STUDIES ON THE TRANSMISSION OF SEMLIKI FOREST VIRUS BY *ANOPHELES FREEBORNI, A. STEPHENSI, A. LABRANCHIAE ATROPARVUS* AND *A. SUNDAICUS*

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Semliki Forest virus (SFV) has been shown to be transmitted by *Aedes aegypti* (Davies and Yoshpe Purer, 1954, and Woodall and Bertram, 1959) and by *Aedes togoi* (Nye and Lien, 1960) using suckling mice as the transmission animal. The virus has also been shown to be transmitted by *Anopheles albimanus* and *A. quadrimaculatus* (Collins, 1963), using wet baby chicks as the transmission animal.

Reported here are the results of attempts to infect *Anopheles freeborni, A. stephensi, A. labranchiae atroparvus* and *A. sundaicus* with SFV and to determine the transmissibility of SFV by these mosquitoes.

METHODS AND PROCEDURES. The virus was Semliki Forest virus (SFV), strain R-1-1, mouse brain passage 12, obtained through the courtesy of Dr. Telford Work, Communicable Disease Center, Atlanta, Georgia.

The *A. freeborni* (F-1 strain) was from Marysville, California, and has been maintained in the laboratory since 1944. The *A. sundaicus,* originally from Java, the *A. stephensi* from Delhi, India, and the *A. labranchiae atroparvus,* from England, were obtained from the London School of Hygiene and Tropical Medicine, London, England, through the courtesy of Mr. G. Davidson in 1962 and 1963 and have been maintained in the laboratory since that time.

Mosquitoes were infected by allowing them to feed through a Baudruche (untreated) membrane on a SFV heparinized rabbit blood pool. Virus pools were prepared by harvesting the brains of four mice dying from SFV infection. The brains were ground in four milliliters of Bacto-heart infusion broth (Difco) and centrifuged for 15 minutes at 1500 r.p.m. The supernatant was then diluted 1:3 in the heparinized rabbit blood, warmed to 37° C. and placed on the membrane which formed the bottom of a half-pint feeding cup. The cup was then placed on top of the cage containing the mosquitoes. After a 15-minute feeding period, the engorged mosquitoes were transferred to holding cages and stored in an incubator at 25° C. to 26° C. The mosquitoes were fed 5 percent Karo solution daily on a cotton pledget.

After 10 to 20 days of extrinsic incubation, mosquitoes were allowed to feed individually on wet baby chicks. Approximately 48 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 feeding and at periodic intervals thereafter. 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 one ml. of Bacto-heart infusion broth containing 1000 units of penicillin and 2 milligrams of streptomycin per ml. 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).

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RESULTS. The results of studies on the development of SFV in *A. freeborni*, and *A. stephensi* are shown in Table 1. Mosquitoes were sampled 2, 4, 6, 8, and 10 days after feeding. It appeared that the infection rates in these two species of *Anopheles* were high and that the viral titers were highest from the sixth day onward. There also appeared to be wide variations in the viral titers on any one particular day.

TABLE 1.—Infectivity of Semliki Forest virus to *Anopheles freeborni* and *Anopheles stephensi*.

Days post inf.	*A. freeborni*		*A. stephensi*	
	Pos. mosq./ no. titrated	Pos. virus titers (mouse log IC LD_{50})	Pos. mosq./ no. titrated	Pos. virus titers (mouse log IC LD_{50})
0	4/4	7.2, 6.5, 6.1, 5.9	4/4	6.0, 5.8, 5.6, 5.3
2	4/4	4.9, 4.1, 3.7, 2.3	3/4	4.7, 4.2, 4.1
4	4/4	5.9, 5.0, 4.0, 3.3	4/4	5.1, 4.3, 3.3, 3.0
6	3/4	7.0, 6.2, 5.0	4/4	6.5, 6.1, 4.7, 4.0
8	4/4	7.0, 6.3, 6.1, 6.1	4/4	6.9, 6.8, 3.8, 2.8
10	8/9	6.2, 6.0, 6.0, 6.0, 5.7, 4.2, 4.0, 3.9	23/24	6.8, 6.5, 6.5, 6.3, 6.2, 6.1, 6.1, 6.1, 6.0, 6.0, 6.0, 6.0, 5.9, 5.9, 5.5, 5.2, 5.2, 5.2, 5.1, 5.0, 4.9, 4.7, 4.3

The results of studies on the development of SFV in *A. labranchiae atroparvus* and *A. sundaicus* are shown in Table 2. The *A. labranchiae atroparvus* mosquitoes were sampled 2, 4, 6, 8, and 10 days after feeding, and the *A. sundaicus* were sampled 2, 4, 6, 8, 10, and 13 days after feeding. It appeared that the infection rates in these two species were not as high as in the previous two. In addition, the positive viral titers in *A. sundaicus* appeared to decrease after the eighth day.

TABLE 2.—Infectivity of Semliki Forest virus to *Anopheles labranchiae atroparvus* and *Anopheles sundaicus*.

