VECTOR COMPETENCE OF *AEDES ALBOPICTUS* FROM PINE BLUFF, ARKANSAS, FOR A ST. LOUIS ENCEPHALITIS VIRUS STRAIN ISOLATED DURING THE 1991 EPIDEMIC[1,2]

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ABSTRACT. The vector competence of *Aedes albopictus* from Pine Bluff, AR, was assessed for a St. Louis encephalitis (SLE) virus strain isolated during the 1991 epidemic. *Aedes albopictus* were fed on hamsters with viremia levels of $10^{4.6}$–$10^{4.9}$ Vero cell plaque-forming units (PFU)/ml. At 7 and 15 days postbloodfeeding, transmission trials were conducted using individual suckling mice. Three of 313 *Ae. albopictus* were determined to be infected with SLE virus with titers of $10^{6.3}$–$10^{7.0}$ PFU/mosquito. At 15 days postbloodfeeding, one of 209 *Ae. albopictus* that refed transmitted virus resulting in a 15-day population transmission rate of 0.5%. The infection threshold (i.e., the amount of virus required to infect from 1 to 5% of mosquitoes) was determined to be approximately $10^{2.3}$ PFU/mosquito. Virus inoculated intracoelomically into *Ae. albopictus* replicated and reached mean titers above $10^{6.0}$ PFU/mosquito on day 6. The combination of low susceptibility to infection and a mammalophilic bloodfeeding pattern suggests that *Ae. albopictus* is unlikely to play a significant role in SLE transmission.

INTRODUCTION

St. Louis encephalitis (SLE) virus is the leading cause of epidemic viral encephalitis in the United States. In July and August of 1991, the town of Pine Bluff experienced the first localized epidemic of SLE recorded in the state of Arkansas. Twenty-five cases occurred in Pine Bluff, a town of 57,000 residents. Four cases were fatal and 4 patients suffered profound residual neurologic deficits (Marfin et al. 1993).

Epidemiologic (Marfin et al. 1993) and entomologic (Savage et al. 1993b) investigations of the outbreak in Pine Bluff were conducted from August 13 to 24, 1991. Investigations to determine the avian hosts of SLE virus in Pine Bluff (McLean et al. 1993), and additional entomologic studies were conducted from August 30 to September 5, 1991.

A total of 20,352 adult female mosquitoes, representing 16 species and 5 additional taxa, were collected in 1991 and processed for virus

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isolation. Eleven isolates of SLE virus were obtained from 169 pools of 6,768 *Culex quinquefasciatus* Say resulting in a minimum infection rate of 1.6 per 1,000 (Savage et al. 1993b). One additional SLE isolate was made from a pool of 22 mosquitoes identified as *Culex (Cux.)* spp. *Aedes albopictus* (Skuse) was the fourth most common species in adult collections, and testing of 1,234 specimens failed to yield virus isolations.

In 1991, 480 larval specimens were collected from 28 habitats during a larval survey conducted at 12 randomly selected residences and additional nonrandomly selected sites in Pine Bluff (Savage et al. 1993b). Seven species in 4 genera were represented. However, only 2 species, *Ae. albopictus* and *Cx. quinquefasciatus*, were frequently encountered: *Ae. albopictus* was present in 17 (61%), and *Cx. quinquefasciatus* in 13 (46%) of the 28 larva-positive habitats. Results of the larval survey in Pine Bluff were similar to those found in a large survey of American cities, which showed that *Ae. albopictus* and members of the *Culex pipiens* complex were much more frequently encountered in eastern and midwestern cities than other mosquito taxa (Moore et al. 1990).

The following studies to assess the vector competence of *Ae. albopictus* for SLE virus were prompted by the common occurrence of *Ae. albopictus* in residential areas of the eastern and midwestern USA, its ability to serve as a vector for a number of other arboviruses (Mitchell 1991), the recent isolation of eastern equine encephalitis virus from this species in Florida (Mitchell et al. 1992), and the propensity of *Ae. albopictus* to feed readily on humans and occasionally on birds (Savage et al. 1993a, Niebylski et al. 1994).

