EXPERIMENTAL STUDIES TO DETERMINE THE SUSCEPTIBILITY TO INFECTION WITH ST. LOUIS ENCEPHALITIS VIRUS OF FIVE SPECIES OF PANAMANIAN MOSQUITOES

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ABSTRACT. The susceptibility to infection with a Panamanian isolate of St. Louis encephalitis (SLE) virus was evaluated in *Culex quinquefasciatus*, *Haemagogus equinus*, *Mansonia dyari*, *Sabethes cyaneus* and *Deinocerites pseudes*. When fed on blood-virus suspensions using the hanging drop or pledget technique, the median infective dose (ID_{50}) of SLE virus for colonized strains of *Cx. quinquefasciatus* and *Hg. equinus* was $10^{2.8}$ plaque forming units (PFU)/2 µl and $10^{3.5}$ PFU/2 µl, respectively. The susceptibility of F_1 and F_2 *Cx. quinquefasciatus* females was similar to the colonized strain by the same technique. When fed on viremic chicks, the ID_{50} for both *Cx. quinquefasciatus* and *Hg. equinus* was $10^{0.5}$ PFU/2 µl. These results and successful transmission trials indicate that both of these species have the potential to serve as vectors of SLE virus in Panama. Because of their poor feeding response, complete susceptibility profiles were not obtained for the other 3 species tested. They appear to be less susceptible than *Cx. quinquefasciatus* and *Hg. equinus*, although *Ma. dyari* was readily infected after engorging on a blood-virus suspension with a titer of $10^{3.0}$ PFU/2 µl.

INTRODUCTION

The ecology of St. Louis encephalitis (SLE) virus remains poorly defined in the tropical Americas. Although SLE virus has been isolated from a number of different mosquito species in this region, their importance as vectors remains unclear (Spence 1980, Tsai and Mitchell 1988). In Panama, SLE virus has been isolated from 7 genera of mosquitoes. Nine isolates have been made from *Mansonia dyari* (Belkin, Heinemann and Page), four from *Sabethes chloropterus* (Humboldt) and a single isolate each from *Haemagogus lucifer* (Howard), *Culex nigripalpus* Theobald, *Deinocerites pseudes* (Dyar and Knab), *Wyeomyia* spp., *Trichoprosopon* spp. and *Sabethes* spp. (Galindo et al. 1959, 1964; Grayson et al. 1967, Gorgas Memorial Laboratory, unpublished data). *Culex quinquefasciatus* Say, a well known vector of SLE in the eastern United States (Tsai and Mitchell 1988), also is abundant in Panama City (Galindo 1978). The importance of any of these mosquitoes as well as other possible vector mosquitoes in the transmission cycle of SLE in Panama has not been determined. Because of this situation, the present study was undertaken with several indigenous mosquito species to determine their susceptibility to infection with SLE virus.

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METHODS

Mosquitoes: The following colonized species were used in these experiments: *Cx. quinquefasciatus*, Panama City colony established in 1975; *Hg. equinus* Theobald, Maje colony established in 1975; *De. pseudes*, Playa Gorgona colony established in 1966; and *Sa. cyaneus* (Fabricius), Ipeti/Maje colony established in 1979. In addition to these colonies, we evaluated *Cx. quinquefasciatus* F_1 and F_2 females from Panama City and Juan Diaz, respectively, and F_1 *Ma. dyari* females from Rio Chagres.

Viruses: The strain of SLE virus used was PaAr 4336 isolated from *Ma. dyari* collected at Altos de Maje, Panama, in 1977. Two virus stocks were prepared: 1) a 10% suckling mouse brain (SMBr) suspension at the 2nd laboratory passage level, and 2) a mosquito suspension prepared by the parenteral inoculation of the original *Ma. dyari* pool directly into *Toxorhynchites theobaldi* (Dyar and Knab) adults. After holding for 12 days at 27°C, 80 inoculated *Tx. theobaldi* adults were triturated in 80 ml of medium 199 with 10% fetal calf serum. The supernatant from this suspension was used as the virus stock.

Infection of mosquitoes: To determine oral susceptibility, females from each mosquito species were exposed to viremic chicks and to SLE virus suspensions prepared in defibrinated chicken blood. In the latter technique, serial 10-fold dilutions of the SMBr virus stock were prepared in the defibrinated blood to determine the median infective dose (ID_{50}) for each species. After warming to 37°C, drops of the blood-virus suspension were placed directly on the nylon mesh tops of the cartons containing the females, or

small cotton gauze pledgets placed on the nylon mesh were soaked with the blood-virus suspension. The females were allowed to feed at 27°C for 1–2 hours. Pre- and postfeeding interval samples of the blood-virus suspensions were frozen at −70°C for later virus titration. All females that engorged during feeding were incubated for 14–21 days at 27°C and 80–90% RH, and were then frozen at −70°C.

