# VECTOR COMPETENCE OF AEDES ALBOPICTUS FROM HOUSTON, TEXAS, FOR DENGUE SEROTYPES 1 TO 4, YELLOW FEVER AND ROSS RIVER VIRUSES

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ABSTRACT. A combination of virus infection and transmission experiments showed that a Houston, Texas strain of Aedes albopictus is a competent vector for dengue (DEN), yellow fever (YF) and Ross River (RR) viruses. However, at 14 days incubation, DEN virus infection rates in a Puerto Rican strain of Aedes aegypti were significantly higher for each of the four DEN serotypes, except DEN-1, than in Houston Ae. albopictus fed simultaneously on the same virus suspensions. The degree of correlation between disseminated DEN infection rates in Houston Ae. albopictus and transmission to an in vitro system ranged from 42 to 88% for the four DEN serotypes. No significant difference was noted in YF virus infection rates or transmission rates in the two mosquito species fed on the same virus suspensions and incubated for the same time period. Also, RR virus infection and transmission rates in Houston and Hawaiian strains of Ae. albopictus were generally comparable.

# INTRODUCTION

Since the discovery of Aedes albopictus (Skuse) in Harris County, Texas (Sprenger and Wuithiranyagool 1986), its presence has been documented in 12 states in the United States and in three states in Brazil (Anonymous 1986a, 1986b; Forattini 1986; CDC, unpublished data). Aside from its significance as a pest species, Ae. albopictus in the Western Hemisphere has aroused the interest of public health authorities because of its known and potential vector relationship with several arboviruses of public health importance (Shroyer 1986). Of chief concern in the southern United States, and Central and South America is the impact that Ae. albopictus may have on the transmission and maintenance of dengue (DEN) and vellow fever (YF) viruses. We report here on experimental infection and transmission studies with these viruses and a strain of Ae. albopictus from Houston, Texas. In addition, we have included Ross River (RR) virus, an alphavirus that has recently extended its range into the Pacific basin and for which several geographic strains of Ae. albopictus have been shown to be efficient experimental vectors (Mitchell and Gubler 1987).

### MATERIALS AND METHODS

Viral and mosquito strains. The sources and passage histories of the viral strains are shown in Table 1. The Houston Ae. albopictus colony was established from 48 females collected as adults and approximately 12 females and a few males reared from larvae, all collected in Houston, Harris County, Texas, during March 1986.

Only F<sub>2</sub> and F<sub>3</sub> laboratory generation females were used in our experiments. The Hawaii Ae. albopictus colony used for comparative purposes in the RR virus experiments was from Makiki. Oahu, Hawaii, and in the F<sub>19</sub> and F<sub>20</sub> laboratory generations; its vector competence for RR virus had been determined previously (Mitchell and Gubler 1987). The Ae. aegypti (Linn.) strain was from the Rexville, Puerto Rico colony maintained at the Centers for Disease Control (CDC) laboratory in San Juan. The generation history of the parent colony is unknown, but the strain had been colonized for about 4 years with periodic additions of field-collected Ae. aegypti. A subcolony was established in our Fort Collins insectary during March 1986, and F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> generations from this colony were used in the experiments.

Experimental procedure. All mosquitoes were reared at 26.7 (±0.5°C), 80% RH, and a photoperiod of L:D 16:8. Three- to five-day-old females were used in the feeding trials. Mosquitoes were allowed to feed on suspensions consisting of fresh DEN virus grown in Toxorhynchites amboinesis (Doleschall) or YF virus grown in C6/36 cells or suckling-mouse brains, harvested on the day of feeding, diluted as appropriate, and mixed with equal volumes of washed human red blood cells. Procedures for preparing and feeding the YF virus suspension were described in detail (Miller and Mitchell 1986); the following procedure for preparing the DEN virus is that used at the CDC, San Juan Laboratories. Recently emerged Tx. amboinesis females were inoculated with seed virus, incubated for 7 days at 26.7°C, and cold-anesthetized mosquitoes were triturated live in heat-inactivated calf serum at ratios of 0.1 to 0.2 ml per mosquito, depending on the number of mosquitos available and the volume of feeding suspension required.

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Table 1. Sources and passage histories of viral strains.