Table 1. Infection and transmission rates in *Aedes albopictus* from Pine Bluff, AR, for a St. Louis encephalitis virus strain, AR91-2783, isolated during the 1991 epidemic.

Meal titer ($\log_{10}$ PFU/ml)	7 days after feeding		15 days after feeding		
	No. infected/ no. tested (%)	No. transmitting/no. that refed (%)	No. infected/ no. tested (%)	No. transmitting/ no. that refed (%)	No. transmitting/no. infected (%)
4.6	0/5 (0)	0/5 (0)	1/57 (1.8)	1/40 (2.5)	1/1 (100)
4.8	0/9 (0)	0/9 (0)	0/52 (0)	0/41 (0)	NA
4.8	0/10 (0)	0/10 (0)	0/58 (0)	0/40 (0)	NA
4.9	0/13 (0)	0/13 (0)	1/56 (1.8)	0/44 (0)	0/1 (0)
4.9	0/6 (0)	0/6 (0)	1/47 (2.1)	0/44 (0)	0/1 (0)
Total	0/43 (0)	0/43 (0)	3/270 (1.1)	1/209 (0.5)	1/3 (33.3)

MATERIALS AND METHODS

Mosquito colony: Adult *Ae. albopictus* attracted to humans were collected by aspirator on July 22, 1993, in Pine Bluff, and immediately mailed to the Division of Vector-Borne Infectious Diseases (DVBID), Ft. Collins, Colorado. Mosquitoes were maintained in an insectary at a temperature of 26.8°C, a photoperiod of 16:8 (L:D), and RH of approximately 85%. Adults were provided with 5% sucrose, and colony mosquitoes were fed on hamsters at regular intervals. Eggs were collected and hatched as needed. Larvae were reared at the above conditions on a diet of rabbit pellets and liver powder. Twelve to 16 h prior to virus transmission trials, sucrose sources were removed from cages and replaced with gauze pads dampened with water.

Preparation of stock virus and determination of viremia profile in hamsters: Stock virus was prepared by intracoelomic inoculation of F_1 *Cx. pipiens* Linn. from Ft. Collins, CO, with a primary SLE isolate, AR91-2783, obtained from a pool of *Cx. quinquefasciatus* collected during the Pine Bluff epidemic. Inoculated *Cx. pipiens* were maintained in the insectary for 7 days, frozen at -70°C, and ground in 1 ml of BA-1 with a mortar and pestle. The suspension was centrifuged, and the supernatant was diluted to 7.0 ml and dispensed into 0.5-ml aliquots. Aliquots were titrated by plaque assay in Vero cell culture using a double overlay (Mitchell et al. 1987).

Ten golden Syrian hamsters, 4 wk old, were subcutaneously inoculated with *ca.* $10^{3.4}$ plaque-forming units (PFU) of stock virus. Hamsters were anesthetized with Metaphane and bled daily for 5 days by cardiac puncture. Blood samples were processed as described by Mitchell et al. (1990) and titrated in Vero cell culture to determine virus profiles.

Transmission experiments: Experiments used F_1 *Ae. albopictus* from Pine Bluff and stock virus

prepared as described above. Five groups of 3–5-day-old F_1 *Ae. albopictus* were fed on 5 hamsters during the period of peak viremia, which was determined to be about 72 h postinoculation. Hamsters were 26 days old on the day of inoculation and each received *ca.* $10^{3.5}$ PFU of virus. Five freshly fed *Ae. albopictus* from each group, and a postfeeding blood sample from each hamster were titrated in Vero cell culture. At 7 and 15 days postbloodfeeding, transmission trials were conducted by feeding *Ae. albopictus* individually on 1–3-day-old suckling mice. Mice were marked and returned to their mothers. Each mosquito and brain suspensions from dead or sick mice were tested in Vero cell culture to verify the presence of virus and to calculate titers.

