Susceptibility Parameters of *Aedes albopictus* to Per Oral Infection with Eastern Equine Encephalitis Virus

CARL J. MITCHELL, ROBERT G. McLEAN, ROGER S. NASCI, WAYNE J. CRANS,[1] GORDON C. SMITH, AND DONALD F. CACCAMISE[1]

Medical Entomology–Ecology Branch, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, P.O. Box 2087, Fort Collins, CO 80522

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ABSTRACT *Aedes albopictus* (Skuse) mosquitoes were fed on snowy egrets, *Egretta thula* (Thayer and Bangs), that had been infected by subcutaneous inoculation of eastern equine encephalitis (EEE) virus. Freshly fed mosquitoes were frozen and tested to determine how much virus they had ingested. Other fed mosquitoes from the same lots were incubated for 7 d at 27°C before testing. Seven lots of *Ae. albopictus* fed on viremic birds. Based on average amounts of virus ingested and day 7 virus infection rates in mosquitoes from the same lots, the amount of virus required to infect 50% of the mosquitoes was calculated to be $10^{2.8}$ Vero cell plaque-forming units (PFU). The infection threshold (i.e., the amount of virus required to infect from 1 to 5% of mosquitoes) was determined to be ≤10 PFU per blood meal. These parameters indicate that *Ae. albopictus* is sufficiently susceptible to infection with EEE virus to enable it to acquire infectious doses from a wide variety of viremic birds and possibly from equines.

KEY WORDS *Aedes albopictus*, eastern equine encephalitis, arbovirus

THE RECENT ISOLATION of 14 strains of eastern equine encephalitis (EEE) virus from *Aedes albopictus* (Skuse) collected in Polk County, FL, has increased concerns that the mosquito may become an epizootic and epidemic vector of EEE virus (Mitchell et al. 1992). Previously, Scott et al. (1990) showed that a strain of *Ae. albopictus* from Houston, TX, could become infected with EEE virus by feeding on viremic chicks and could transmit the virus by bite in the laboratory. In that study, the EEE virus titers in the infectious bloodmeal were $>10^8$ baby hamster kidney tissue culture 50% infective doses (BHK $TCID_{50}$). Consequently, 100% of the mosquitoes that fed became infected and no information was obtained on the threshold of infection (i.e., the lowest concentration of virus capable of causing an infection in ≈1–5% of specimens ingesting it [Chamberlain et al. 1954]), or on the virus dose required to infect 50% of the mosquitoes (ID_{50}).

We had the opportunity to feed *Ae. albopictus* on snowy egrets, *Egretta thula* (Thayer and Bangs), from New Jersey that had been inoculated with EEE virus for determination of viremia profiles (R.G.M., personal communication). Our objective was to determine whether *Ae. albopictus* could become infected by feeding on snowy egrets with various levels of viremia. Our results shed light on the infection threshold and on the ID_{50}.

Materials and Methods

Snowy egrets 6–8 wk old were inoculated subcutaneously with $10^{2.9}$ to $10^{5.2}$ Vero cell plaque-forming units (PFU) of EEE virus (strain NJO/60). A Lake Charles, LA, strain of *Ae. albopictus* used in the feeding trials was colonized in 1987 and field material was added to the colony annually from 1988 to 1991. Larvae were reared at a temperature of 27°C, a photoperiod of 9:15 (L:D) h, and were fed a standard diet of liver powder. Adults were provided with 5% sucrose from the time of emergence until the evening before blood feeding. Females used in the feeding trials were 5–17 d old.

Mosquitoes were allowed to feed in groups of 80–100 by placing them in a mosquito cage that contained a single egret restrained by taping the bird's legs to its body. The beak also was taped to prevent the bird from eating the mosquitoes. Mosquitoes were allowed to feed for 2 h 15 min, or less, if large numbers engorged sooner. Five freshly fed *Ae. albopictus* from each lot were frozen at −70°C at the end of each feeding period and subsequently tested to determine the amount of virus ingested. The remaining engorged mosquitoes were transferred to clean cages, given 5% sucrose, and incubated for 7 d at

[1] Department of Entomology, Rutgers University, New Brunswick, NJ 08903.