To feed mosquitoes on viremic animals, 1-day-old chicks were inoculated intramuscularly with 0.1 ml of a 1:10 dilution of the *Tx. theobaldi* stock preparation. Female mosquitoes were exposed for 4 h to the chicks at different intervals after inoculation. Pre- and postfeeding blood samples were taken from each chick for viral assay. Females that engorged were incubated as before for 14–21 days and then frozen for viral assay.

Transmission trials: To determine transmission efficiency, infected mosquitoes were held from 8–29 days at 27°C and individually exposed to 1-day-old chicks. After exposure for 1–2 h, any females containing blood were frozen for assay. The chicks were bled at 24–36 h after removal from the cartons to detect viremia, and were rebled 2–3 weeks later to detect antibodies to SLE virus.

Virus and antibody assay: For viral assay, individual mosquitoes were triturated in 2 ml of medium 199 with 5% fetal calf serum and antibiotics and centrifuged at 1,000 rpm for 15 min. The supernatant was tested by plaque assay in Vero cell cultures (Earley et al. 1967). The ID_{50} of SLE virus for each mosquito species was calculated by the method of Spearman and Karber (Finney 1971). To determine the titers of blood-virus suspensions and chick bloods, serial 10-fold dilutions were prepared and inoculated on monolayers of Vero cells. The dose of SLE virus imbibed by each mosquito was calculated as the mean of the pre and post feeding titers/2 μl of the blood-virus suspensions or chick bloods. Antibodies to SLE virus in chick blood were measured by plaque reduction neutralization assay in Vero cell cultures (Earley et al. 1967), Seymour et al. 1983).

RESULTS

The ID_{50} for the 3 populations of *Cx. quinquefasciatus* were 2.5–7.9 fold lower than the ID_{50} for *Hg. equinus* when PaAr 4336 was ingested from hanging drops/pledgets (Table 1). The long established Panama City *Cx. quinquefasciatus* colony was as susceptible as the F_1 and F_2 progeny of wild females collected from the city or a nearby suburban area.

The ID_{50} of PaAr 4336 for the other 3 species of mosquitoes could not be determined. However, when *Ma. dyari* and *De. pseudes* females fed on a high titered blood-virus suspension ($10^{3.0}$ PFU/2 μl), 20/20 (100%) and 10/29 (34.5%) of the engorged females became infected, respectively. *Culex quinquefasciatus* females fed simultaneously on the same dose also had a 100% (19/19) infection rate. The susceptibility of *Sa. cyaneus* could not be determined because the females did not engorge.

Culex quinquefasciatus (F_1 Panama City) was more susceptible to infection with PaAr 4336 when infected by feeding on viremic chicks (Table 2) compared with feeding on blood-virus suspensions (Table 1). The ID_{50} by the chick feeding route was $10^{0.5}$ PFU/2 μl, almost 400 times less than that by pledget feeding. The ID_{50} of PaAr 4336 for *Hg. equinus* also was $10^{0.5}$ PFU/2 μl when the females engorged on viremic chicks. *Mansonia dyari, Sa. cyaneus* and *De. pseudes* did not become infected after engorging on viremic chicks; however, *Ma. dyari* were exposed only to chicks with low titered viremias (Table 3).

Table 1. Median infective dose (ID_{50}) of SLE virus* for *Culex quinquefasciatus* and *Haemagogus equinus* from Panama determined by feeding on blood-virus suspensions.

Species**	History	ID_{50} ± SE
Cx. quinquefasciatus		
Panama City	Colony	2.8 ± 0.14***
Panama City	F_1	3.1 ± 0.10
Juan Diaz	F_2	2.6 ± 0.15
Hg. equinus	Colony	3.5 ± 0.12

* Panamanian strain of SLE virus (PaAr 4336) isolated from *Ma. dyari*, 1977.

** For each serial virus dilution used, at least 20 females that had engorged were assayed.

*** Log^{10} PFU/2 μl.

Table 2. Susceptibility of *Culex quinquefasciatus* to infection with SLE virus by feeding on viremic chicks.

Titer	No. positive/total	% positive
2.0–2.3*	34/171	19.9
2.4–3.0	23/62	37.1
3.2–3.8	32/46	69.6
4.4–5.0	77/93	82.8
5.5–5.8	34/35	97.1

* Log_{10} PFU/ml of blood.
$ID_{50} = 10^{0.5}$ PFU/2 μl.

Table 3. Susceptibility of 4 species of Panamanian mosquitoes to SLE virus by feeding on viremic chicks.

Species	Titer of chick blood	No. positive/ total	% positive
Hg. equinus	2.6–3.0*	1/100	1.0
	3.4–4.3	31/35	88.6
	4.5–5.4	38/43	88.4
Ma. dyari	2.3–2.8	0/70	0
Sa. cyaneus	2.9–3.5	0/99	0
De. pseudes	2.5–4.5	0/44	0

* Range of the mean pre- and postfeeding titers expressed as Log_{10} PFU/ml of blood.

