VECTOR COMPETENCE OF GEOGRAPHIC STRAINS OF *AEDES ALBOPICTUS* AND *AEDES POLYNESIENSIS* AND CERTAIN OTHER *AEDES* (*STEGOMYIA*) MOSQUITOES FOR ROSS RIVER VIRUS

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ABSTRACT. The vector competence of geographic strains of *Aedes albopictus* and *Ae. polynesiensis* and Fiji strains of *Ae. pseudoscutellaris* and *Ae. aegypti* was assessed for Ross River (RR) virus, the etiologic agent of epidemic polyarthritis. Strains of *Ae. polynesiensis* from Fiji, Rarotonga, Samoa and Tahiti were the most susceptible to infection *per os* ($MID_{50} \leq 10^{1.2}$ Vero cell plaque-forming units [PFU]/blood meal). Virus transmission data were variable, but all strains except the one from Fiji transmitted virus at 14 to 21 days postinfection. Shanghai and Hawaii *Ae. albopictus* and Fiji *Ae. pseudoscutellaris* were also highly susceptible to *per os* infection with RR virus (MID_{50} $10^{2.0}$ to $10^{2.6}$ PFU). Singapore and Sri Lanka *Ae. albopictus* and Fiji *Ae. aegypti* were the least susceptible (MID_{50} $10^{4.0}$ to $10^{4.2}$ PFU). With one exception, virus transmission rates for *Ae. pseudoscutellaris* and *Ae. aegypti* and the four strains of *Ae. albopictus* ranged from 52 to 100%.

A total of 4,718 third- and fourth-instar larvae from the second gonotrophic cycle of potentially infected females were tested for RR virus in 39 pools. Infection rates in parental females ranged from 87 to 100% in *Ae. albopictus*, *Ae. pseudoscutellaris* and *Ae. polynesiensis* and 40 to 48% in *Ae. aegypti*. Virus was not isolated from larval progeny.

INTRODUCTION

Ross River (RR) virus is an alphavirus and the etiologic agent of epidemic polyarthritis (EPA). Until 1979, the known geographic distribution of RR virus was limited to Australia, New Guinea, New Britain and the Solomon Islands, all in the western half of the Pacific basin (Tesh et al. 1975). In April 1979, cases of EPA were reported from an area close to Nadi International Airport, Viti Levu, Fiji. The epidemic spread throughout the island group, resulting in an estimated 50,000 clinical cases (Aaskov et al. 1981). Several thousand additional cases resulted from further spread to American Samoa in August 1979, to Futuna and Wallis Islands in November 1979, and to New Caledonia, Tonga, and the Cook Islands in January-February 1980 (Marshall and Miles 1984).

Although RR virus has been isolated from several species of wild-caught mosquitoes in Australia and several species have been shown to be capable of transmitting the virus experimentally, *Aedes vigilax* (Skuse) and *Culex annulirostris* Skuse have been considered to be the principal vectors (Kay et al. 1982). The distribution of the latter species coincides with enzootic-endemic RR virus activity in Australia and New Guinea, and it occurs on all the Pacific island groups where cases have been reported. However, Marshall and Miles (1984) suggest that *Cx. annulirostris* may be more important in maintaining primary cycles than as an epidemic vector. *Aedes vigilax* probably was the main vector of RR virus in Fiji and New Caledonia (Austin et al. 1979, Marshall and Miles 1984), but its distribution in the Pacific does not extend eastward beyond the Fiji Islands. *Aedes polynesiensis* Marks was the probable vector of RR virus in the Cook Islands (Rosen et al. 1981, Gubler 1981), and it is the only mosquito species from which virus was isolated during the 1979-1980 outbreaks in the Pacific. Gubler (1981) showed that a strain of *Ae. polynesiensis* from Rarotonga, Cook Islands, is an efficient vector of RR virus under experimental conditions. This new virus-vector association and epidemiologic data suggesting a man-mosquito cycle for RR virus in the outbreaks in American Samoa and Rarotonga (Tesh et al. 1981, Rosen et al. 1981), are indicative of the potential for further spread of the virus.

We report here on the vector competence of several geographic strains of *Ae. albopictus* (Skuse) and *Ae. polynesiensis* and one strain each of *Ae. pseudoscutellaris* (Theobald) and *Ae. aegypti* (Linn.) from Fiji. Each of these species feeds readily on humans, has the potential for rapid population increases and, with the exception of *Ae. albopictus*, is present in areas where outbreaks of EPA have occurred. Among these species, *Ae. aegypti* and *Ae. polynesiensis* previously have been shown to be capable of transmitting RR virus under experimental conditions (Kay et al. 1979, 1982; Gubler 1981).

