RECENTLY INTRODUCED *AEDES ALBOPICTUS* IN THE UNITED STATES: POTENTIAL VECTOR OF LA CROSSE VIRUS (BUNYAVIRIDAE: CALIFORNIA SEROGROUP)[1]

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ABSTRACT. A population of *Aedes albopictus* collected in 1986 in Harris County, Texas, was evaluated for its vector competence with 4 California serogroup viruses (Jamestown Canyon, Keystone, La Crosse and trivittatus). Rates of midgut infection, dissemination of virus beyond the midgut and oral transmission to suckling mice were markedly different for the 4 viruses in a pattern representative of the antigenic relationships known for the California serogroup. Only La Crosse virus was shown to be efficiently transmitted by this recently introduced mosquito population. The results suggest that populations of *Ae. albopictus* originating from the Harris County population might well be as efficient in transmitting La Crosse virus as are populations of the natural mosquito vector, *Aedes triseriatus*, from the midwestern La Crosse virus enzootic region. The public health implications of these results are discussed in relation to the rapid spread of *Ae. albopictus* throughout the eastern half of the United States and into regions where La Crosse virus is known to be enzootic.

INTRODUCTION

The recent introduction of *Aedes albopictus* (Skuse) into the southern United States (Sprenger and Wuithiranyagool 1986) from one or more northern Asia sources (Hawley et al. 1987) has heightened concerns about the possibility of enhanced future transmission of dengue virus serotypes in the southern United States. Both the indigenous *Aedes aegypti* (Linn.) and introduced populations of *Ae. albopictus* in the United States have been shown capable of transmission of 1 to 4 dengue serotype viruses (Boromisa et al. 1987, Mitchell et al. 1987).

The dissemination of introduced populations of *Ae. albopictus* into the upper Midwest (Nawrocki and Hawley, 1987), evidence showing these populations capable of successfully overwintering in the upper Midwest (Hawley et al. 1989) and the previous demonstration that La Crosse virus (LACV) could persist in and be transovarially transmitted by *Ae. albopictus* parenterally inoculated with this virus (Tesh 1980, Tesh and Gubler 1975) raised additional speculation that this second arbovirus might be transmitted in the future by introduced *Ae. albopictus* populations moving into and competing with *Ae. triseriatus* for habitats in the numerous midwestern LACV enzootic/endemic foci.

This report documents the vector competence of a recently introduced population of *Ae. albopictus* for LACV compared to its greatly reduced vector competence for 3 other California serogroup viruses tested.

MATERIALS AND METHODS

Mosquito strains and colony maintenance: *Aedes albopictus* (HARRIS strain) was originally collected in Harris County (Houston), Texas, in the spring of 1986. The F_3 generation was tested for vector competence for the 4 California serogroup viruses. The vector competence of 2 populations of *Ae. albopictus* collected at tire dump locations in Evansville (DUNN strain) and Indianapolis (INDY strain), Indiana, was subsequently evaluated (both in the F_2) for only LACV. A (continuously maintained) laboratory colony of *Ae. triseriatus* (WALTON strain) was used as a control in the trials with LACV and Jamestown Canyon virus (JCV) because: 1) this colony had been used with numerous fresh *Ae. triseriatus* field populations in earlier studies to evaluate the vector competence of geographically diverse populations of that species for LACV (Grimstad and Haramis 1984, Grimstad et al. 1977), and 2) as a control to ensure that expected rates of infection and transmission in each trial with LACV would be observed before accepting the HARRIS population results. Immature mosquitoes were reared in enamel basins (200 first-instar larvae/basin) containing 2.0 liters of tap water and fed a standardized liver powder (National Biochemicals, Cleveland, OH) diet in a manner earlier described for *Ae. aegypti*

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and *Ae. triseriatus* (Munstermann and Wasmuth 1985a, 1985b). A minimum of 450 females were produced for each *Ae. albopictus* population tested. The rearing and isolation insectaries were both maintained at 27°C, 80% RH, with a 16:8 h light:dark photoperiod. Honey-soaked cellucotton pads and 50 cc water-filled cups (with paper toweling strips for oviposition) were placed in the 3.8 liter (1 gallon) plastic holding cages to provide a source of carbohydrates and water for the emerging adults.