Virus Stra	source	Location	Year	Passage
DEN-1 1620	Human serum	Puerto Rico	1985	Mosquito-1
DEN-2 1618	Human serum	Puerto Rico	1985	Mosquito-1
DEN-3 155'	Human serum	Mozambique	1985	Mosq1, C6/36-1
DEN-4 1633	Human serum	Puerto Rico	1985	Mosquito-1
YF 7883	9 Haemagogus spegazzini	Trinidad	1978	C6/36-2
RR	Human serum	Rarotonga	1980	Mosquito-1

Mosquito suspensions were centrifuged at 4,000 rpm for 30 min at 4°C, and the supernatant was removed, diluted as appropriate, and mixed with equal volumes of washed red blood cells. Generally, suspensions containing freshly harvested DEN or YF viruses were fed undiluted and at 10- and 100-fold dilutions. The feeding suspensions were warmed for 4 min at 37°C, and drops were placed directly on nylon netting covering pint-sized cages containing the mosquitoes. Mosquitoes were exposed for 15 min, and fully engorged specimens were sorted, placed in cages, given 5% sugar water, and incubated for appropriate intervals at 26.7°C and 80% RH. Mosquitoes ingested RR virus by feeding on viremic hamsters infected by subcutaneous inoculation 48 to 96 hr prior to feeding (Mitchell and Gubler 1987).

Yellow fever and RR virus transmission trials were conducted by allowing mosquitoes to feed on 2-day-old suckling mice. Mice were monitored for 14 days for signs of illness or death. Dengue virus transmission was determined by the in vitro feeding technique described by Aitken (1977). Capillary tubes were loaded with approximately 5 µl of calf serum, and the proboscis of a test mosquito was inserted in a tube following removal of the mosquito's wings. The capillary tube was fixed in a Styrofoam® rack, and each mosquito was left in place for at least 30 min. The amount of feeding suspension ingested was recorded, and the remainder of the suspension was expressed onto a microscope slide, loaded into a calibrated capillary needle, and injected parenterally into five, or occasionally fewer, Rexville Ae. aegypti. These mosquitoes were given 5% sugar water and incubated at 26.7°C for 7 days. They were frozen at -70°C until assayed for virus.

Mosquito infection with DEN and YF viruses was determined by examining head squashes for viral antigen by the direct fluorescent antibody test (DFAT) (Kuberski and Rosen 1977). Sometimes associated carcasses were sonicated and tested for YF virus by plaque assay in Vero cell culture. All assays for RR virus in mosquitoes were done in Vero cell culture by methods recently described (Mitchell and Gubler 1987).

Dengue stock virus and feeding suspensions were titrated by inoculating Tx. ambionensis

with 0.17  $\mu$ l each of tenfold dilutions (Rosen and Gubler 1974) and calculating the mosquito infectious dose<sub>50</sub> (MID<sub>50</sub>)/ml (Reed and Muench 1938). Titrations of YF virus (Miller and Mitchell 1986) and RR virus (Mitchell and Gubler 1987) were done by plaque assay in Vero cell culture.

#### RESULTS

The susceptibility of Houston Ae. albopictus to per os infection with DEN viruses 1-4 is compared with that of Rexville Ae. aegypti in Table 2. Generally, DEN viral antigen was detectable in head tissues by day 7 of incubation in both species; however, Ae. albopictus did not have detectable DEN-2 antigen at that time. Aedes aegypti was not tested for DEN-4 antigen on day 7 because unexplained mortality in this cohort reduced the sample size, and we wished to keep the remainder for a longer incubation period.

Disseminated DEN virus infection rates were compared in both species for each DEN serotype. There was no significant difference in infection rates for any DEN serotype in Ae. aegypti and Ae. albopictus incubated less than 14 days. However, at 14 days' incubation, the infection rates in Ae. aegypti were significantly greater ( $P \le 0.05$ , Fisher's exact test) for each DEN serotype except DEN-1.

Titers of DEN virus feeding suspensions that would be expected to result in 50% infection rates in Ae. aegypti and Ae. albopictus that fed on these suspensions were calculated from the data in Table 2 for DEN-1, DEN-2 and DEN-3, i.e., log<sub>10</sub> MID<sub>50</sub>/ml. DEN-4 was not included because of the paucity of data points. These titers were virtually identical for DEN-1, 7.19 and 7.20 in Ae. aegypti and Ae. albopictus, respectively. However, the titer of the DEN-2 feeding suspension required to infect 50% of Ae. aegypti per os (6.57) was significantly lower (P ≤ 0.05, probit analysis) than for Ae. albopictus (7.67). A significant difference ( $P \le 0.05$ ) also was noted for DEN-3 virus, where the titers required to infect 50% of Ae. aegypti and Ae. albopictus were 7.20 and 9.16, respectively.