Intracoelomic inoculation and virus growth curve: A group of 2–6-day-old F_1 *Ae. albopictus* were intracoelomically inoculated with a large dose, *ca.* $10^{3.5}$ PFU, of virus. A 2nd group of 3–8-day-old F_2 *Ae. albopictus* were similarly inoculated with a small dose, *ca.* $10^{2.0}$ PFU, of virus. Both groups were maintained on sucrose and incubated under standard insectary conditions. Five specimens from each group were frozen immediately after inoculation and 5–8 specimens were frozen daily for 9–11 days. Each mosquito was ground in 1 ml of BA-1 with TenBroeck grinders, and supernatants were titrated in Vero cell culture.

Viremia profiles for 4 bird species: Five adult northern cardinals (*Cardinalis cardinalis*) from Memphis, TN, and 9 2–3-month-old, American robins (*Turdus migratorius*) from Ft. Collins, CO, were captured and transported to the DVBID (McLean et al. 1985). Sixteen 3-wk-old bobwhite quail (*Colinus virginianus*) and 6 3-day-old chickens (*Gallus gallus*) were obtained from commercial sources. Birds were maintained in the laboratory under conditions described elsewhere (McLean et al. 1983). Birds were inoculated subcutaneously with *ca.* $10^{3.3}$–$10^{3.6}$ PFU of
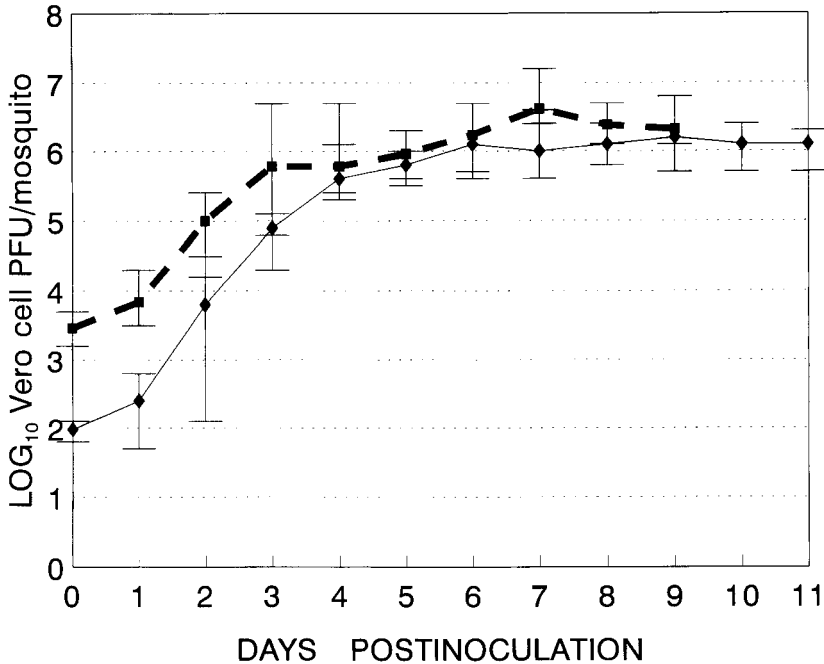
Fig. 1. Growth of SLE virus strain AR91-2783 in *Aedes albopictus* from Pine Bluff, AR, following intracoelomic inoculation at 2 different doses. Group 1, represented by a broken line, was inoculated with *ca.* $10^{3.5}$ PFU; Group 2, represented by a solid line, was inoculated with *ca.* $10^{2.0}$ PFU. Daily values are the geometric mean, maximum, and minimum values for groups of 5–8 specimens.

SLE virus, and bled daily for 7 days following inoculation. Baby chicks were inoculated with SLE virus strain 79V10028, whereas birds of the other 3 species were inoculated with virus strain MSI-7. Blood samples were tested and titrated by plaque assay in Vero cell culture to determine daily viremia titers (McLean et al. 1985).

RESULTS

Of 10 hamsters inoculated with *ca.* $10^{3.4}$ PFU of SLE virus to establish a virus profile, 2 were viremic with titers of $10^{2.0}$–$10^{2.8}$ PFU/ml at day 1 postinoculation. Peak viremia, $10^{5.7}$ PFU/ml, was reached on day 3, and on days 3 and 4 postinoculation 10 of 10 hamsters were viremic with titers of $10^{2.7}$–$10^{5.7}$ PFU/ml, $\bar{x} = 10^{4.8}$ PFU/ml, $n = 20$. On day 5, titers began to fall, with a range of $10^{3.7}$–$10^{5.2}$ PFU/ml, and 7 of 10 hamsters remained viremic.