Virus strains: The following stock viruses grown in suckling mouse brains (SMB) were used in vector competence trials: LACV, Indiana isolate GW-1978 (Pinger et al. 1983) in the 3rd SMB passage; JVC, Indiana isolate 800245 (Boromisa and Grimstad 1986) in the 3rd SMB passage; Keystone virus (KEYV), B64-5587.05 (provided by N. Karabatsos, VBDD/CDC, Ft. Collins, CO) in the 5th SMB passage; and trivittatus virus (TVTV), Indiana isolate CMWA-1978 (Pinger et al. 1983) in the 2nd SMB passage.

Infectious blood feeding and assay of vector competence: To minimize age-related differences in infection and transmission, all mosquitoes were orally exposed to virus 4–6 days after emergence. Stock virus was diluted in freshly defibrinated rabbit blood and then placed into a heated (37°C) membrane feeder (Rutledge et al. 1964) fitted with a Baudruche membrane. The virus dose used approximated that found in viremic chipmunks needed to infect all susceptible *Ae. triseriatus* (Patrican et al. 1985) with an adjustment for reduced efficiency of virus infection seen with artificial membrane feeding. Approximately 125–150 adults were placed in each of four 3.8-liter plastic cages, and one by one the cages were rotated under the feeder at 10-min intervals for a total exposure time of 1.5 hours. Only fully engorged females were transferred to 3.8 liter holding cages and supplied with a carbohydrate source and oviposition substrate as described above and retained for subsequent refeeding on suckling mice. At the end of each feeding period an aliquot of the virus-blood mixture was removed from the feeder and the titer of infectious virus determined in Vero cell culture.

After a 14- or 21-day extrinsic incubation period, female mosquitoes were refed individually on a 2- to 3-day-old suckling mouse (ICR strain, Harlan Sprague Dawley, Indianapolis, IN). Mice were observed for 7 to 10 days for signs of morbidity and mortality. Mice showing signs of infection were euthanized and stored at −70°C for further assay to confirm the presence of virus in the brain tissues. Individual mosquito midguts and heads and mouse brains were assayed for virus in Vero cell culture by plaque assay as previously described (Grimstad and Haramis 1983). Presence or absence of virus in the mosquito midgut, mosquito head and mouse brain indicated the level of virus dissemination and confirmed oral transmission, establishing the proportion of females of each test population with barriers to virus infection and transmission (Hardy et al. 1983).

RESULTS

In the first trial, *Ae. albopictus* HARRIS and *Ae. triseriatus* WALTON were fed on a LACV-rabbit blood mixture that titered 4.2 $\log_{10}$ $TCID_{50}$/0.025ml. Forty females from each sample were refed 14 days later on suckling mice and a portion of the remaining females allowed to refeed on day 21.

No significant differences were seen with either *Ae. albopictus* HARRIS or *Ae. triseriatus* WALTON in infection, dissemination (virus beyond the midgut) and transmission based on day of refeeding (Table 1). Therefore, subsequent transmission trials with *Ae. albopictus* HARRIS and JCV, KEYV and TVTV were conducted after a 14-day extrinsic incubation period.

The transmission rate of LACV by the *Ae. albopictus* HARRIS population, whether expressed as the *population* (no. transmitting/total no. refeeding that were tested) or *modified* (no. transmitting/no. refeeding that had disseminated infections) rate (Table 1), was significantly greater than that seen for any of the other 3 viruses in pairwise comparisons ($P < 0.05$; Chi-square 2 × 2 Contingency Table Analysis or Fisher's Exact Test). In contrast, pairwise comparisons of this population's rates of disseminated infection with LACV, JCV and KEYV did not differ significantly. However, disseminated infection with TVTV occurred at a significantly ($P < 0.005$) lower rate than with LACV, JCV or KEYV. No significant differences were detected ($P > 0.05$) in pairwise comparisons of rates of transmission and infection with JCV vs. KEYV (Table 1). The relevance of these comparisons and the relative prevalence of midgut infection, midgut escape and salivary gland barriers (Fig. 1) is addressed below in relation to the antigenic relationships of these 4 viruses within the California serogroup.