We also tested four groups of Ae. albopictus for their ability to transmit each of the four

Table 2. Susceptibility of Rexville Aedes aegypti and Houston Aedes albopictus to infection per os with dengue viruses.

	Days incu- bation		Infection rates <sup>b</sup>			
Dengue virus		Titer <sup>a</sup>	Ae aegypti		Ae. albopictus	
		feeding suspension	No. tested	% pos.	No. tested	% pos.
1	7	9.0	20	45	20	60
		9.2	0		19	58
	14	6.6	60	38	60	23
		7.6	58	67	60	70
		8.9	0		25	100
		9.0	57	75	68	85
_		9.2	21	100	40	98
2	7	7.6	20	25	Õ	•
		7.6	0		20	0
	13	6.3	31	10	60	12
		7.4	11	36	54	26
		9.0	5	40	37	23
	14	5.6	33	24	34	3
		6.6	14	7	35	17
		7.6	27	74	32	53
		7.6	20	85	38	45
		8.4	25	92	23	74
3	7	8.3	20	10	0	
		8.4	0		20	15
	14	6.3	32	41	60	27
		7.4	60	68°	59	22
		8.1	25	64	15	53
		8.3	60	47°	53	38
		8.4	7	71	34	41
4	7	8.0	0		20	0
		9.2	0		20	5
	13	6.2	13	0	60	7
		7.4	14	14	60	13
		8.0	11	45	59	39
	14	7.9	25	76	19	43
		9.2	6	50	42	29

<sup>&</sup>lt;sup>a</sup>  $log_{10}$  MID<sub>50</sub>/ml.

DEN viruses following exposure to infection per os and 14 days of incubation. Correlation between disseminated infection rates, i.e., mosquitoes with positive head squashes, and virus transmission rates ranged from 42 to 88% (Table 3).

Yellow fever virus infection and transmission rates in Ae. aegypti and Ae. albopictus are compared in Table 4. Both species had comparable infection (70 and 80%) and transmission (46 and 55%) rates 11 days after feeding on a meal containing  $10^{6.7}$  MID<sub>50</sub>/ml of YF virus. No significant differences (P > 0.05, Fisher's exact test) were noted in the infection rates or transmission rates of the two species following ingestion of similar amounts of virus and incubation for the same time period.

In addition to the data on YF summarized in Table 4, data were obtained on infection thresholds and virus dissemination rates in Ae. aegypti

Table 3. Day-14 infection and transmission rates in the Houston strain of *Aedes albopictus* orally exposed to the four serotypes of dengue virus.

Dengue serotype	Meal titer log <sub>10</sub> MID <sub>50</sub> /ml	No. infected*/ no. tested	No. trans- mitting <sup>b</sup> / no. infected
1	8.9	25/25 (100%)	21/24 (88%)
2	8.4	23/25 (92%)	17/23 (74%)
3	8.1	16/25 (64%)	8/15 (53%)
4	7.9	19/25 (76%)	8/19 (42%)

<sup>&</sup>lt;sup>a</sup> Based on detection of dengue viral antigen in mosquito head tissues by DFAT.

and Ae. albopictus fed simultaneously on a meal containing  $10^{4.9}$  MID<sub>50</sub>/ml of YF virus. On day 11 of incubation, 4 of 25 (16%) Ae. aegypti bodies contained virus as compared to 1 of 25 (4%) Ae.

<sup>&</sup>lt;sup>b</sup> Based on detection of DEN viral antigen in head tissues by DFAT.

<sup>&</sup>lt;sup>c</sup> Records for the numbers fed and tested from these groups suggest that a labelling error occurred; therefore, these data probably should be reversed.

<sup>&</sup>lt;sup>b</sup> Determined by the *in vitro* feeding technique of Aitken (1977).

Table 4. Infection and transmission rates in Aedes aegypti and Aedes albopictus fed simultaneously
on vellow fever virus suspensions.