MATERIALS AND METHODS

Virus strain. We used a Rarotonga strain of RR virus obtained from Dr. Leon Rosen. It was isolated from human serum by inoculation into

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Table 1. Mosquito species and strains used in Ross River virus infection and transmission experiments.

Species	Geographic origin	Laboratory colony generation
Aedes albopictus	Makiki, Oahu, Hawaii	F_2, F_{16}, F_{17}
	Colombo, Sri Lanka	F_2
	Singapore	F_2
	Shanghai, China	Beijing insectary colony, F_{120}
	Shanghai, China	F_2
Ae. polynesiensis	Fiji	colonized since 1981[a]
	Rarotonga	colonized since 1980[a]
	Samoa	colonized since 1978[a]
	Tahiti	colonized since 1969[a]
Ae. pseudoscutellaris	Fiji	colonized since 1981[a]
Ae. aegypti	Fiji	colonized since 1981[a]

[a] Generation history unknown.

Toxorhynchites amboinensis (Doleschall) in March 1980. Our virus seed pools were prepared in heat-inactivated fetal calf serum (FCS) following one further passage in *Tx. amboinensis.*

Mosquitoes. Four strains of *Ae. polynesiensis,* one strain each of *Ae. pseudoscutellaris* and *Ae. aegypti,* and five strains of *Ae. albopictus* were used. Their origins and colonization histories are shown in Table 1. Mosquitoes were reared in environmental chambers at 26 ± 1°C. Humidity in the chambers was maintained at 80 ± 5% RH, and the light:dark cycle was set at 16L:8D.

Experimental procedure. Viremia curves were determined in 6- to 10-week-old Golden Syrian hamsters inoculated subcutaneously with approximately 10^4 Vero cell plaque-forming units (PFU) of RR virus. Ten hamsters were inoculated with virus, and two were sacrificed each day and bled by cardiac puncture for viremia determinations. Subsequently, 3- to 5-day-old mosquitoes were allowed to feed for 1 hr or less on viremic hamsters that had been anesthetized with 0.15 cc of Ketamine HCl (100 mg/ml). Hamsters were bled by heart puncture at the completion of feeding, and sera were collected and frozen at −70°C until titrated. Engorged mosquitoes were sorted and placed in gallon cages, provided with oviposition dishes containing strips of moist paper, and incubated at 26.7 ± 0.5°C and 80% RH. Some groups of mosquitoes were fed simultaneously on the same viremic hamster. A single anesthetized hamster was placed on top of three Plexiglas® cages (15.2 × 7.6 × 2.5 cm) covered with netting and containing the mosquitoes. A few mosquitoes were infected by feeding on suckling mice that had been inoculated subcutaneously with approximately 10^5 to 10^6 $TCID_{50}$ 24 hr earlier.

To assay for virus transmission, we allowed mosquitoes to refeed individually on 1- to 3-day-old suckling mice following appropriate periods of incubation. The mosquitoes were frozen, and mice that were bitten were marked and returned to their mothers and observed for 14 days. Infected mice typically displayed hind leg paralysis between days 6 and 9 postinfection.

In one experiment, progeny of six lots of infected mosquitoes were tested to determine whether transovarial transmission (TOT) of RR virus had occurred. Following an infective feed, mosquitoes were allowed to oviposit and were then refed on uninfected hamsters on day 14 of incubation. Eggs from the second oviposition cycle were collected 17 to 20 days following the infective meal, conditioned in the usual manner, hatched, and the resultant larvae were reared in an incubator at 20°C and 15L:9D.

Adult mosquitoes were disrupted by sonic energy in 1 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,[3] and 1 μg/ml Fungizone). Suspensions were centrifuged at 2,000 rpm for 20 min. The supernatant was frozen at −70°C until tested. Third and fourth instar larvae were rinsed twice in M199 + 20% FCS and ground in pools of 125 or less in 2 ml of the same diluent, then centrifuged for 20 min at 2,000 rpm. Aliquots were frozen and subsequently tested in Vero cell culture. Blood samples from hamsters were taken by heart puncture, allowed to clot, and centrifuged at 1,500 rpm for 15 min. Sera were dispensed into screw-cap vials and stored at −70°C.

