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Infection of *Mansonia perturbans* and *Psorophora ferox* Mosquitoes with Venezuelan Equine Encephalomyelitis Virus. (22216)

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Past studies have indicated Venezuelan equine encephalomyelitis (VEE) to be a mosquito-borne disease. In Trinidad, Gilyard(1) transmitted VEE from donkey to donkey by bite of *Mansonia titillans* (Walker). In Ecuador, Levi-Castillo(2) reported transmission by *Aedes taeniorhynchus* (Wied.), *M. titillans,* and *Culex quinquefasciatus* (Say). Recent work in the United States(3-5) has shown *A. triseriatus* (Say) to transmit efficiently in the laboratory.

The present report concerns studies on the VEE vector potentials of two additional species of mosquitoes, *M. perturbans* (Walker) and *Psorophora ferox* (Humboldt).

Materials and methods. The strain of VEE virus used, and methods of virus titration and of handling, feeding, incubating, and processing the mosquitoes were the same as reported previously(3,4). The *M. perturbans* were collected alive in Louisiana as adults, and were used in experiments 5 days later. The *P. ferox* were reared from larvae collected near Montgomery, Ala., and were 7-10 days old when used. Adult guinea pigs inoculated intraperitoneally with 1,000-10,000 mouse LD_{50} of virus 48 hours previously served as sources for mosquito infection. After incubation at 80°F, the mosquitoes were refed individually upon normal 3-week-old guinea pigs to determine their ability to transmit infection by bite.

Results. M. perturbans. Forty females engorged upon blood having a mouse intraperitoneal virus titer of $10^{8.4}$. A total of 16 egg rafts were laid by these specimens 6-13 days later. These rafts were ground in 2 ml of diluent each and titrated for virus. On the 13th day of incubation 16 of 29 mosquitoes remaining alive refed individually upon normal guinea pigs. These mosquitoes, as well as those which did not refeed, were then titrated to determine the amount of virus each contained. Table I summarizes the infection and transmission data.

P. ferox. Fifty females were fed upon blood containing virus titering $10^{6.3}$. Several hundred eggs laid 6-11 days later were pooled, ground in 2 ml of diluent, and inoculated into mice to test for virus.

On the 14th day of incubation the surviving mosquitoes were given opportunity to refeed individually upon normal guinea pigs. Subsequently, all mosquitoes were titrated to determine the amount of virus contained per

TABLE I. Infection of *M. perturbans* and *P. ferox* with VEE Virus.

Species	Titer of virus in blood ingested (mouse i.p. LD_{50})	% mosquitoes infected	Mouse i.p. LD_{50} contained/infected mosquito	% mosquitoes transmitting by bite	Virus recovered from mosquito eggs
M. perturbans	$10^{8.4}$	83 (24/29)	$10^{8.9}$ to $10^{6.0}$	38 (6/16)	+, 7 of 16 rafts*
P. ferox	$10^{6.3}$	84 (32/38)	$10^{1.5}$ to $10^{5.2}$	11 (2/18)	—

* No more than 300 mouse i.p. LD_{50} of virus were recovered from any of the rafts.

specimen. Infection and transmission results are included in Table I.

Discussion. The finding of virus in 7 of 16 *M. perturbans* egg rafts is of interest but as yet of unknown significance. Inability to rear this species in the larval stage prevented determining whether a second generation of infected females could be reared from these eggs. A similar study with eastern equine encephalomyelitis in *M. perturbans* has shown 2 out of 19 egg rafts laid following an infected meal to contain virus(6). It is possible, of course, that the virus may have been located only on the surface of the eggs despite rinsing with serum-saline solution. However, these findings are reported here in the hope that they may stimulate a more complete investigation.

The infection and transmission rates show *M. perturbans* and *P. ferox* to be susceptible to VEE infection and capable of transmitting it. Both of these species are abundant and widely distributed in the United States and are fierce biters upon man. Therefore, they must be considered as potentially dangerous vectors of VEE infection should this virus be introduced into the United States.

Summary. Two species of mosquitoes, *Mansonia perturbans* (Walker) and *Psorophora ferox* (Humboldt), were allowed to feed upon guinea pigs infected with the virus of Venezuelan equine encephalomyelitis. After 2 weeks incubation at 80°F, the infection rate and guinea pig transmission rate for *M. perturbans* were found to be 83% and 38%, respectively, and for *P. ferox*, 84% and 11%, respectively. Virus was isolated from 7 of 16 *M. perturbans* egg rafts, but not from the eggs of *P. ferox*. The significance of the infected eggs is still unknown.

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