Figure 1 shows the proportion of *Ae. albopictus* HARRIS tested with each virus that had either a midgut infection, midgut escape, or salivary gland barrier or orally transmitted to suckling mice. *Aedes albopictus* HARRIS midgut barriers appeared to be of little importance in reducing the transmission rate with LACV, JCV or KEYV, while the salivary gland barrier reduced

Table 1. Summary of vector competence trials with *Aedes albopictus*, *Aedes triseriatus* and 4 California serogroup viruses (La Crosse, Jamestown Canyon, Keystone and trivittatus).

Population and virus tested (day*)	No. tested	Transmission		Disseminated infection	Uninfected at the midgut level
		Population**	Modified		
Ae. albopictus HARRIS:					
La Crosse (day 14)	40	45.0 (18/40)	46.1 (18/39)	97.5 (39/40)	2.5 (1/40)
La Crosse (day 21)	40	42.5 (17/40)	47.2 (17/36)	90.0 (36/40)	7.5 (3/40)
Jamestown Canyon (day 14)	30	6.7 (2/30)	7.7 (2/26)	86.7 (26/30)	3.3 (1/30)
Keystone (day 14)	37	0 (0/37)	0 (0/31)	83.8 (31/37)	8.1 (3/37)
trivittatus (day 14)	50	0 (0/50)	0 (0/12)	24.0 (12/50)	72.0 (36/50)
Ae. albopictus DUNN:					
La Crosse (day 14)	9	22.0 (2/9)	33.0 (2/6)	67.0 (6/9)	11.0 (1/9)
Ae. albopictus INDY:					
La Crosse (day 14)	10	10.0 (1/10)	16.6 (1/6)	60.0 (6/10)	20.0 (2/10)
Ae. triseriatus WALTON (Control strain):					
La Crosse (day 14)	40	80.0 (32/40)	88.9 (32/36)	90.0 (36/40)	5.0 (2/40)
La Crosse (day 21)	10	100 (10/10)	100 (10/10)	100 (10/10)	0.0 (0/10)
Jamestown Canyon (day 14)	40	0.0 (0/40)	0.0 (0/1)	2.5 (1/40)	82.5 (33/40)
Jamestown Canyon (day 21)	50	0.0 (0/50)	0.0 (0/0)	0.0 (0/50)	94.0 (47/50)

* Day refed after initial infectious bloodmeal had been taken. Infectious bloodmeal titers ($\log_{10}$ $TCID_{50}$/0.025 ml in Vero cell culture): LACV = 4.2; JCV = 4.4; KEYV = 3.5; TVTV = 5.8 for *Ae. albopictus* HARRIS and *Ae. triseriatus* WALTON trials; LACV = 4.5 for *Ae. albopictus* DUNN and INDY.

** Population transmission rate = no. transmitting/total no. refeeding; Modified transmission rate = no. transmitting/no. refeeding that had disseminated infections.

potential transmission by 54, 92 and 100%, respectively, in mosquitoes infected with the 3 viruses (Fig. 1). In contrast the midgut infection barrier appeared to be the primary factor that contributed to the poor vector competence of this population for TVTV, while a salivary gland barrier effectively blocked transmission by those relatively few (24% of the population) *Ae. albopictus* HARRIS females that had a disseminated TVTV infection (Table 1, Fig. 1). The relatively high proportion of *Ae. triseriatus* WALTON with a midgut infection barrier to JCV demonstrated why this mosquito is a poor vector of this virus (Fig. 1).

Results of transmission trials with LACV and *Ae. albopictus* DUNN and *Ae. albopictus* INDY indicate both were readily infected after ingestion of virus but transmitted virus to mice at lower rates than did *Ae. albopictus* HARRIS (Table 1).