Seven days after feeding on viremic hamsters with moderate viremias of between $10^{4.6}$–$10^{4.9}$ PFU/ml, 43 *Ae. albopictus* refed on individual suckling mice. All 43 *Ae. albopictus* were tested individually in cell culture and determined to be uninfected, and all suckling mice survived (Table 1).

Fifteen days after feeding on viremic hamsters,

209 of 270 (77%) *Ae. albopictus* refed on individual suckling mice. Three of the 270 *Ae. albopictus*, or 1.1% (Table 1), were infected with SLE virus with titers of $10^{6.3}$–$10^{7.0}$ PFU/mosquito. One of 209 mosquitoes that refed transmitted virus resulting in a 15-day population transmission rate of 0.5% (Chamberlain et al. 1954). All 3 of the infected mosquitoes refed, and one of the 3 (33%) transmitted SLE virus (Table 1).

Twenty-five mosquitoes, 5 from each hamster, were frozen immediately after bloodfeeding. Individual mosquitoes ingested from $10^{1.5}$ to $10^{2.7}$ PFU per mosquito ($\bar{x} = 10^{2.3}$ PFU, $n = 25$), based on an estimated blood meal volume of 5 μl.

Mosquitoes inoculated intracoelomically with large doses of virus, *ca.* $10^{3.5}$ PFU, replicated virus and reached a mean value of $10^{5.5}$ PFU/mosquito on day 3, and a mean value of $10^{6.0}$ PFU/mosquito by day 6: mean titers remained at or above this level through day 9 when the experiment was terminated (Fig. 1). Mosquitoes inoculated with smaller doses of virus, *ca.* $10^{2.0}$ PFU, replicated virus and obtained a mean value of $10^{5.5}$ PFU/mosquito on day 4, and by day 6 postinoculation, mean titers were above $10^{6.0}$ PFU/mosquito and remained at or above this level through day 11 (Fig. 1).
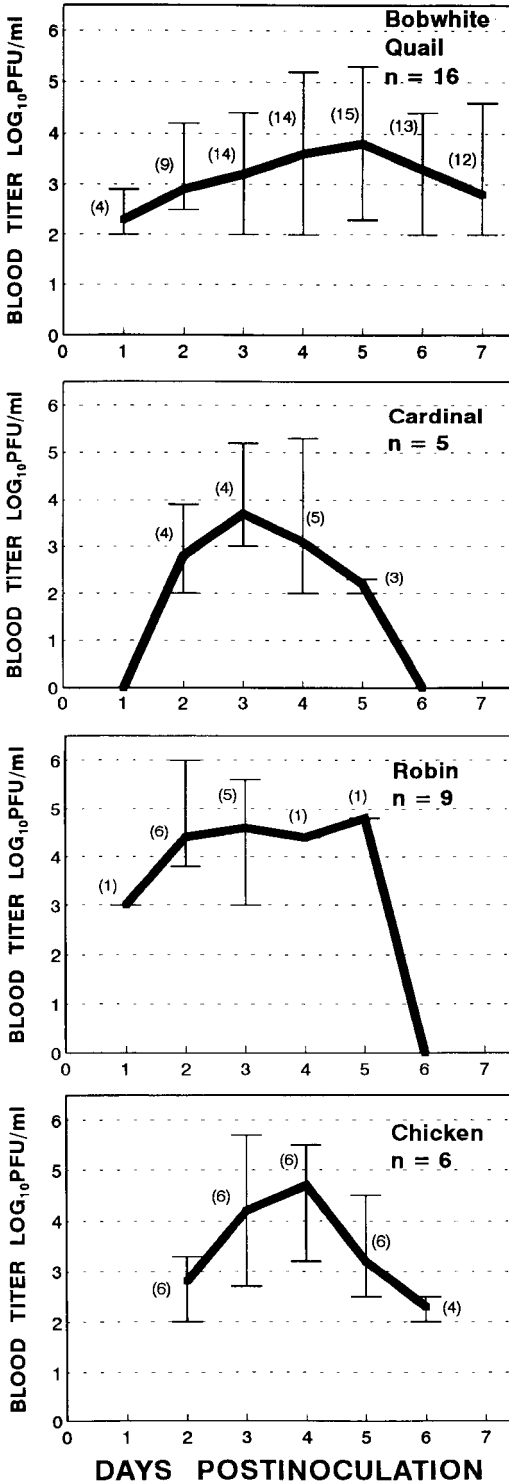
All birds of the 4 species inoculated with SLE virus became viremic. Robins and young chickens displayed the highest viremia titers, whereas bobwhite quail had the longest duration of viremia. Daily mean viremia titers in robins ranged between $10^{4.4}$ and $10^{4.8}$ PFU/ml on days 2–5 postinoculation, and daily mean titers in baby chicks of $10^{4.2}$–$10^{4.7}$ PFU/ml occurred on days 3–4 postinoculation. Daily mean titers in bobwhite quail and cardinals remained at values below $10^{4.0}$ PFU/ml, although individual birds experienced higher titers (Fig. 2).

