STUDIES OF SINDBIS VIRUS IN *ANOPHELES ALBIMANUS* AND *AEDES AEGYPTI*

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Sindbis virus was first isolated from pools of *Culex* sp. mosquitoes in the region of Sindbis village, Egypt, by Taylor and Hurlbut (1953). This virus has been shown to belong to Casal's arthropod-borne virus group A. Isolations of this agent were also made from *Culex antennatus, C. univittatus* and *Anopheles pharoensis*. The virus has also been isolated by Weinbren *et al.* (1956) from culicine mosquitoes collected near Johannesburg, South Africa, and by Shah *et al.* (1960) in India. Rudnick *et al.* (1962) have reported the isolation of this virus from *C. bitaeniorhynchus* collected in the Philippines.

Hurlbut (1953) was able to demonstrate the experimental transmission of Sindbis virus by *C. pipiens* and *C. univittatus* mosquitoes to suckling mice. Mosquitoes were infected by feeding on infected suckling mice or by the thrusting of steel pins wet with the virus suspension through the wall of the thorax.

Transmission of this virus by *Aedes aegypti* mosquitoes exposed as larvae to a suspension of Sindbis virus has been reported by Peleg (1965).

In the present investigation, *Anopheles albimanus* and *Aedes aegypti* mosquitoes were tested to determine their ability to transmit Sindbis virus experimentally.

MATERIALS AND METHODS. The virus was Sindbis virus Ar-1055, obtained through the courtesy of Dr. Phillip Coleman, Communicable Disease Center, Atlanta, Georgia, U.S.A.

The *An. albimanus* mosquitoes were the A-9 strain originally obtained from San Salvador and maintained in our laboratory since 1960.

The *Ae. aegypti* were the CD strain originally obtained from Technical Development Laboratories, CDC, Savannah, Georgia, and maintained in our laboratory since 1959.

Mosquitoes were infected by allowing them to feed through a Baudruche (untreated) membrance on a virus suspension in heparinized rabbit blood. The brains of 19 suckling mice previously inoculated with the seventh mouse passage of Sindbis

virus were ground in three milliliters of 20 percent chicken serum in Bacto-heart infusion broth (Difco). After centrifugation for 15 minutes at 1500 r.p.m., one milliliter of the supernatant was added to 8 milliliters of freshly drawn heparinized rabbit blood.

For the mosquito feeding, the suspension was warmed to approximately 37°C and placed on the membrane which formed the bottom of a ½ pint feeding cup. The cup was then placed on top of the cage containing the mosquitoes. The feeding period was 10 minutes, after which time the engorged mosquitoes were transferred to holding cages and stored in an incubator at 25° to 26° C. The mosquitoes were fed 5 percent Karo solution daily on a cotton pledget.

After 12, 14 and 19 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, diluted 1:10 in bacto-heart infusion broth and stored frozen at —68° C. The samples were later thawed and inoculated intracerebrally into 1-day-old mice using one litter of mice per sample. Presence of virus in the chick blood, as indicated by the death of the suckling mice from Sindbis virus, constituted evidence of virus transmission by the mosquito.

Mosquitoes were collected and killed by freezing immediately after feeding. These were stored at —68° 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 2 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. One litter of 1-day-old suckling mice was inoculated intracerebrally (0.02 ml. per mouse) per dilution and the LD_{50}s calculated by the method of Reed and Meunch (1938).

RESULTS. Two separate experiments were performed. The first involved a paired comparison of the infectivity of Sindbis virus to *Ae. aegypti* and *An. albimanus* mosquitoes and included 12- and 19-day transmission attempts. The second experiment involved *An. albimanus* only.

The results of the first experiment are shown in Table I. The *Ae. aegypti* mosquitoes had a 25 percent infection rate after 12 and 19 days of extrinsic incubation. The positive virus titers ranged from 4.9 to 7.1 mouse $1/Log_{10}$ IC LD_{50} per mosquito. The median positive virus titer was approximately 6.6. The total of three transmissions by this mosquito indi-

TABLE 1.—Infectivity of Sindbis virus to *Aedes aegypti* and *Anopheles albimanus* mosquitoes.

Experiment	Mosquito species	Days post infection	Positive mosq./ number titrated	Positive virus titers (mouse $1/Log_{10}$ IC LD_{50})
I	*Aedes aegypti*	0	5/5	5.7, 5.2, 5.0, 4.7, 4.3
		12	3/12	7.1*, 6.1, 4.9
		19	3/12	7.1, 6.7, 6.5
	Anopheles albimanus	0	5/5	6.0, 5.5, 5.5, 5.1, 4.9
		12	11/20	6.7, 6.3, 5.3, 5.3, 5.3, 5.2, 4.7, 4.5, 4.3, 4.1, 3.6
		19	7/8	6.3, 5.5, 5.5, 5.5, 5.4, 5.2, 5.2
II	*Anopheles albimanus*	0	4/4	6.4, 6.4, 6.2, 6.1
		14	10/10	7.1, 7.0, 6.3, 6.2, 6.2, 6.2, 6.1, 6.0, 5.7, 5.1

* Underlined titers are those of mosquitoes which transmitted the virus to baby chicks.

cated a 50 percent transmission rate per positive mosquito.

The *An. albimanus* mosquitoes had a 64 percent infection rate after 12 and 19 days of extrinsic incubation. The positive virus titers ranged from 3.6 to 6.7 mouse $1/\text{Log}_{10}$ IC LD_{50} with the median positive virus titer being approximately 5.3. There was only one transmission by this species.

The results of the second experiment are also shown in Table I. This test was designed to repeat the transmission of Sindbis virus by *An. albimanus* mosquitoes. The median virus titer originally ingested was higher than in the first experiment (6.3 verus 5.5). After 14 days of extrinsic incubation, 10 out of 10 mosquitoes were positive for the virus with a median virus titer of 6.2. Only one mosquito transmitted the infection to baby chick.

It is apparent from the results of these two experiments that *An. albimanus* mosquitoes are readily infected with Sindbis virus using the membrane feeding technique. However, the transmission rate to baby chicks after 12 to 19 days of extrinsic incubation was very low in view of the high virus titers present in these mosquitoes.

On the other hand, the *Ae. aegypti* mosquitoes had a much lower infection rate but the transmission rate was higher.

It is of interest that this is the third Group A virus which has been experimentally transmitted by *An. albimanus* mosquitoes, the others being Semliki Forest virus (Collins, 1963) and eastern equine encephalitis virus (Collins, *et al.*, 1965). This work demonstrates the possibility of using *A. albimanus* as a vector of the members of this group. Further studies are needed to determine its potential as a vector of viruses belonging to other arthropod-borne virus groups.

Aedes aegypti is an established experimental and natural vector of a number of arthropod-borne viruses. Its transmission of Sindbis virus using the membrane infection technique, although not previously reported, was anticipated.

SUMMARY. *Aedes aegypti* and *Anopheles albimanus* mosquitoes were infected with Sindbis virus using a membrane feeding technique. Transmission of the virus to baby chicks was obtained using both species of mosquito after 12 to 19 days of extrinsic incubation. Although *An. albimanus* had a higher infection rate, the *Ae. aegypti* had a higher transmission rate. This is the third Group A arthropod-borne virus which has been experimentally transmitted by *An. albimanus* mosquitoes.

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