DISCUSSION

The HARRIS population of *Ae. albopictus* was competent in transmitting LACV to a vertebrate at rates equal to or greater than a number of *Ae. triseriatus* field populations from the midwestern LACV endemic region (mean 46% (105/226), median 43%, range 27–65%; Grimstad et al. 1977). This indicates that introduced *Ae. albopictus* populations must be considered potentially able to transmit LACV as efficiently as fresh *Ae. triseriatus* field populations. The 44% (35/80) rate of transmission of LACV by the *Ae. albopictus* HARRIS population (Table 1) was not significantly different ($P > 0.05$) from the mean LACV transmission rate of 46% reported for the 7 fresh midwestern *Ae. triseriatus* field populations (Grimstad et al. 1977). However, the 94% (75/80) infection rate with HARRIS (Table 1) was significantly greater ($P < 0.005$) than the mean (72%; 162/226) of the 7 *Ae. triseriatus* field populations and exceeded all of the latter's population infection rates by 13 to 44% (see Table 2 in Grimstad et al. 1977).

Few conclusions could be drawn with regard to the two Indiana *Ae. albopictus* field populations due, in part, to the small sample sizes (Table 1). However, the infection rates of DUNN and INDY were not significantly different from rates of infection seen with LACV endemic region *Ae. triseriatus* populations ($P > 0.05$) [i.e., 67 and 60% for DUNN and INDY (Table 1) vs. a mean of 72% (162/226; see Table 2 in Grimstad et al. 1977)]. Despite our producing >600 females of each population and placing them under the feeder, less than 20 of each *Ae. albopictus* DUNN and INDY population fully engorged. However, the surviving females all refed, or at least probed, on a suckling mouse on day 14 (Table 1). In subsequent membrane feeding experiments with these 2 populations and in attempts to maintain them by offering a human arm (G.B.C.) as a blood source, we have noticed an extreme reluctance to engorge in the laboratory. This may change with extended colonization. In contrast, both the DUNN and INDY populations fed avidly on collectors at the tire dump sites.

The barrier depiction in Fig. 1 suggests that

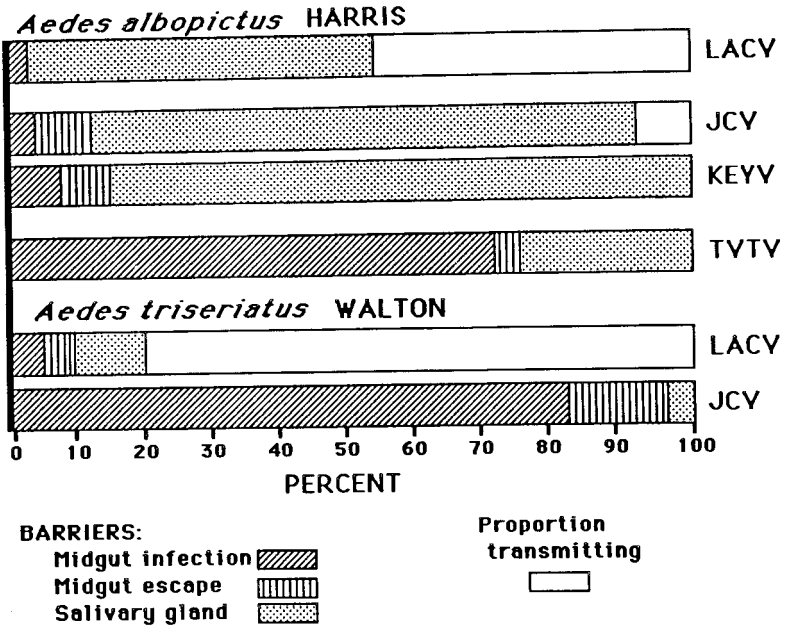

Fig. 1. Proportion of *Aedes albopictus* HARRIS and *Aedes triseriatus* WALTON having barriers to infection and transmission of California serogroup viruses 14 days after oral exposure. Abbreviations: LACV = La Crosse virus; JCV = Jamestown Canyon virus; KEYV = Keystone virus; TVTV = trivittatus virus. The bar graphs depict the proportion of each species with a *midgut infection barrier* (no infectious virus persisting in the midgut, presumably the result of a failure to infect the midgut epithelial cells), *midgut escape barrier* (infectious virus persisting only in the midgut tissues and not disseminating beyond that organ), *salivary gland barrier* (infectious virus disseminated beyond the midgut into the head and presumably to the salivary glands with either no infection of the salivary gland tissues, or if infected, then no shedding of the virus occurs into the saliva; these 2 events describe a salivary gland infection barrier and a salivary gland escape barrier (Grimstad et al. 1985)), and the *proportion orally transmitting* each virus to suckling mice.