DISCUSSION

These experiments demonstrate that *Ae. albopictus* is not highly susceptible to peroral infection with SLE virus. At blood meal titers of $10^{4.6}$–$10^{4.9}$ PFU/ml, only 3 of 313 (1.0%) of mosquitoes were infected. The low infection rate, combined with data on the titer of SLE virus in individual mosquitoes frozen immediately after bloodfeeding on infected hamsters, provides information on the infection threshold, that is, the amount of virus required to infect 1–5% of mosquitoes (Chamberlain et al. 1954). Virus ingested at these blood meal titers ranged from $10^{1.5}$ to $10^{2.7}$ PFU per mosquito, with a mean of $10^{2.3}$ PFU per mosquito ($n = 25$). In contrast, the infection threshold for 2 different *Cx. quinquefasciatus* populations from California, fed on 2 groups of viremic chicks inoculated with different SLE virus strains from California, was shown to be less than 1 PFU per blood meal (Meyer et al. 1983). In a similar study using cardinals as the blood meal source of SLE virus strain MSI-7 (McLean et al. 1985), the infection threshold for *Cx. quinquefasciatus* colony originating from Missouri was less than 2 PFU per blood meal. Thus, the amount of SLE virus required to infect 1–5% of *Ae. albopictus* specimens is at least 2 orders of magnitude greater than that required to infect a principal vector of SLE virus.

Although *Ae. albopictus* is not readily susceptible to infection, SLE virus replicates to high titers, $10^{6.3}$–$10^{7.0}$ PFU/mosquito ($n = 3$), following peroral infection, and infected *Ae. albopictus*

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Fig. 2. St. Louis encephalitis virus viremia profiles for 4 bird species. Bird species and sample size are provided on each graph. See the Materials and Methods section for information on age of birds, virus strain, and inoculation procedures. Blood titers are expressed in PFU in Vero cell culture per ml of blood. Daily values are the geometric mean, maximum, and minimum values for viremic birds. Number of viremic birds on each day is indicated in parentheses.

can transmit virus by bite at a rate of 33% based on our limited sample (Table 1). St. Louis encephalitis virus intracoelomically inoculated into *Ae. albopictus* replicated to high titers, $>10^{6.0}$ PFU/ml (Fig. 1). The replication of SLE virus to high titers in both intracoelomically inoculated and perorally infected specimens, combined with the low susceptibility to peroral infection, suggests the presence of a mesenteronal infection barrier (Hardy 1988).