Days post inf.	*A. labranchiae atroparvus*		*A. sundaicus*	
	Pos. mosq./ no. titrated	Pos. virus titers (mouse log IC LD_{50})	Pos. mosq./ no. titrated	Pos. virus titers (mouse log IC LD_{50})
0	4/4	5.7, 5.7, 5.0, 4.3	4/4	5.5, 4.3, 4.1, 3.7
2	2/4	4.8, 2.4	4/4	4.3, 4.3, 3.8, 3.7
4	2/4	4.3, 4.0	4/4	4.8, 3.5, 2.5, 2.3
6	1/4	3.3	4/4	5.0, 4.5, 4.5, 2.5
8	2/4	6.9, 2.8	3/4	5.4, 3.4, 3.0
10	..	..	3/4	3.7, 3.3, 3.3
13	..	..	1/6	3.3

The results of the transmission experiments are shown in Table 3. The *A. freeborni* were infected on five different occasions and transmission feedings were made after 10 to 12 days of extrinsic incubation. A total of 76 mosquitoes were fed upon baby chicks with four resultant transmissions of SFV (5 percent). Subsequent titration indicated, however, that 35 of these mosquitoes actually contained SFV so that the transmission rate for the infected individual mosquitoes was approximately 11 percent. The titers of the transmitting mosquitoes ranged from 5.3 to 6.0 mouse log IC LD_{50}.

The *A. stephensi* were infected on four different occasions and transmission feed-

TABLE 3.—Transmission of Semliki Forest virus by *Anopheles freeborni, A. stephensi, A. l. atroparvus,* and *A. sundaicus mosquitoes.*

Mosq. species	Initial SFV titers (mouse log IC LD_{50})			Days post inf.	Trans./ attempts	SFV titers (mouse log IC LD_{50})		
	Test no.	Pos./tot.	Pos. titers			Pos./tot.	Mean pos.	Titer of trans. mosq.
A. freeborni	1	3/3	4.1, 4.0, 3.0	12	1/12	4/12	5.7	5.3
	2	2/3	5.0, 5.0	12	2/20	6/20	6.0	6.1, 5.5
	3	4/4	5.5, 5.3, 4.8, 4.0	10	0/20	11/20	4.5	...
	4	4/4	6.3, 5.8, 5.1, 5.0	10	0/15	6/15	4.9	...
	5	4/4	7.2, 6.5, 6.1, 5.9	10	1/9	8/9	5.2	6.0
A. stephensi	1	4/4	5.0, 5.0, 4.7, 4.3	9	0/5	4/5	3.7	...
				13	0/10	10/10	4.8	...
	2	4/4	6.0, 5.8, 5.0, 4.7	10	0/10	9/10	5.6	...
	3	4/4	6.0, 5.8, 5.6, 5.3	10	5/24	23/24	5.7	6.8, 6.5, 6.3, 6.1, 6.0
	4	4/4	6.3, 5.9, 5.9, 5.7	9	1/7	7/7	5.8	6.2
A. l. atroparvus	1	4/4	6.2, 6.1, 6.0, 5.7	10	1/20	4/20	5.1	5.0
				20	1/18	5/15	5.6	5.5
A. sundaicus	1	4/4	5.6, 5.3, 5.2, 5.2	10	0/15	3/15	3.3	...

ings were made between days 9 and 13 post-infection. A total of 56 feedings were made with six resultant transmissions of SFV (11 percent). Subsequent titration indicated that 53 of these mosquitoes actually contained SFV so that the transmission rate for the infected individual *A. stephensi* mosquitoes was also approximately 11 percent. The titers of the transmitting mosquitoes ranged from 6.0 to 6.8 mouse log IC LD_{50}.

The transmission studies with *A. labranchiae atroparvus* were made 10 and 20 days post-infection. A total of 35 mosquitoes were fed with two transmissions (6 percent). Subsequent titration indicated that nine of these mosquitoes actually contained SFV. The transmission rate for the infected mosquitoes was approximately 22 percent. The virus titers of the transmitting mosquitoes were 5.0 and 5.5 mouse log IC LD_{50}.

Only 15 transmissions were attempted using the *A. sundaicus* mosquitoes with no resultant transmissions. Only three of these mosquitoes contained virus and the titers were all quite low. Further studies with this species were prevented by the absence of available mosquitoes.

DISCUSSION. All of the species of *Anopheles* thus far investigated appear to be readily susceptible to infection by SFV using the membrane feeding technique. The virus titers of the transmitting mosquitoes reported here ranged from 5.0 to 6.8 mouse log IC LD_{50}. This is in close agreement with the results reported previously (Collins, 1963 and 1963a and Collins *et al.*, 1964) and would indicate that anopheline mosquitoes must have a SFV titer of 5.0 or greater before they are capable of transmitting SFV to baby chicks.

The total transmission rate for the *A. stephensi* was the highest reported here. When only the positive mosquitoes were considered, however, the *A. freeborni* and *A. stephensi* had approximately the same transmission rate.

The low infection rates in *A. labranchiae atroparvus* and *A. sundaicus* would indicate a reduced vector potential. However, those *A. labranchiae atroparvus* which were still infected after 10 days of extrinsic incubation had a relatively high mean titer and were capable of transmitting SFV.

SUMMARY. *Anopheles freeborni, A. stephensi, A. labranchiae atroparvus* and *A. sundaicus* were readily infected with Semliki Forest virus by means of the membrane feeding technique.

Transmission of the virus to wet baby chicks was obtained with the first three species after extrinsic incubation periods of 9 to 20 days. The *A. stephensi* appeared to be the most susceptible to infection and also had the highest transmission rate.

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