if the salivary gland barrier to LACV were somehow "broken down" in *Ae. albopictus* HARRIS, that population would presumably transmit at rates exceeding 95%. The same would be true for JCV and KEYV, but to a lesser extent (i.e., > 85%). Grimstad and Haramis (1984) have shown that nutritional deprivation of larval *Ae. triseriatus* results in adult females having a significantly enhanced vector competence for LACV. In that study they demonstrated the "breakdown" of the barriers to infection and transmission, including the salivary gland barrier in nutritionally deprived samples. The trials we have reported here with *Ae. albopictus* used well-fed larvae and adults. In a field situation nutritional resource limitations, especially as they occur in container habitats used by both *Ae. albopictus* and *Ae. triseriatus*, might well alter the vector competence of introduced *Ae. albopictus* populations coming into LACV enzootic foci. Competition with *Ae. triseriatus* in tree hole and tire habitats for nutritional resources is a certainty, and the seasonal size reduction already documented for *Ae. triseriatus* (Haramis 1984) will only be exacerbated.

Three specific patterns of barrier occurrence and transmission were seen for *Ae. albopictus* HARRIS tested with the 4 California serogroup viruses (Fig. 1). The first pattern—few females with midgut infection and midgut escape barriers, and an equal number with salivary gland barriers and no salivary gland barriers—was seen with LACV. A second pattern—few females with midgut infection and escape barriers, a high proportion with salivary gland barriers and few females able to transmit virus—was seen with JCV and KEYV. The third pattern—a marked number of females with midgut infection and salivary gland barriers with a minimal midgut escape barrier and no transmission—was seen with TVTV. In the California serogroup there are 3 antigenically recognized "viruses" (TVT, Melao and California encephalitis) and numerous serotypes of the latter two (Grimstad 1988). We chose to test LACV as the midwestern and eastern serotype of California encephalitis virus, and JCV and KEYV as midwestern and eastern serotypes of Melao virus; TVTV has no recognized serotypes but is found throughout the eastern half of the United States (Grimstad

1988). The 3 observed patterns (Fig. 1) correspond to the three antigenically recognized California serogroup viruses (Grimstad 1988); indeed, the patterns of the two Melao virus serotypes (JCV, KEYV) are almost identical (Fig. 1).

We recognize that these observations have no ecological significance for the North American California serogroup viruses because they did not evolve through contact with *Ae. albopictus*. However, the relevance of this may lie in the fact that serotypes of each of the viruses are very similar on a molecular basis, particularly the G1 envelope protein; however, molecular differences among the envelope protein(s) of the three serogroup viruses are noticeably different (Gonzalez-Scarano et al. 1983, Ushijima et al. 1980). The relative ability of each virus to attach to the midgut epithelial cell surface and undergo subsequent replication within the midgut epithelial cells and elsewhere in the mosquitoes with disseminated infections would be expected to vary. We believe that aspects of this phenomenon were indirectly shown in our comparison of *Ae. albopictus* HARRIS infected with the 4 viruses (Fig. 1). Therefore, the comparisons may be useful from the standpoint of confirming virus relationships.

Finally, from the standpoint of public health, the demonstrated ability of this introduced population to efficiently transmit LACV at rates equal to or greater than those seen with the natural vector in the field suggests that when *Ae. albopictus* invades LACV foci in the eastern United States, that it will become a second vector. The movement of this introduced species into suburban areas and into tree holes where *Ae. triseriatus* already is established means that the threat of a parallel vector situation throughout the eastern and midwestern states must be taken seriously. Monitoring for the introduction of *Ae. albopictus* in the "La Crosse belt" should be increased.

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