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INFECTION AND TRANSMISSION THRESHOLDS OF EASTERN ENCEPHALITIS VIRUS TO *AEDES AEGYPTI* AS DETERMINED BY A MEMBRANE FEEDING TECHNIQUE

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The use of a membrane feeding technique to determine infection and transmission thresholds of Semliki Forest virus to mosquitoes has been previously reported (Collins *et al.*, 1964). The application of this technique to other mosquito virus systems would appear indicated.

Reported here are the results of studies with *Aedes aegypti* mosquitoes and eastern encephalitis virus (EEE) using the membrane feeding technique.

MATERIALS AND METHODS. The eastern encephalitis virus (EEE strain NJO-60), was obtained through the courtesy of Dr. Telford Work, Communicable Disease Center, Atlanta, Georgia.

The *A. aegypti* mosquitoes (CD strain) were originally obtained from Technical Development Laboratories, Communicable Disease Center, Savannah, Georgia.

Mosquitoes were infected by allowing them to feed through a Baudruche (untreated) membrane on serial 10-fold dilutions of EEE in fresh heparinized rabbit blood. The brains of six moribund mice previously inoculated with EEE, NJO-60, MP-4, were ground in four milliliters of Bacto heart infusion broth (Difco) and centrifuged for 15 minutes at 1500 r.p.m. Serial 10-fold dilutions of the supernatant were made in broth. For mosquito feeding, one part of each dilution was added to four parts of blood. This was warmed to 37° C. and placed on the membrane which formed the bottom of a ½ pint feeding cup. The feeding period was 15 minutes, after which time the engorged mosquitoes were transferred to holding cages and stored in an incubator at 25°C. to 26° C. The mosquitoes were fed five percent Karo solution daily on a cotton pledget.

After 11 and 25 days of extrinsic incubation, mosquitoes were allowed to feed individually on wet baby chicks. Approximately 24 hours later, blood samples were taken by cardiac puncture and the blood inoculated intracerebrally into five mice. Presence of virus in the chick blood constituted evidence of virus transmission by the mosquito.

Samples of mosquitoes were collected and killed by freezing immediately after initial feeding and after varying periods of extrinsic incubation. These were stored in a mechanical freezer at —65° C. to

—70° C. until titrated. To determine virus titers, mosquitoes were ground individually in a mortar with a one milliliter aliquot of Bacto-heart infusion broth containing 1000 units of penicillin and two milligrams of streptomycin per milliliter. The suspension was centrifuged for 15 minutes at 1500 r.p.m. and serial 10-fold dilutions were made in the broth. Five three-week old mice were inoculated intracerebrally per dilution and the LD_{50}s calculated by the method of Reed and Meunch (1938).

RESULTS. The relationship between virus ingested by *A. aegypti* and that present after 11, 25 and 32 days of extrinsic incubation is shown in Table 1. On day 11, the mean titer of virus ingested which gave initial infection (infection threshold) was 4.5 mouse log IC LD_{50}. On day 32, however, one mosquito was positive which had fed on the EEE virus pool which gave a mean initial titer of 3.4 mouse log IC LD_{50}. Based on the results of all three days examined, the 50 percent infection threshold was at approximately 4.9 mouse log IC LD_{50}.

The quantity of virus present in the infected mosquitoes indicated only minor variation in these levels on the three different days. It is of interest that once infected, these mosquitoes had comparable virus titers even though the mean quantity of virus ingested ranged from 3.4 to 7.4 mouse log IC LD_{50}.

The results of the transmission studies are shown in Table 2. In these tests, a total of 16 mosquitoes transmitted EEE virus. The transmission threshold was equal to or less than 4.9 mouse log IC LD_{50}. The titers of the transmitting mos-

TABLE 1.—Relationship between eastern encephalitis virus ingested by *Aedes aegypti* mosquitoes and that present after 11, 25 and 32 days of extrinsic incubation.

