PRELIMINARY STUDIES OF *AEDES BAHAMENSIS* AS A HOST AND POTENTIAL VECTOR OF ST. LOUIS ENCEPHALITIS VIRUS[1]

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ABSTRACT. *Aedes bahamensis*, a recent introduction to the state of Florida, was studied to determine its capability to serve as a host or vector of St. Louis encephalitis (SLE) virus. Females were readily infected by inoculation, and had whole body virus titers as high as $10^{9.4}$ mosquito infectious $doses_{50}$. Sixty percent of females that had been infected by inoculation were capable of orally transmitting virus. Nearly half of the females feeding on viremic chickens became infected, but not all of these infections disseminated to the head. Vertical transmission of SLE virus to progeny of inoculated females was also demonstrated.

INTRODUCTION

The peridomestic mosquito *Aedes (Howardina) bahamensis* Berlin, known previously only from the Bahamas, has invaded south Florida and has an expanding distribution (Pafume et al. 1988, O'Meara et al. 1989). This mosquito utilizes tires and other artificial containers as larval habitats in Florida. Females are commonly autogenous for their initial gonotrophic cycle, but subsequent gonotrophic cycles are not initiated without blood feeding (O'Meara et al. 1989). The species is known to feed on man (O'Meara et al. 1989).

An earlier study of *Ae. bahamensis* (incorrectly referred to as *Aedes albonotatus* (Coquillett) in that paper, which predated the formal description of *Ae. bahamensis*) concluded that chikungunya and dengue-2 viruses did not persist or replicate to high titer after parenteral inoculation (Llewellyn et al. 1970). Because *Ae. bahamensis* now inhabits a part of Florida that is endemic for St. Louis encephalitis (SLE) virus, a pilot study was undertaken to assess the vector competence of *Ae. bahamensis* for this virus. This study was also motivated by a desire to establish whether or not the reported insusceptibility of *Ae. bahamensis* to parenteral infection with dengue-2 virus was indicative of a general resistance to flaviviruses.

MATERIALS AND METHODS

Mosquitoes: The *Ae. bahamensis* used were colonized from mosquitoes collected in Broward and Dade counties, FL, and were used in laboratory generations 2 and 3. After oviposition of autogenous eggs, females were allowed to feed on restrained chickens to initiate additional egg production. Rearing was at 28°C and a 15:9 L:D photophase plus 45-min crepuscular periods. Mosquitoes used as bioassay hosts were laboratory-reared *Culex quinquefasciatus* Say, DISNEY strain, which originated from mosquitoes collected in Orange Co., FL.

Virus: The SLE virus strain used (77V-12908) was isolated from Florida *Cx. nigripalpus* Theobald during an epidemic period in 1977. It had experienced one passage each in suckling mice, C6/36 *Aedes albopictus* (Skuse) cells and *Toxorhynchites amboinensis* (Doleschall) mosquitoes.

Virus assay: Individual female mosquitoes that had been infected by ingesting viremic blood were first examined by indirect immunofluorescent staining (IFAT) of head squashes (Kuberski and Rosen 1977). Anti-SLE hyperimmune mouse ascitic fluid from the National Institutes of Health and commercial fluorescein isothiocyanate-conjugated goat anti-mouse gamma globulin (Antibodies, Inc., Davis, CA) were used in the IFAT. Body remnants of individuals with SLE-negative heads were then triturated in 0.5-ml diluent (phosphate buffered saline + 25% heat-inactivated calf serum + 0.5% gelatin + 600 units/ml penicillin + 600 mg/ml streptomycin) and centrifuged for 5 min at 10,000 × g. Supernatant fluids were assayed for virus. All assays and titrations were accomplished by parenteral inoculation of *Cx. quinquefasciatus* females, head squashes of which were examined by IFAT after 10–12 days incubation at 28° C. Five head squashes were examined per inoculum. Titration endpoints were calculated by the method of Karber (Hawkes 1979). Mosquitoes have been used extensively as bioassay hosts in other studies (e.g., Gubler et al. 1979, Rosen 1981), an approach that is especially useful for flaviviruses such as SLE. *Culex* vector species have been shown to be as suitable for SLE virus assay as *Toxorhynchites*

[1] This study was conducted in institutionally approved facilities, in accordance with the *Guide for the Care and Use of Laboratory Animals*, as promulgated by the National Research Council. University of Florida, Institute of Food and Agricultural Sciences, Experiment Station Journal Series No. R-00331.

amboinensis, and more sensitive than plaque assay in Vero cell cultures (Hardy et al. 1984; D. A. Shroyer, unpublished data). *Aedes bahamensis* females with SLE-negative heads, but with detectable virus in body remnants, were scored as "infected-nondisseminated"; mosquitoes with SLE-positive heads were scored as "infected-disseminated."

Infection of mosquitoes: Mosquitoes were infected by inoculation (Rosen and Gubler 1974) or by feeding on viremic baby chickens. Exposures to infected chicks were timed to coincide with estimated periods of peak viremia. Chick viremia was determined by allowing 15 *Aedes aegypti* (Linn.) females to engorge on each chick during a 30-min period immediately before exposure to test *Ae. bahamensis.* This approach to measurement of host viremia has been used by others (Gubler 1981, Mitchell et al. 1983). Fully engorged *Ae. aegypti* were frozen at −85° C immediately after feeding; 5 females were later thawed and triturated together in 0.5-ml diluent, then titrated in *Cx. quinquefasciatus* hosts. Chick blood titers were calculated by assuming that the *Ae. aegypti* ingested 4 μl of blood (Klowden and Lea 1980); calculated logarithmic titers are relatively insensitive to errors in estimated blood meal volume.