Culex quinquefasciatus efficiently transmitted SLE virus to chicks by 16 days post-feeding (Table 4). *Haemagogus equinus* infected by feeding on a viremic chick ($10^{1.5}$ PFU/2 μl) also were able to transmit SLE virus (5/5 females) after 16 days incubation. Transmission ability was not evaluated for the other 3 species.

DISCUSSION

Although SLE virus has not been isolated from either *Cx. quinquefasciatus* or *Hg. equinus* in Panama, this study shows that both species could be vectors. Poor feeding responses did not allow as complete an evaluation of the other 3 species, but the data obtained suggest that they are less susceptible to infection with SLE virus. *Culex quinquefasciatus* has been identified as an important vector of epidemic SLE virus in the Eastern United States where it is involved in an urban transmission cycle with wild birds as the amplifying host (Tsai and Mitchell 1988), and it may play a similar role in Panama. Although epidemics have not been reported, approximately 7% of Panama City residents have been found seropositive to SLE virus (Galindo 1978). St. Louis encephalitis virus also has been isolated from wild birds captured in urban areas of Panama (Galindo et al. 1964, Galindo 1978).

Haemagogus equinus could serve as a vector of SLE virus in the Panamanian forest where infection has been documented in man, wild mammals and birds (Galindo et al. 1959, Rodaniche and Johnson 1961, Rodaniche and Galindo 1961, Seymour et al. 1983). This is the most ubiquitous *Haemagogus* species in the forest and feeds readily on man and a number of forest dwelling vertebrates (Galindo et al. 1950, Galindo et al. 1951, Trapido et al. 1955). *Haemagogus equinus* also could serve as a disseminating vector to transfer SLE virus from an enzootic forest cycle into the urban habitat since it lives in cleared areas on the forest periphery and has been reported from peridomestic habitats (Galindo et al. 1951, Trapido and Galindo 1956).

The vector competence studies reported herein used laboratory models for the unknown natural vertebrate hosts. The hanging drop/pledget technique may not accurately estimate the susceptibility of a vector (Turell 1988). Both *Cx. quinquefasciatus* and *Hg. equinus* were less susceptible when fed by this method compared with feeding on viremic chicks. In a previous study, however, colonized females from a Panama population of *Cx. quinquefasciatus* also were found to be less susceptible to SLE virus infection when fed on viremic sloths, *Choloepus hoffmanni* and *Bradypus variegatus*, which are suspected natural hosts of SLE virus in Panama, compared with viremic chicks (Seymour et al. 1983). Unfortunately, a direct comparison between the level of susceptibility found in that experiment and in our experiment cannot be made because different assay systems were used to determine viral titers.

An adequate dose-response profile was not obtained for *De. pseudes*, *Sa. cyaneus* or *Ma. dyari* with either technique. However, 100% of the *Ma. dyari* females that fed on a high titered blood-virus suspension did become infected, and presumably would be just as susceptible if exposed to a viremic host. This laboratory finding supports field data implicating this species as an epizootic SLE virus vector in Panama under appropriate ecological conditions. The 9 SLE virus isolates from wild *Ma. dyari* females were obtained at a dry tropical forest site over a period of a few months in 1977 when seroconversions also were recorded in sentinel animals and anti-SLE virus antibody rates were high in wild mammals and birds (Gorgas Memorial Laboratory, unpublished data). Ecological changes at this site, caused by the formation of a large man-made lake, resulted in a tremendous population explosion of *Ma. dyari* during the time of the SLE virus outbreak.

The vector competence of other species from which SLE virus has been isolated in Panama

Table 4. Transmission of SLE virus to 1-day-old chicks by infected *Culex quinquefasciatus* (Panama City colony)* after different incubation periods at 27°C.

Days post-infection	No. transmitting/ no. feeding	% transmitting
8	0/9	0
16	20/22	91
26–29	11/12	92

* Females were infected by feeding on a blood-virus suspension (10^6 PFU/2 μl) or on a viremic chick ($10^{0.8}$ PFU/2 μl).

needs to be determined. *Culex nigripalpus* is an important vector in Florida, and SLE virus has been isolated from this species in other Latin American countries (Tsai and Mitchell 1988). Although only a single SLE virus isolate has been obtained from this mosquito in Panama, it is abundant in coastal lowland areas where SLE activity has been documented in sentinel animals (Gorgas Memorial Laboratory, unpublished data). Both *Hg. lucifer* and *Sa. chloropterus* have been strongly implicated in the tropical forest transmission cycle of yellow fever virus in Panama (Galindo 1979) and may play a similar role in SLE virus transmission, particularly among arboreal vertebrates. Because SLE virus transmission in Panama has been documented in environments as ecologically different as tropical rain forest and coastal lowland, several mosquito species probably are important vectors.

ACKNOWLEDGMENTS

We thank Layla Perez and Dora Tablate for technical assistance. This research was partially funded by grant AI-16433 from the National Institute of Allergy and Infectious Diseases.

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