Initial EEE Ingested		Days Post-Infection	Pos./Tested	% Infected	Positive EEE Titers (Mouse log IC LD_{50})
Mean Titer	Individual Titers				
7.4	8.0, 7.7, 7.2, 7.0, 7.0	11	9/12	75	6.9, 6.8, 6.4, 6.1, 6.0, 6.0, 5.9, 5.5, 5.2
		25	9/10	90	6.5, 6.3, 6.1, 6.1, 6.0, 5.5, 5.3, 4.8, 4.7
		32	4/4	100	6.8, 5.0, 5.0, 4.4
6.1	6.8, 6.2, 6.1, 5.9, 5.7	11	8/12	67	7.0, 6.7, 6.0, 6.0, 6.0, 5.3, 5.1, 3.9
		25	9/10	90	6.3, 6.1, 6.1, 6.0, 6.0, 5.9, 5.7, 5.3, 5.1
		32	9/10	90	6.8, 6.0, 5.3, 5.2, 5.1, 5.0, 4.8, 4.7, 4.1
4.9	5.3, 5.1, 5.0, 4.8, 4.1	11	5/10	50	6.8, 6.7, 6.3, 6.3, 6.0
		25	4/10	40	6.2, 6.2, 6.0, 5.5
		32	6/10	60	5.9, 5.9, 5.5, 5.5, 5.3, 5.2
4.5	4.9, 4.5, 4.5, 4.3, 4.1	11	2/10	20	5.5, 5.5
		32	1/10	10	5.7
3.4	4.0, 3.8, 3.1, 3.1, 2.8	11	0/10	0	
		32	1/10	10	5.0

TABLE 2.—Effect of initial EEE titer on virus transmission by *Aedes aegypti*.

Initial EEE Ingested	Day 11		Day 25	
	Trans./ Attempts	EEE titer in Trans. Mosq.	Trans./ Attempts	EEE titer in Trans. Mosq.
7.4	3/12	6.8, 6.4, 6.1	5/10	6.5, 6.1, 6.1, 6.0, 4.7
6.1	3/12	7.0, 6.7, 6.0	3/10	6.1, 6.0, 5.3
4.9	1/10	6.3	1/10	5.5

quitoes ranged from 4.7 to 7.0 mouse log IC LD_{50}.

DISCUSSION. The EEE infection thresholds in *A. aegypti* were studied by Chamberlain *et al.* (1954) using either infected suspensions of chick embryo or mouse brain sweetened with 1 gram of sugar per 8.0 milliliters, or upon animals circulating various concentrations of virus in their blood. The *A. aegypti* animal blood virus infection threshold was around $10^{3.0}$ and the 50 percent infection level $10^{6.0}$.

In the present investigation using unsweetened blood and membrane feeding, the 50 percent infection threshold was 4.9 mouse log IC LD_{50} and the 5 percent infection threshold (combining results of day 11 and 32) was 3.4 mouse log IC LD_{50}.

It was reported by Chamberlain *et al.* (1954) that *A. aegypti* mosquitoes which fed upon chicks having blood virus titers of from $10^{8.0}$ to $10^{8.5}$ gave a transmission rate of 56 percent (14 out of 25) after 13 days of extrinsic incubation. It was estimated that the mosquitoes ingested blood virus titers of from $10^{7.0}$ to $10^{7.5}$.

In the present investigation, the transmission rate of 50 percent was obtained here after 25 days of extrinsic incubation with those mosquitoes having an initial virus titer of 7.4. This is almost identical with that reported by these previous investigators. The rates of 30 percent and 10 percent for the succeeding 10-fold dilutions indicate relationship between the amount of virus ingested and the transmission rate.

It is of interest that although the percentage of infection decreased with a decrease in the amount of virus ingested, the virus titers present in individual mosquitoes were essentially the same. The titers of infected mosquitoes fed on the two lowest dilutions (3.4 and 4.5 mouse log IC LD_{50}) were 5.7, 5.5, 5.5 and 5.0 mouse log IC LD_{50}. All of these exceeded the highest individual initial titer for these groups by from 0.6 to 1.0 mouse log IC LD_{50}. It would appear that once infected, the *A. aegypti* mosquitoes support the production of EEE virus to a relatively high level.

SUMMARY. Serial 10-fold dilutions of an Eastern encephalitis virus suspension were fed upon by *Aedes aegypti* mosquitoes using a Baudruche membrane. The 50 percent infection threshold was 4.9 mouse log IC LD_{50} and the 5 percent infection threshold was around 3.4. The 50 percent transmission threshold was 7.4 and the initial transmission threshold was approximately 4.9.

The results using this membrane feeding technique corresponded closely with those previously reported by workers using animal feeding techniques.

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