VERTICAL TRANSMISSION OF DENGUE VIRUSES BY STRAINS OF *AEDES ALBOPICTUS* RECENTLY INTRODUCED INTO BRAZIL

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ABSTRACT. Three strains of *Aedes albopictus* from Brazil were examined for their ability to vertically transmit dengue 1 (DEN-1) and dengue 4 (DEN-4) viruses. Parental females were uniformly infected by parenteral inoculation of virus, and 8,121 F_1 progeny from DEN-1 and DEN-4 infected mothers were pooled in lots of approximately 50 and tested for virus. Seven of 60 pools were positive for DEN-1 virus, and 1 of 121 pools was positive for DEN-4 virus. In DEN-1 assays, the minimum infection rate (MIR) for larvae (2 pools tested) was 1:84. Among positive cohorts of adults, pooled by sex and by geographic strain of mosquito, the MIR ranged from 1:193 to 1:626 for males and from 1:187 to 1:311 for females. Only a single pool of adult females was positive for DEN-4 virus (MIR 1:1022 for an adult female cohort from Santa Teresa). These results indicate that Brazilian *Ae. albopictus* have the potential to play a role in the maintenance of dengue viruses in nature.

INTRODUCTION

Aedes albopictus (Skuse) became established in the western hemisphere relatively recently (Hawley 1988), and Forattini (1986) has reported an infestation in and around São Paulo, Brazil. Recent outbreaks of dengue in Rio de Janeiro (Schatzmayr et al. 1986) and the demonstration that *Ae. albopictus* from Cariacia City, Espirito Santo State, Brazil, can transmit the 4 serotypes of dengue virus under experimental conditions (Miller and Ballinger 1988) suggests that this mosquito species may become a vector of dengue viruses in future outbreaks in Brazil. Perhaps equally important is the potential for infected females to transmit virus to their progeny and act as maintenance reservoirs for dengue viruses. Rosen et al. (1983) demonstrated vertical transmission of all 4 dengue serotypes by a strain of *Ae. albopictus* from Oahu, Hawaii. We tested 3 geographic strains of *Ae. albopictus* from Brazil for their ability to vertically transmit dengue 1 (DEN-1) and dengue 4 (DEN-4) viruses. Dengue 1 virus was involved in the Rio de Janeiro outbreak (Schatzmayr et al. 1986), and DEN-4 has become endemic in the Americas since its introduction in 1981 (Gubler 1987).

MATERIALS AND METHODS

Colonies of 3 geographic strains of *Ae. albopictus* were established in our insectary from eggs collected in Anchieta and Santa Teresa, Espirito Santo State, and São Paulo, Brazil, during 1987 and 1988. One-hundred-fifty females of each strain from the F_3 laboratory generation were given an opportunity to mate and then were parenterally inoculated with approximately 0.17 μl of viral suspension (Rosen and Gubler 1974) at 4 to 5 days of age. Stock viruses of DEN-1 (1620) and DEN-4 (1632), isolated from human serum specimens obtained in Puerto Rico during 1985 and passed once in adult *Toxorhynchites amboinensis* (Doleschall) were used. Inoculated females were fed 10% sucrose and incubated at 26.7 (±0.5°C), 80% RH, and a photoperiod of L:D 16:8. The females were fed once on anesthetized hamsters on the 8th day after inoculation. Moist oviposition papers were placed in each cage 2 days after feeding, left for 3 days and then removed and dried overnight. The following day, i.e., day 14 postinoculation, parental females were frozen at −70°C until head squashes were prepared and examined for viral antigen using a direct fluorescent antibody test (DFAT) (Kuberski and Rosen 1977).

Eggs from the infected females were hatched within a few weeks of the time they were laid. Larvae were reared under insectary conditions described above and were fed a standard diet (Lea 1964) supplemented with rabbit chow. Adults were separated by sex and tested for the presence of viral antigen, usually in pools of 50 or less and always in pools of fewer than 100 specimens. A few larvae and pupae also were pooled and tested. Each pool was triturated in 1.0 ml of BA-1 diluent (0.2 M Tris, pH 8.0, 0.15 M NaCl, 1% BSA, 10 mg/liter phenol red, 50 μg/ml gentamicin[1] and 1 μg/ml Fungizone[1]). Pools of larvae and pupae were washed once in clean water and twice in BA-1 diluent before being triturated. Suspensions were centrifuged at 2,500 × g for 20 min. Supernatants were decanted, stored at −70°C and subsequently injected into mosquitoes for virus amplification and antigen detection by DFAT.