Specimens were screened for virus or were titrated as appropriate. Viral assays were done by inoculating Vero cell cultures and counting plaques. Briefly, tenfold dilutions were made in BA-1, and samples (0.1 ml) were inoculated into Vero cell cultures in six-well plates, adsorbed for 1 hr at 37°C, and overlaid with 1% Noble agar in M-199 supplemented with 2% FCS, 2.0 g/liter of $NaHCO_3$, 150 μg/ml of DEAE-dextran,

[3] 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.

and 1:4,000 neutral red. Cell cultures were then examined for 10 days for characteristic plaques. Blood samples from viremic suckling mice used to infect mosquitoes were assayed in the C6/36 clone of *Ae. albopictus* cells using methods previously described (Rosen et al. 1981, Gubler 1981). Tests were not done to determine the relationship between $TCID_{50}$ and Vero cell PFU for RR virus; however, this information would not alter the conclusions drawn.

The virus infection rate in mosquitoes, expressed as a percentage, is the proportion of mosquitoes tested that contained virus. The ID_{50} value was estimated using probit regression. The virus transmission rate, also expressed as a percentage, is the proportion of infected mosquitoes that transmitted virus upon refeeding after a suitable extrinsic incubation period. Differences in infection rates between species fed simultaneously on the same viremic hamster were tested for significance by Fisher's Exact Test (Snedecor and Cochran 1967).

RESULTS

Golden Syrian hamsters, 6 to 10 weeks old, were viremic on days 1 through 4 following subcutaneous inoculation of RR virus. Titers in Vero cell PFU/ml were $10^{2.7}$ and $10^{3.5}$ on day-1, $10^{6.0}$ and $10^{6.1}$ on day-2, $10^{6.6}$ and $10^{7.3}$ on day-3, and $10^{5.5}$ and $10^{6.6}$ on day-4 postinoculation. Virus was not detected on day-5.

The results of experiments concerning the susceptibility of mosquito species and strains to *per os* infection with RR virus are summarized in Tables 2 and 3. Since infection rates are static and since infected individuals are readily detectable in our assay system by day 7 postfeeding, no distinction is made between groups with different incubation periods when analyzing the infection rate data. Where possible, MID_{50} values were calculated by probit regression for each species and strain fed on viremic hamsters. The limited data for *Ae. polynesiensis* fed on suckling mice (Table 2) are excluded because mouse blood samples were titrated in C6/36 cells, and titers were expressed as $TCID_{50}$. The MID_{50} values are based on the assumption that each mosquito ingested 5 μl of blood.

The MID_{50} values could not be determined for strains of *Ae. polynesiensis* because 83% or more became infected after feeding on the lowest titered blood meals taken (Table 2). However, it is obvious that these values would be below $10^{1.2}$ Vero cell PFU for each of the four strains tested. MID_{50} values (and 95% confidence limits) for the other species and strains follow: *Ae. pseudoscutellaris* $10^{2.0}$ ($10^{1.6}$, $10^{2.4}$); *Ae. aegypti* $10^{4.2}$ ($10^{3.9}$, $10^{4.5}$); Beijing *Ae. albopictus* $10^{2.5}$ ($10^{1.7}$, $10^{3.1}$), Hawaii $10^{2.6}$ ($10^{1.7}$, $10^{3.2}$), Shanghai $10^{2.4}$ ($10^{0.9}$, $10^{3.6}$), Singapore $10^{4.0}$ ($10^{3.3}$, $10^{4.8}$), and Sri Lanka $10^{4.1}$ ($10^{3.1}$, $10^{5.5}$). Paired comparisons between MID_{50} values indicate that those of Beij-

Table 2. Ross River virus infection rates in geographic strains of *Aedes polynesiensis* and one strain each of *Ae. pseudoscutellaris* and *Ae. aegypti* fed on viremic hamsters or suckling mice.

Titer of infective meal[a]	*Ae. aegypti*	*Ae. pseudoscutellaris*	*Ae. polynesiensis*			
	Fiji	Fiji	Fiji	Rarotonga	Samoa	Tahiti
	n (% infect.)	n (% infect.)	n (% infect.)	n (% infect.)	n (% infect.)	n (% infect.)
2.4		19[b] (5)				
3.4					8[b] (88)	
3.5				18[b] (83)		
4.0		43[b] (37)				
4.5			15[b] (93)			7[b] (100)
4.6				5[b] (100)		
4.7						21[b] (100)
5.5			2[b] (100)			
5.7		7[b] (100)				15 (93)
6.0	48 (40)				6 (100)	
6.2						
6.6	48 (48)					8 (100)
6.8			18 (100)			
6.9			6 (100)	9 (100)		
7.0		71 (99)				
7.3		5 (100)			23 (100)	
8.3						
8.5	30 (83)	9 (100)	8 (100)	5 (100)		

[a] Titers from 2.4 through 7.3 are $\log_{10}$ Vero cell PFU/ml from viremic hamsters; titers 8.3 and 8.5 are $TCID_{50}$/ml from viremic suckling mice.

