ARTICLES

STUDIES OF TENSAW VIRUS IN *ANOPHELES QUADRIMACULATUS, A. ALBIMANUS* AND *A. MACULATUS*

WILLIAM E. COLLINS AND ANDREW J. HARRISON

National Institutes of Health, National Institute of Allergy and Infectious Diseases
Laboratory of Parasite Chemotherapy, Chamblee, Georgia

Tensaw virus, an arbovirus of the Bunyamwera group, was isolated from anopheline mosquitoes in the Southeastern United States by Chamberlain, *et al.* (1966). Sudia, *et al.* (1966) transmitted the virus through *Anopheles quadrimaculatus* using dogs as the infecting animal and suckling mice as the transmission animal.

Reported here are the results of studies on the infectivity of Tensaw virus to *A. quadrimaculatus, A. albimanus* and *A. maculatus* using a membrane feeding technique. The results of transmission studies using *A. quadrimaculatus* and *A. albimanus* are also reported.

MATERIALS AND METHODS. The Tensaw virus (strain A-9 171b) was obtained through the courtesy of Dr. Telford Work, Communicable Disease Center, Atlanta, Georgia. The *A. quadrimaculatus* mosquitoes were the Q-1 strain which was obtained from Technical Development Laboratories, CDC, Savannah, Georgia, and maintained in our laboratory since 1959. The *A. albimanus* mosquitoes were the A-9 strain originally obtained from San Salvador, and maintained in our laboratory since 1960. The *A. maculatus* were obtained from Kuala Lumpur, Malaysia, and have been maintained in our laboratory since 1964.

Mosquitoes were infected by allowing them to feed through a Baudruche (untreated) membrane on dilutions of Tensaw virus in fresh heparinized rabbit or chicken blood. The virus suspensions were prepared by grinding in a mortar the brains of 15 to 20 dead or moribund mice in six milliliters of Bacto heart infusion broth (Difco) and centrifuging for 15 minutes at 1500 r.p.m. For the mosquito feeding, one part of each virus dilution was added to four parts of blood; this was then warmed to 37° C. and placed on the membrane which formed the bottom of a half-pint ice cream cup. The cup was then placed on top of the cage containing the mosquitoes. The feeding period was 15 minutes, after which time the engorged mosquitoes were transferred to holding cages and placed in an incubator at 25° to 26° C. The mosquitoes were fed 5 percent Karo solution daily on a cellulose pledget.

In the transmission studies mosquitoes were allowed to feed individually on one-day-old suckling mice, after 10 to 14 days of extrinsic incubation. The brains of mice dying 4 to 7 days after mosquito feeding were harvested and individually ground in a mortar with 2 milliliters of broth containing 1000 units of penicillin and 2 milligrams of streptomycin per ml. This suspension was centrifuged for 15 minutes at 1500 r.p.m., and serial 10-fold dilutions of the supernatent were inoculated intracerebrally into three-week-old mice. Death of these mice in a typical Tensaw pattern was considered evidence of transmission of the virus to the suckling mouse.

To determine virus titers, mosquitoes were killed by freezing 1) immediately after initial feeding, 2) immediately after transmission attempt, or 3) after various periods of extrinsic incubation. These were stored in a mechanical freezer at −65° to −70° C. until titrated. At this time each mosquito was ground individually in a mortar with 1 ml. aliquot of broth containing penicillin and strepto-

mycin. The suspension was centrifuged for 15 minutes at 1500 r.p.m. and serial 10-fold dilutions were made in broth. Five three-week-old mice were inoculated intracerebrally per dilution and the LD_{50} calculated by the method of Reed and Meunch (1938).

