also used for *in vitro* transmission attempts, using capillary tubes containing fetal bovine serum (FBS). After 15 days, extrinsic incubation, legs and wings were removed from the mosquitoes, the proboscis was inserted into a finely drawn tip on the capillary tube, and both mosquito and capillary tube were pressed against double-sided adhesive tape on a glass slide. Mosquitoes were left in place for approximately 45 min. After the mosquito was removed, the sera in the capillary tube was expressed into 0.2 ml of BA-1 diluent. Both mosquito and BA-1 diluent suspension were stored at −70°C until tested.

Virus assay: Each specimen was screened or titrated for virus content, as appropriate, by plaque-assay in Vero cell culture grown in 6-well plates. Briefly, 0.1 ml of each sample was inoculated into duplicate wells and adsorbed for 1 h at 37°C. Cultures were then overlayed with 1% Noble agar in medium-199 containing 2% heat-inactivated FBS, 2.2 g/liter $NaHCO_3$, 150 μg/ml DEAE-dextran, 8 ml of a 1:300 dilution of neutral red vital stain/liter, and antibiotics, including penicillin, streptomycin, gentamicin and amphotericin B. Cell cultures were observed daily for 10 days for plaques.

Whole mosquitoes and mosquito remnants were ground individually in 1-ml grinders. Specimens containing salivary gland and midgut material were disrupted with high frequency sonication. Suspensions of each were tested for virus as described. All positive specimens were retested and titrated for total virus content.

Baby mice found dead or moribund after exposure to the infected mosquitoes during virus transmission attempts were tested for virus by making a 10% (w/v) suspension of the brain tissue in BA-1 diluent and then assayed in Vero cell culture. The presence of MAY virus was verified using an indirect immunofluorescence test.

RESULTS

Hamster viremia: Neither virus was recovered from blood samples taken 24 h postinoculation. Mean titers of MAY virus in samples collected at 48, 72 and 96 h postinoculation had respective $\log_{10}$ geometric mean titers (±SE) of 4.2 ± 0.1 (n = 3), 5.6 ± 0.1 (n = 3) and 4.7 ± 0.2 (n = 3) PFU/ml of whole blood. Blood samples were not collected 96 h postinoculation from hamsters inoculated with ORO virus, but samples collected at 48 and 72 h postinoculation had respective $\log_{10}$ mean titers of 5.5 ± 2.0 (n = 2) and 7.1 ± 0.5 (n = 2) PFU/ml of whole blood.

Mosquito infection rates: Infection and transmission rates for mosquitoes fed on viremic MAY- or ORO-infected hamster are summarized in Table 1. The infection rates of mosquitoes feeding on a hamster circulating 5.3 $\log_{10}$ PFU/ml of MAY virus was 16, 9 and 11% on days 6, 13 and 20 postfeeding, respectively. The $\log_{10}$ mean virus titers for infected specimens increased during incubation and were 4.5 ± 0.4 PFU on day 6, 5.0 ± 1.0 PFU on day 13 and 5.6 ± 0.1 PFU on day 20. The increase in titer between day 6 and 20 was statistically significant (t-test, P = 0.03).

The infection rates of mosquitoes feeding on an ORO virus-infected hamster with a $\log_{10}$ titer of 7.3 PFU/ml were 13, 5 and 3% after 6, 13 and 20 days incubation, respectively. Log_{10} mean titers of the infected mosquitoes were 2.0 ± 0.2, 2.8 ± 0.7 and 3.1 on days 6, 13 and 20, respectively.

Refeeding and transmission: A total of 129 mosquitoes refed of the 231 exposed to individual baby mice during the 3 transmission attempts; however, only 11 mosquitoes that refed were subsequently shown to be infected (6 MAY and 5 ORO). The $\log_{10}$ titers of virus recovered from the 6 mosquitoes infected with MAY virus were 5.1 and 4.5 on day 6, 3.0 and 6.3 on day 13, and 5.7 and 5.5 on day 20. No virus transmission occurred on day 6, but at day 13 the mosquito containing 6.3 $\log_{10}$ PFU transmitted virus, and on day 20 both mosquitoes transmitted virus (Table 1). All mosquitoes containing ≥5.5 $\log_{10}$ PFU transmitted MAY virus, whereas none of the mosquitoes containing ≤5.1 $\log_{10}$ PFU transmitted virus.

The 5 ORO infected mosquitoes that refed had $\log_{10}$ titers of 1.5, 2.0, 2.4 and 1.6 on day 6 and 3.1 on day 20. None of the infected mosquitoes transmitted virus (Table 1).

Table 1. Infection and transmission of Mayaro and Oropouche viruses by *Aedes albopictus* (São Paulo strain).

Virus	Titer of infective blood meal	Infection rate (days extrinsic incubation)			Transmission rate (days extrinsic incubation)		
		6	13	20	6	13	20
Mayaro	5.3[a]	5/32[b] 4.5 ± 0.4[d]	3/32 5.0 ± 1.0	5/47 5.6 ± 0.1	0/2[c]	1/2	2/2
Oropouche	7.3	5/40 2.0 ± 0.2	2/40 2.8 ± 0.7	1/40 3.1	0/4		0/1

[a] Log_{10} PFU/ml of infected hamster blood.
[b] Number infected mosquitoes/number mosquitoes tested.
[c] Number mosquitoes transmitting/number infected mosquitoes feeding.
[d] Geometric mean titer ± SE of infected mosquitoes in PFU/ml.