Table 1. EEE virus infection rates and representative titers in *Ae. albopictus* immediately after feeding and on day 7 after feeding

Egret host	Freshly fed		Day 7 after feeding		
	No. positive/ no. tested	Avg amount of virus ingested[a]	No. Positive/ no. tested	% Positive	Representative virus titers[b]
2,042	1/5	≤1.0	1/34	2.9	4.1
127	5/5	2.7	19/40	47.5	4.2, 5.0, 5.3
149	5/5	3.1	26/43	60.5	5.0, 5.3, 6.2
2,050[c]	5/5	1.9	11/47	23.4	5.0, 5.8, 6.2
126	2/5	≤1.0	1/41	2.4	6.7
128	5/5	1.4	0/19	0	—
2,050[c]	1/5	≤1.0	0/29	0	—

[a] Log_{10} Vero cell PFU/female; only positive females included.
[b] Log_{10} Vero cell PFU/female.
[c] Mosquitoes were fed on this host on 2 d successively.

a temperature of 27°C, a photoperiod of 9:15, and 85% RH.

Freshly fed mosquitoes and mosquitoes that had been incubated for 7 d were processed and tested for virus using techniques previously described (Mitchell et al. 1990). Briefly, mosquitoes were disrupted by sonication in 1 ml each of BA-1 diluent. The suspensions were clarified by centrifugation and supernatants were stored in screw-cap vials at −70°C until tested. Specimens were screened for virus and titrated by plaque assay in Vero cell culture.

Results

Nine lots of *Ae. albopictus* were fed on eight egrets; one egret was used as a host on successive days. Virus was detectable in at least some freshly fed mosquitoes in seven of the nine lots and ranged from an average dose of ≤10–1,360 PFU per female in each lot. Average EEE virus titers in freshly fed mosquitoes, day 7 postfeeding infection rates, and representative virus titers are summarized in Table 1. Based on average amounts of virus ingested and day 7 virus infection rates, the ID_{50} is $10^{2.8}$ PFU (95% confidence limits: $10^{2.6}$–$10^{3.1}$) as determined by probit analysis. The infection threshold is ≤10 PFU per blood meal. Representative virus titers in mosquitoes on day 7 postinfection ranged from $10^{4.1}$–$10^{6.7}$ PFU per mosquito.

Discussion

The ID_{50} of $10^{2.8}$ PFU, based on an estimated blood meal volume of 5 μl, is equivalent to a titer of $10^{5.1}$ PFU/ml. Such titers are occasionally attained by experimentally infected large birds such as the white ibis, *Eudocimus alba* (Linnaeus) (Kissling et al. 1954a) and the snowy egret (R.G.M., personal communication, and this study) and are frequently exceeded in a variety of passerine birds (Kissling et al. 1954a). Moreover, viremia titers in experimentally infected equines sometimes approach or exceed $10^{5.1}$/ml (Kissling et al. 1954b, Sudia et al. 1956, Byrne et al. 1964). In fact, Sudia et al. (1956) found that a virus titer of $10^{5.2}$ mouse intracerebral LD_{50} units in an experimentally infected horse was sufficient to infect 41% of *Aedes sollicitans* (Walker) that fed upon it. The infection threshold for *Ae. sollicitans* was estimated to be $\approx 10^{3.2}$ mouse intracerebral LD_{50} units.

Our results, demonstrated that *Ae. albopictus* has an infection threshold and ID_{50} comparable with that of *Ae. sollicitans*, a probable epidemic vector of EEE virus in coastal New Jersey (Crans et al. 1986). Our results also showed that EEE virus can replicate to high titer ($10^{6.7}$ PFU) in *Ae. albopictus* following ingestion of small amounts of virus (≤10 PFU) by the mosquito. Although we did not demonstrate EEE virus transmission by bite in the current study, the relatively high titers achieved 7 d after ingesting small quantities of virus suggest that such transmission probably was possible at least by some of the infected mosquitoes. The mammalophilic feeding habits of *Ae. albopictus* (Tempelis et al. 1970, Savage et al. 1993), its low threshold of infection for EEE virus, and demonstrated EEE viremia levels in equines (Kissling et al. 1954b, Sudia et al. 1956, Byrne et al. 1964) make *Ae. albopictus*-equine-*Ae. albopictus* transmission of EEE virus a distinct possibility. Certainly, the per-oral susceptibility of *Ae. albopictus* to infection with EEE virus makes it possible for this mosquito to acquire infectious doses of virus from a wide variety of avian hosts.

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