Meal titer	Extrinsic	No. infected*/no. tested		No. transmitting <sup>b</sup> /no. infected	
log <sub>10</sub> PFU/ml	incubation	Ae. aegypti	Ae. albopictus	Ae. aegypti	Ae. albopictus
6.7	11 days	20/25 (80%)	21/30 (70%)	6/13 (46%)	6/11 (55%)
6.7	14 days	$1/1 \ (100\%)$	34/46 (74%)	0/1	4/28 (14%)
5.9	11 days	10/24 (42%)	19/30 (63%)	3/8 (38%)	1/14 (7%)
5.9	14 days	3/3 (100%)	13/20 (65%)	1/3 (33%)	1/8 (13%)
5.0	14 days	12/30 (40%)	6/20 (30%)	3/7 (43%)	0/6

<sup>&</sup>lt;sup>a</sup> Based on detection of yellow fever viral antigen in mosquito head tissues by DFAT.

albopictus. Yellow fever viral antigen was detected in 1 of 25 Ae. aegypti heads and none of 25 Ae. albopictus heads. On day 14 of incubation. 4 of 10 (40%) Ae. aegypti and 8 of 55 (15%) Ae. albopictus bodies contained YF virus, and 1 of 10 (10%) and 1 of 55 (2%) heads were positive for antigen. The observed differences in infection and dissemination rates between the two species are not significant (P > 0.5). All mosquitoes were given an opportunity to refeed on suckling mice to test for virus transmission; however, since infection rates were low, there were few mosquitoes with disseminated infections among those that refed. The single infected Ae. aegypti that refed on day 11 successfully transmitted virus; none of the remaining Ae. aegypti and Ae. albopictus that refed had disseminated infections. On day 14, the single Ae. aegypti with a disseminated infection did not refeed; the single Ae. albopictus with a disseminated infection refed but did not transmit virus.

Ross River virus infection and transmission rates in Houston and Hawaii  $Ae.\ albopictus$  are compared in Tables 5 and 6, respectively. Both strains were susceptible to infection  $per\ os$ , although the Hawaii strain was significantly  $(P \le 0.05)$  more susceptible than the Houston strain in one feeding trial in which the hamster was circulating  $10^{5.2}$  Vero cell plaque-forming units (PFU)/ml in the blood at the time of feeding. Both species could transmit virus on day 7 of incubation, but transmission rates were higher on day 14 (Table 6). In two instances, RR virus transmission rates were significantly higher  $(P \le 0.05)$  among Hawaii  $Ae.\ albopictus$  than among the Houston strain.

#### DISCUSSION

Geographic strains of Ae. albopictus are known to vary in their ability to become infected with dengue viruses by the oral route (Gubler and Rosen 1976). Our results show that the Houston strain of Ae. albopictus is susceptible to infection by each DEN virus by the oral route and that a high proportion of infected mosqui-

toes can transmit these viruses by 14 days post-infection (Tables 2 and 3). Some Ae. albopictus had disseminated infections of DEN-1, DEN-3 and DEN-4 by day 7 postinfection, thus suggesting that a portion of the mosquitoes may be able to transmit virus at this time. The Houston strain of Ae. albopictus was significantly less susceptible to per os infection with DEN-2, DEN-3 and DEN-4 viruses than was Ae. aegypti, but susceptibilities to DEN-1 virus were comparable. The relative susceptibility of the two species can be expected to vary depending on the origin of the geographic strains (Gubler and Rosen 1976, Gubler et al. 1979).

The DEN viruses also varied in their infectivity. In general, DEN-1 and DEN-2 viruses were most infectious, DEN-3 intermediate, and DEN-4 the least infectious. Such variation in the infectivity of DEN viruses has been observed previously (Gubler and Rosen 1976). Transmission rates of the four DEN viruses also were different (Table 3); however, this variation may be due to either viral strain variation or the titer of the infective meal. Experiments were not done to answer this question.

The DEN virus infection rates reported here are generally higher than those found by Gubler and Rosen (1976). The titers of our feeding suspensions were sometimes higher, and the use of fresh virus suspensions that had not been frozen (Miller and Gubler, unpublished data) probably accounts for the observed differences. Several investigators have shown that infection rates for a variety of arboviruses generally are lower in mosquitoes fed virus suspensions when compared with mosquitoes fed directly on viremic hosts with comparable titers (Mitchell 1983). Therefore, data presented here concerning thresholds of infection for DEN and YF viruses cannot be extrapolated and applied directly to field situations. It follows that we cannot draw any firm conclusions about the susceptibility thresholds of Ae. albopictus to infection with DEN and YF viruses in relation to virus titers that might be encountered when feeding on viremic humans. Nonetheless, the compari-

<sup>&</sup>lt;sup>b</sup> Yellow fever virus transmission was assayed by allowing mosquitoes to feed on 2-day-old mice.