Table 2. Mayaro virus dissemination in orally infected *Aedes albopictus*.

Titer of infective blood meal	Tissue tested					
	6 days			13 days		
	Gut	Salivary Gland	Body	Gut	Salivary Gland	Body
5.4[a]	2/10[b] 4.4 ± 1.5[c]	1/10 5.1	1/9 6.6	0/10	0/10	0/10
6.5	1/8 1.3	0/6	0/8	4/9 4.8 ± 0.7	2/10 5.6 ± 0.5	4/10 5.3 ± 1.1
7.7	8/10 3.0 ± 0.5	2/9 3.9 ± 0.3	5/10 3.8 ± 1.0	9/10 4.8 ± 0.5	7/10 4.2 ± 0.6	7/10 5.0 ± 0.6

[a] Log_{10} PFU/ml.
[b] Number positive/number tested.
[c] Geometric mean titer ± SE of infected tissues.

Mayaro virus dissemination: Midgut infection rates of mosquitoes feeding on membrane feeder suspensions containing 5.4, 6.5 and 7.7 $\log_{10}$ PFU/ml of MAY virus were 10 (2/20), 29 (5/17) and 85% (17/20), respectively (Table 2). For mosquitoes feeding on the lowest virus concentration (5.4 $\log_{10}$ PFU/ml) and incubated 6 days, virus was recovered from 2/10, 1/10 and 1/9 midgut, salivary gland and body remnant suspensions, respectively. Midgut titers for the 2 positive specimens were 5.9 and 2.9 $\log_{10}$ PFU. The positive salivary gland and body remnant suspensions were from the mosquito with a midgut titer of 5.9 $\log_{10}$ PFU. No mosquito that was fed the lowest virus concentration and incubated for 13 days was infected. Only 1/8 mosquitoes feeding on the intermediate virus concentration (6.5 $\log_{10}$ PFU/ml) and incubated 6 days had an infected midgut suspension and none had detectable infected salivary gland or body remnant suspensions; however, after 13 days, virus was recovered from 4/9, 2/10 and 4/10 midgut, salivary gland and body remnant suspensions, respectively. For mosquitoes fed on the highest titered virus suspension (7.7 $\log_{10}$ PFU/ml), the proportion of specimens with infected midguts and body remnants were similar for mosquitoes incubated 6 and 13 days [Fisher's exact test (FET) midgut $P = 0.5$, body remnants $P = 0.3$]; however, infection rates in salivary glands were higher (FET, $P = 0.02$) in those mosquitoes incubated 13 days. Although infection rates were higher after 13 days incubation for mosquitoes fed on 7.7 $\log_{10}$ PFU/ml compared with those feeding on 6.5 $\log_{10}$ PFU/ml, the mean virus titer of infected mosquitoes was similar for both groups.

In vitro transmission of Mayaro virus: A total of 13 mosquitoes were used for *in vitro* feeding, all of which were from the lot fed on the highest virus concentration (7.7 $\log_{10}$ /ml). Eleven of the 13 (85%) mosquitoes were infected after 15 days of extrinsic incubation and, of these, 5/11 (46%) transmitted virus to the serum in capillary tubes. The $\log_{10}$ mean virus titer per infected mosquito transmitting virus was 6.2 ± 1.7 PFU and 3.5 ± 0.9 PFU for nontransmitters.

DISCUSSION

The Brazilian strain of *Ae. albopictus* was relatively refractory to infection with MAY virus after feeding on a hamster with a viremia level of 5.3 $\log_{10}$ PFU/ml. Nevertheless, 3/4 infected mosquitoes that refed transmitted virus after 13 or more days incubation. Similarly, 5/11 (46%) infected mosquitoes incubated for 15 days transmitted virus in the *in vitro* virus transmission trial. Virus titers in mosquitoes fed on the viremic hamster and the membrane feeder continued to increase throughout the incubation period. These results demonstrate that infection rates are dose-dependent and once the barrier is breeched, virus disseminates to other organs.

Relevant to this is the amount of virus that mosquitoes may ingest in nature. Hoch et al. (1981) found that marmosets inoculated with MAY virus developed viremia levels of approximately 5.0 $\log_{10}$ $TCID_{50}$/ml. Viremia detected in human patients during MAY epidemics have been similar to those obtained experimentally with marmosets (Pinheiro 1981a). It is possible that patients have higher viremia levels before onset of recognizable clinical disease.

Ecological studies conducted during a Mayaro and yellow fever outbreak in Belterra, Para, Brazil, indicate that *Haemagogus janthinomys* Dyar was the principal vector (Hoch et al. 1981). Biting activity patterns of *Hg. janthinomys* and *Ae. albopictus* are similar in that both species are diurnal, anthrophilic feeders. The primary larval habitats for *Ae. albopictus* are tree holes and man-made containers, with forest fringe ecotones serving as favored habitat (Hawley 1988). Thus, with large *Ae. albopictus* populations and extensive MAY virus amplication, this species could serve as a secondary vector during outbreaks and perhaps as a bridging vector for MAY virus between the forest habitat and settled areas as suggested for yellow fever virus (Monath 1986).

Results of the experimental feeding of *Ae. albopictus* on viremic hamsters infected with ORO virus demonstrate there is little likelihood they would serve as a vector. Our results are similar to those obtained with *Cx. pipiens* complex mosquitoes (Hoch et al. 1987).

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