Aedes bahamensis females tested for oral susceptibility were sugar-starved 1–2 days and allowed to deposit autogenous eggs before feeding on viremic chicks. Females were exposed to restrained viremic chicks for a period of 16–17 h. Engorged *Ae. bahamensis* were then separated from unengorged mosquitoes by aspiration (Shroyer 1989) and incubated at 28° C until being killed for assay or tested for ability to transmit by bite.

RESULTS AND DISCUSSION

Of 40 *Ae. bahamensis* females inoculated with $10^{3.8}$ mosquito infectious $doses_{50}$ (MID_{50}) of SLE virus, all possessed SLE-positive head squashes by day 14. A sample of 3 of these females had geometric mean virus content of $10^{8.8}$ MID_{50}/mosquito (range = $10^{8.2}$–$10^{9.4}$). After *Ae. bahamensis* females were allowed to engorge on viremic baby chickens, they were held 14 days before their heads were examined by IFAT. Figure 1 shows that some individuals developed disseminated infections at each of the oral input titers used.

To determine whether mosquitoes with disseminated infections could transmit virus by bite, females were inoculated with SLE virus and tested 16 days later for ability to transmit by an *in vitro* "capillary tube" method (Beaty and Aitken 1979). Of 20 females so tested, 60%

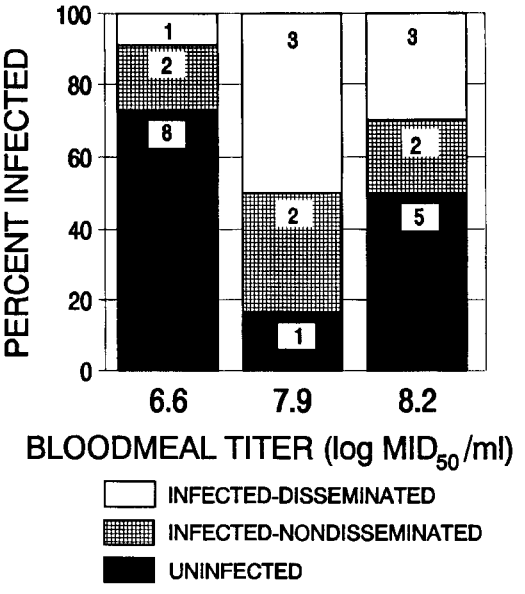

Fig. 1. Oral susceptibility of *Aedes bahamensis* to SLE virus. Numbers within each bar chart section represent sample size.

transmitted virus. Transmission was not evaluated for females with natural disseminated infections established by the oral route, because few females could be induced to engorge on viremic chickens in each experiment, and only some of these subsequently became infected.

One additional experiment shed further light on the suitability of *Ae. bahamensis* as an SLE virus host. After parenteral infection of 148 females 3–8 days post-emergence, autogenous eggs were collected from an unknown number of these females 10–14 days later. Larvae hatched from these eggs were reared at 28° C, and 641 larvae were split into pools of 50–91 when most were 4th instars. Each pool was rinsed in tap water and triturated in 1.0-ml diluent for assay. Of 12 pools tested, one pool of 91 larvae contained at least one vertically infected mosquito; the estimated filial infection rate of all larvae tested was 0.17%, method of Le (1981). This infection rate is somewhat higher than has been reported from *Culex* species (Francy et al. 1981, Nayar et al. 1986).

These results demonstrated that (a) the tested SLE virus strain grew to high titer in *Ae. bahamensis* after parenteral infection, indicating that this species is not refractory to infection with all flaviviruses; (b) *Ae. bahamensis* females were infected by the oral route; (c) most parenterally infected females with disseminated infections transmitted virus by bite, suggesting that some orally infected females probably would be capable of transmission; and (d) SLE virus was ver-

tically transmitted by *Ae. bahamensis* females. Despite the small sample sizes available for each oral susceptibility experiment, it appears that susceptibility of *Ae. bahamensis* is markedly less than normally found in Florida *Cx. nigripalpus* or *Cx. quinquefasciatus*, which routinely display infection rates >95% after ingesting comparable virus doses of the same virus strain (D. A. Shroyer, unpublished data). More exhaustive studies would be needed to assess precisely the oral susceptibility of *Ae. bahamensis*, and to determine whether orally infected females are capable of transmitting virus by bite. Host preference and other biological characteristics of *Ae. bahamensis* are incompletely known, but a reluctance to feed on birds has been noted (Spielman and Feinsod 1979). Reluctance to feed on chickens also contributed to the small sample sizes in the oral susceptibility experiments described above. This preliminary evaluation suggests that *Ae. bahamensis* is not an efficient horizontal transmitter of SLE virus. However, additional biological and behavioral studies would be necessary to determine whether vector competence of this species is sufficient for it to serve as a secondary vector of SLE virus in nature.

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