[b] Infection rates determined on day 7 of incubation; all others determined on days 14 to 21 of incubation.

Table 3. Ross River virus infection rates in geographic strains of *Aedes albopictus* fed on viremic hamsters and incubated 13 to 21 days.

Titer of infective meal[a]	Shanghai F_{120} n (% infect.)	Shanghai F_2 n (% infect.)	Hawaii n (% infect.)	Singapore n (% infect.)	Sri Lanka n (% infect.)
3.3					20 (5)
4.0			53 (23)		
3.7	56 (16)				
4.9	19 (74)	32 (53)	15 (87)[b]		
5.0	40 (65)			50 (4)	
5.2	41 (85)		35 (37)		20 (0)
5.3					30 (7)
5.5					25 (12)
5.6			37 (100)		
5.7	11 (82)	18 (89)	6 (100)[b]		
6.0			39 (87)		
6.2				59 (53)	
6.3	8 (100)	14 (100)	9 (89)[b]		
6.5	60 (100)		51 (47)		
6.7					20 (80)
6.9			24 (96)		
7.6			40 (95)		
7.7				50 (96)	
8.1			36 (97)		
8.3	28 (100)	27 (100)	22 (100)[b]		
8.4	19 (100)	36 (100)	13 (100)[b]		

[a] $\log_{10}$ Vero cell PFU/ml.
[b] Underscored values obtained by simultaneously feeding three mosquito strains on same hamsters.

ing, Hawaii, and Shanghai *Ae. albopictus* and Fiji *Ae. pseudoscutellaris* are significantly lower ($P < 0.05$) than those of Singapore and Sri Lanka *Ae. albopictus* and Fiji *Ae. aegypti*.

Three strains of *Ae. albopictus* (Shanghai F_{120}, Shanghai F_2 and Hawaii) were compared by feeding lots of each simultaneously on each of five viremic hamsters (underscored values in Table 3). Although infection rates in the three strains were quite similar, MID_{50} values could not be determined because none of the lots had infection rates below 50%. No significant difference ($P > 0.05$) in infection rates was found when the results were compared in Fisher's Exact Test.

Virus transmission data for infected mosquitoes that refed on suckling mice 13 to 21 days following the infective feed are summarized in Table 4. Each species and strain tested transmitted virus with the exception of *Ae. polynesiensis* from Fiji and, in this case, the sample size was small (n = 13). The data are inadequate for a rigorous statistical analysis.

A total of 4,718 third and fourth instar larvae from the second oviposition cycle of potentially infected females was tested in 39 pools for RR virus. Infection rates in parental females ranged from 87 to 100% in *Ae. albopictus*, *Ae. pseudoscutellaris*, and *Ae. polynesiensis*, and 40 to 48% in *Ae. aegypti* (Table 5). Virus was not isolated from larval progeny.

DISCUSSION

Among the species and strains tested, the four strains of *Ae. polynesiensis* were the most susceptible to RR virus infection *per os*. Based on an estimated blood meal volume of 5 μl, ingestion of 30 to 160 Vero cell PFU resulted in infection rates of 83 to 100% in the four strains. We did not determine extrinsic incubation periods; however, Kay (1982) found that RR virus proliferated quickly in *Ae. vigilax* infected *per os*. Virus was present in the salivary glands on day 2 postfeeding, initial transmission by bite occurred on day 4, and maximum transmission efficiency was reached after 10 to 13 days. Therefore, we attempted virus transmission with mosquitoes that had been incubated 13 to 21 days. Virus transmission data for *Ae. polynesiensis* are variable (Table 4); however, each strain except the one from Fiji transmitted virus. This exception is probably due to the small number of specimens tested. In general, our results concerning *Ae. polynesiensis* confirm and extend those of Gubler (1981), who showed that the Rarotonga and Samoa strains are efficient experimental vectors of RR virus.

Susceptibility of the remaining species and

Table 4. Ross River virus transmission rates among *Aedes* (*Stegomyia*) mosquitoes infected by feeding on viremic hamsters or suckling mice.