Table 5. Ross River virus infection rates in Hawaiian and Houston strains of Aedes albopictus.

Titer <sup>a</sup> of infective	Day-7 incubation		Day-14 incubation		
	Hawaii	Houston	Hawaii	Houston	
meal	n (% infect.)		n (% infect.)	n (% infect.)	
4.5	b		24 (33)	39 (13)	
5.2	50 (90)°	50 (70)	50 (90)°	50 (70)	
5.8	20 (100)	20 (65)	12 (58)	14 (79)	
7.1	20 (100)	20 (100)	17 (100)	24 (96)	
7.6	50 (98)	50 (100)	46 (98)	50 (98)	

<sup>&</sup>lt;sup>a</sup> Log<sub>10</sub> Vero cell PFU/ml.

Table 6. Ross River virus transmission rates by Hawaiian and Houston strains of Aedes albopictus.

	Day-7 transmission		Day-14 transmission	
Titer <sup>a</sup> of infective	Hawaii	Houston	Hawaii	Houston
meal	n <sup>b</sup> (% trans.) n (% trans.)		n (% trans.)	n (% trans.)
5.2	23 (43)	27 (33)	35 (77)°	25 (52)
5.8	12 (75)	6 (50)	5 (100)	6 (67)
7.1	10 (90)	17 (53)	9 (100)	11 (64)
7.6	27 (67)°	40 (38)	31 (94)	36 (78)

<sup>&</sup>lt;sup>a</sup> Log<sub>10</sub> Vero cell PFU/ml.

sons among species of mosquitoes and strains of virus are quite valid since any reduction in sensitivity attributable to the artificial feeding technique should be the same in the paired comparisons.

Our results show that Houston Ae. albopictus and Rexville Ae. aegypti are readily infected with YF virus by the oral route and that virus transmission rates are similar and substantial (55 and 46%, respectively) on day 11 postinfection. Also, the strains of the two species tested have similar thresholds of infection. Dinger et al. (1929) previously demonstrated that Ae. albopictus from Java could transmit YF virus; however, since mosquitoes were tested in groups, it was not possible to quantify infection and transmission rates.

The Houston strain of Ae. albopictus is also an efficient experimental vector of RR virus. Infection rates approached 100% following the ingestion of high-titered blood meals, and virus transmission rates also were high (52 to 78%) by day 14 postinfection. Both the Hawaii and Houston strains of Ae. albopictus were also capable of transmitting RR virus by day 7 postinfection. These results are in general agreement with those concerning RR virus in Ae. vigilax (Skuse), a primary vector in Australia and perhaps Fiji. Kay (1982) showed that Ae. vigilax infected per os could transmit RR virus by bite

4 days later and that maximum transmission efficiency was reached by 10 to 13 days postinfection. Our results suggest that the Hawaii strain of Ae. albopictus may sometimes be a more efficient experimental vector of RR virus than is the Houston strain. Mitchell and Gubler (1987) previously showed that geographic strains of Ae. albopictus may vary in their vector competence for RR virus.

In view of the known and potential vector relationship of Ae. albopictus with several arboviruses, its establishment in the Western Hemisphere is a justifiable cause for concern. Whether its presence in the United States increases the risk of epidemic dengue transmission is a point that may be debated since Ae. aegypti already is present in many of the same areas. However, the fact that all four DEN viruses can be transmitted transovarially by Ae. albopictus under experimental conditions (Rosen et al. 1983) warrants concern about its potential as a reservoir for endemic dengue.

The situation in Central and South America and the islands of the Caribbean appears more ominous. Aedes albopictus may contribute to dengue transmission and maintenance, and in certain areas, has the potential of bridging the gap between jungle and urban yellow fever cycles. The species may become abundant in the forest fringe and adjacent urban areas. Whether

b Not done.

<sup>&</sup>lt;sup>c</sup> Differs significantly from other strain tested the same day;  $P \le 0.05$  in Fisher's exact test.

<sup>&</sup>lt;sup>b</sup> Number of infected mosquitoes that refed.

<sup>&</sup>lt;sup>c</sup> Differs significantly from other strain tested the same day;  $P \le 0.05$  in Fisher's exact test.

it might also become established in jungle foci of *Haemagogus*-transmitted YF remains to be seen.

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