[1] Use of trade names or commercial sources is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

Initially, each sample to be assayed for virus was injected parenterally into 5 or more *Toxorhynchites amboinensis* mosquitoes as described elsewhere (Rosen and Gubler 1974). Each mosquito received an inoculum of 0.17 μl, was incubated for 14 days at 26.7 ± 0.5°C and then examined by the head-squash technique (Kuberski and Rosen 1977). Head squashes from at least 5 mosquitoes inoculated with each sample were usually examined; if negative, the test was not considered satisfactory unless at least 3 were examined. Unsatisfactory tests were repeated by reinoculating the mosquitoes with original samples that had been thawed and refrozen following the first test. Due to insufficient numbers of *Tx. amboinensis*, some of these tests were done by inoculating *Ae. aegypti* (Linn.) from the Rexville, Puerto Rico, colony. This mosquito strain was known to be equally susceptible to the strains of dengue viruses used in our experiments (Mitchell et al. 1987). Mosquito head squashes were examined for viral antigen by DFAT, utilizing polyvalent conjugates (1818 and 1909, 1:100) prepared from human dengue convalescent sera.

RESULTS

Eight or more parental females from each geographic strain, and from each virus cohort, tested positive for DEN antigen by DFAT, thus indicating that parental females were uniformly infected. A total of 2,512 F_1 progeny of females infected with DEN-1 were tested in 60 pools. Three pools of Anchieta progeny, including 1 of only 2 larval pools tested, and 4 Santa Teresa pools were positive for DEN-1 viral antigen (Table 1). None of 11 pools of São Paulo progeny were positive. Among the positive pools from Anchieta and Santa Teresa adult mosquitoes, 2 were from males and 4 from females. The minimum infection rate (MIR) for larvae was 1:84, and among positive groups of adults the MIR ranged from 1:193 to 1:626 for males and from 1:187 to 1:311 for females.

A total of 5,609 F_1 progeny of females infected with DEN-4 were tested in 121 pools. Only a single positive pool, from Santa Teresa adult females, was detected (Table 1). The MIR was 1:1022.

DISCUSSION

Previous studies on the vertical transmission of dengue viruses by *Ae. albopictus* (Rosen 1987a, 1987b; Rosen et al. 1983, Tesh 1980) used a mosquito strain from Oahu, Hawaii. Our results represent the only evaluation of vertical transmission of dengue viruses by other geographic strains of this mosquito species. Rosen et al. (1983) found that the rates of vertical transmission varied with the serotype and the strain of virus. In their study, the highest rates were observed with strains of DEN-1 virus (MIR 1:73 to 1:579); however, rates also were high with strains of DEN-4 virus (MIR 1:194 to 1:535). Some of these rates are for larvae, pupae and adults combined, and others are for larvae and pupae combined. Nonetheless, the range in vertical transmission rates of DEN-1 virus by Anchieta and Santa Teresa *Ae. albopictus* (MIR 1:84 to 1:626) that we observed is remarkably similar to MIRs reported by Rosen et al. (1983). In contrast, the MIR for DEN-4 among Santa Teresa females (1:1022), or males and females combined (1:1906), indicates less efficient ver-

Table 1. Dengue virus infection rates among different stages and sexes of F_1 progeny of *Aedes albopictus* females infected with DEN-1 or DEN-4 viruses.

Mosquito strain	F_1 stage and sex	No. of specimens	Pools positive/ total	Minimum infection rate
Parent females infected with DEN-1 virus				
Anchieta	Larvae	84	1/2	1:84
	Pupae	82	0/2	<1:82
	Adult male	193	1/5	1:193
	Adult female	187	1/5	1:187
Santa Teresa	Adult male	626	1/14	1:626
	Adult female	932	3/21	1:311
São Paulo	Adult male	198	0/6	<1:198
	Adult female	210	0/5	<1:210
Parent females infected with DEN-4 virus				
Anchieta	Adult male	743	0/16	<1:743
	Adult female	1,141	0/23	<1:1,141
Santa Teresa	Adult male	884	0/19	<1:884
	Adult female	1,022	1/23	1:1,022
São Paulo	Adult male	799	0/18	<1:799
	Adult female	1,020	0/22	<1:1,020

tical transmission of DEN-4 virus than reported by Rosen et al. (1983) for Oahu *Ae. albopictus*. Such results support the view that vertical transmission rates can vary depending on the serotype and strain of virus, and on the geographic strain of mosquito.

The relatively high susceptibility of *Ae. albopictus* to oral infection with dengue viruses (Gubler and Rosen 1976, Gubler et al. 1979, Rosen et al. 1985, Mitchell et al. 1987, Miller and Ballinger 1988), and the degree of vertical transmission demonstrated by Rosen et al. (1983) and by this study, indicate that this species has the potential to play a role in the maintenance of dengue viruses in nature. Consequently, control measures directed at *Ae. albopictus* may not only reduce the risk of dengue transmission during epidemics but also lessen the chances of dengue viruses becoming established in new endemic foci.

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