Mosquito species	Strain	Titer of infective meal[a]	Days incub.	Transmission[b] n	Transmission[b] %
Aedes aegypti	Fiji	6.0	21	15	53
		6.6	21	20	85
		8.5[a]	14	11	55
Ae. pseudoscutellaris	Fiji	7.0	21	23	57
		7.3	21	1	100
		8.5[a]	14	9	0
Ae. polynesiensis	Fiji	6.8	21	5	0
		8.5[a]	14	8	0
	Rarotonga	6.9	17	4	75
		8.5[a]	14	4	0
	Samoa	6.2	17	4	100
		8.3[a]	14	23	13
	Tahiti	5.7	17	10	40
		6.6	21	2	100
Ae. albopictus	Hawaii	6.0	21	25	76
		7.6	21	20	85
	Sri Lanka	5.3	15	1	100
	Singapore	6.2	14	23	52
		6.5	13	16	69
	Shanghai F_{120}	4.7	14	6	83
		5.0	14	24	71
		5.2	14	26	92
		6.5	14	47	92

[a] Titers marked are for suckling mice and are expressed in $TCID_{50}$/ml; all other titers are for hamsters and are expressed as $\log_{10}$ Vero cell PFU/ml.

[b] Transmission rates are based only on the number of infected mosquitoes that refed (n). Discrepancies between these numbers and the number of infected mosquitoes listed in Tables 2 and 3 are due to the fact that some mosquitoes did not refeed during the virus transmission attempts.

Table 5. History of mosquito progeny tested for transovarial transmission of Ross River virus.

		Parental females			
Mosquito species	Strain	Titer of infective meal[a]	Infection rate (%)[b]	No. refed for second oviposition	No. of larvae tested
Aedes albopictus	Hawaii	6.0	87.2	64	668
		7.6	95.0	84	1,500
Ae. aegypti	Fiji	6.0	39.6	83	800
		6.6	47.9	74	1,250
Ae. pseudoscutellaris	Fiji	6.0	98.6	38	250
Ae. polynesiensis	Fiji	6.8	100.0	22	250

[a] $\log_{10}$ Vero cell PFU/ml.

[b] Based on samples of 20 or more individuals tested from lots that survived the second oviposition cycle and an extrinsic incubation period of 21 days.

strains to *per os* infection with RR virus falls into two groupings. Shanghai and Hawaii *Ae. albopictus* and Fiji *Ae. pseudoscutellaris* were highly susceptible, and Singapore and Sri Lanka *Ae. albopictus* and Fiji *Ae. aegypti* were less so. It should be emphasized that only Shanghai and Hawaii strains of *Ae. albopictus* were compared directly by feeding them simultaneously on the same viremic hamsters and that no significant difference in susceptibility was found when comparisons were done in this manner. The Shanghai strains (F_{120} and F_2 generations) appear to be equally susceptible to *per os* infection with RR virus despite the great difference in their colonization history (Table 2). Unfortunately, the Singapore and Sri Lanka colonies were lost, and direct comparisons with these particular strains are no longer possible. In any event, with the possible exception of the Sri Lanka *Ae. albopictus* strain, each of the species and strains tested could reasonably be expected to become infected after feeding on viremic humans with titers comparable to those demonstrated during the Rarotonga outbreak (Rosen et al. 1981). In

general, Fiji *Ae. aegypti* and *Ae. pseudoscutellaris* and the four strains of *Ae. albopictus* used in the virus transmission experiments (Table 4) were efficient transmitters of RR virus.

Our attempt to demonstrate transovarial transmission of RR virus by orally infected adults during the second oviposition cycle met with negative results. Kay (1982) found 2/51 female and 0/71 male progeny of seven intrathoracically inoculated female *Ae. vigilax* to contain RR virus. Apparently, this is the only evidence of transovarial transmission of this virus.

Aedes polynesiensis is one of the several closely related species in the subgenus *Stegomyia* that make up the *scutellaris* group. The majority of species in this group have been recognized from the South Pacific, where one or more members occur on every major inhabited island group except New Caledonia, the Loyalties, and New Zealand (Belkin 1962). *Aedes albopictus,* sometimes considered to be a member of the *scutellaris* group in the broad sense (Belkin 1962), does not occur in the South Pacific but is widespread in the Oriental and Indomalayan regions and is well established in Hawaii. More recently, this species has been reported in the United States from Memphis, Tennessee (Reiter and Darsie 1984), Harris County, Texas (Sprenger and Wuithiranyagool 1986), and several southeastern states and in Brazil (Morbidity and Mortality Weekly Report 1986). Vector competence studies with the Harris County strain are in progress, and the results will be reported separately. *Aedes pseudoscutellaris* is also a member of the *scutellaris* group and is known only from the Fiji Islands. *Aedes aegypti* has a cosmotropical distribution and is present in Hawaii and several island groups of the South Pacific. Obviously, each of these species has the potential for playing a role in future outbreaks of RR virus in the Pacific basin and, in the case of *Ae. aegypti* and *Ae. albopictus*, in other areas as well. Knowledge concerning the vector competence of these species and strains for RR virus may be useful in developing control strategies in the event of future outbreaks.

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