The primary vertebrate hosts responsible for amplification of SLE virus in the United States are passeriform and columbiform birds. The American robin, house sparrow (*Passer domesticus*), and mockingbird (*Mimus polyglottos*) were implicated as the primary vertebrate amplifying hosts of SLE virus during the Pine Bluff epidemic (McLean et al. 1993). Vector species important in SLE virus transmission to humans must readily obtain an infectious dose of SLE virus from birds and subsequently transmit virus to humans after a suitable incubation period. *Aedes albopictus* has an estimated infection threshold for SLE virus of $10^{2.3}$ PFU which corresponds to a 5-μl blood meal from a host with a viremia level of $10^{4.6}$ PFU/ml. At host viremia levels of $10^{4.6}$–$10^{4.9}$ PFU/ml, only 1.0% of *Ae. albopictus* specimens ($n = 313$) became infected, and only one of 209 (0.5%) transmitted virus at 15 days post-bloodfeeding (Table 1). A small proportion ($<5\%$) of *Aedes albopictus* could acquire infectious doses of SLE virus from some adult birds of a number of bird species that are experiencing peak levels of viremia (McLean and Bowen 1980, Hardy and Reeves 1990, Fig. 2); however, less than peak viremia levels in most adult birds (McLean and Bowen 1980), including house sparrows (Bowen et al. 1980), would not be sufficient to infect an epidemiologically significant number of *Ae. albopictus*. Although SLE virus profiles for many relevant bird species, including the mockingbird, are lacking, the American robin appears to be one of the few species for which mean peak titers greater than $10^{4.6}$ PFU/ml are observed in adult birds (McLean et al. 1985, Fig. 2). Mean peak blood titers of $10^{4.6}$ PFU/ml in native North America animals are thought to occur commonly only in nestling birds, and mean peak titers at or above this level have been documented in 0.5–3-day-old chickens (Sudia and Chamberlain 1959, McLean et al. 1985, Fig. 2), and 5–10-day-old house sparrows (Bowen et al. 1980, Ludwig et al. 1986). Although peak SLE virus titers for individual adult birds for a number of important bird species reach titers sufficient to infect a small proportion of *Ae. albopictus*, the mean titers in adult birds for nearly all species that have been studied fall below the SLE virus infection threshold for *Ae. albopictus*.

Experimental studies of SLE virus infection in all native North American mammals studied thus far indicate that mean peak blood titers fall below the level of the infection threshold for *Ae. albopictus* (i.e., $10^{4.6}$ PFU/ml). However, experimental studies (McLean et al. 1985, Hardy and Reeves 1990) indicate that 4 species of rodents, the Nelson's antelope squirrel (*Ammospermophilus nelsoni*), least chipmunk (*Eutamias minimus*), cotton rat (*Sigmodon hispidus*), and the Fresno kangaroo rat (*Dipodomys nitratoides*), may obtain individual peak blood titers of $10^{4.3}$ to $10^{5.1}$ PFU/ml. Although these experimental results suggest that peak viremia levels in these mammals would be sufficient to infect a small proportion of *Ae. albopictus,* a natural role for these species in SLE virus transmission has never been demonstrated.

To assess the vectorial capacity of *Ae. albopictus* for SLE virus, data on infection and transmission rates, the infection threshold, and virus titers in the blood of vertebrate hosts must be combined with information on bloodfeeding habits. To date, blood meal hosts for *Ae. albopictus* have been analyzed at 9 eastern and midwestern sites (Savage et al. 1993a, Niebylski et al. 1994). Feeding on birds was documented at 4 of the 9 sites, and the percentage of positive feeds on birds at the 9 sites ranged from 0 to 16%. The average percentage of positive feeds on birds for the 429 specimens tested from all 9 sites was 7%. Knowledge of the biology and unique environmental features of the 9 study sites suggests that bird-feeding rates for *Ae. albopictus* will commonly fall between 0 and 4%. The combination of low susceptibility to infection and a mammalophilic bloodfeeding pattern suggests that *Ae. albopictus* is an unlikely vector for SLE virus, and is unlikely to play a significant role in SLE transmission in most areas. However, the opportunistic nature of bloodfeeding by *Ae. albopictus* may result in significant spatial and temporal variation in host utilization, and *Ae. albopictus* could become involved in the amplification and transmission of SLE virus in particular situations where large *Ae. albopictus* populations are present near a large number of nestling birds.

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