RESULTS. The relationship between the virus ingested by *A. quadrimaculatus* mosquitoes and that present after 5 to 25 days of extrinsic incubation is presented in Table 1. This table contains the results of 17 different feedings. The data are presented in relation to the amount of virus originally ingested by a given lot of mosquitoes. Five mosquitoes were individually titrated after each initial feeding so that the group with an initial titer of 4.8 actually represents four separate feeding tests, and the group with a titer of 4.1 represents eight separate feeding tests. The median titers after 14 to 15 days of extrinsic incubation illustrate that initial titers of from 2.3 to 4.8 mouse $1/Log_{10}$ IC LD_{50} all resulted in approximately the same median titer (4.0 to 4.3). A ten-fold dilution from the initial median titer of 2.3 infected only 3 of 5 mosquitoes tested and resulted, after 14 days, in a 30 percent infection rate with relatively low virus titers. An additional 10-fold dilution of the virus failed to infect mosquitoes.

The relationship between the virus ingested by *A. albimanus* mosquitoes and that present after 10 to 14 days of extrinsic incubation is presented in Table 2. This table contains the results of 10 different feedings. Although the initial median titers of 4.9 and 3.9 gave subsequent titers comparable to those found in the *A. quadrimaculatus,* the median titers resulting from the lower concentrations initially ingested were lower in the *A. albimanus.* The ten-fold dilution from the initial median titer of 2.1 at first infected no mosquitoes but after 14 days of incubation resulted in a 22 percent infection rate, indicating a similar infection threshold for these two mosquito species.

To follow the development of Tensaw

TABLE 1.—Relationship between Tensaw virus ingested by *Anopheles quadrimaculatus* and that present after 5 to 25 days of extrinsic incubation.

Initial			Post-Incubation				
Pos./ Tested	Median Pos. Virus Titer*	Range	Day	Pos./ Tested	Percent Infected	Median Pos. Virus Titer	Range
20/20	4.8	4.0–5.4	12	9/9	100	4.0	3.0–4.7
			14	15/15	100	4.0	3.3–4.9
			18	3/3	100	3.8	3.3–4.3
40/40	4.1	3.5–4.8	5	75/75	100	3.9	2.1–5.5
			10	80/80	100	4.2	2.5–5.3
			15	40/41	98	4.1	2.8–5.3
			20	10/10	100	4.0	3.3–4.7
			25	15/16	94	4.5	3.7–4.8
5/5	3.8	3.3–4.1	14	16/16	100	4.2	3.7–5.0
			18	1/2	50	4.5	4.5
5/5	2.7	2.5–3.0	14	15/15	100	4.0	3.1–5.0
			18	4/4	100	3.3	3.0–3.5
5/5	2.3	2.2–3.1	10	6/10	60	2.9	2.3–3.7
			14	3/3	100	4.3	3.0–4.3
3/5**	2.1	2.1	10	1/10	10	5.3	5.3
			14	3/10	30	2.3	2.2–2.7
0/5**	...	...	10	0/10	0	...	...
			14	0/10	0	...	...

* All titers expressed as the mouse $1/Log_{10}$ IC LD_{50}.
** Ten-fold dilution of virus from previous concentration.

TABLE 2.—Relationship between Tensaw virus ingested by *Anopheles albimanus* and that present after 10 to 14 days of extrinsic incubation.

Initial			Post-Incubation				
Pos./ Tested	Median Pos. Virus Titer*	Range	Day	Pos./ Tested	Percent Infected	Median Pos. Virus Titer	Range
5/5	4.9	4.7–5.0	10	5/5	100	3.9	3.3–4.2
			13	7/7	100	4.0	2.9–4.7
20/20	3.9	3.0–4.1	10	39/40	98	3.9	2.1–5.0
			12	32/34	94	4.3	3.0–5.7
			13	15/15	100	3.3	2.7–4.8
			14	18/21	86	3.1	2.1–5.3
10/10	2.9	2.3–3.9	10	10/10	100	3.1	2.8–4.9
			14	7/8	88	2.8	2.2–4.3
7/10	2.1	2.1–2.3	10	5/5	100	3.1	2.3–4.8
			14	8/14	57	2.9	2.5–4.0
0/5**	...	...	10	1/10	10	3.7	3.7
			14	2/9	22	2.8	2.7–3.0

* All titers expressed as the mouse $1/Log_{10}$ IC LD_{50}.
** Ten-fold dilution of virus from previous concentration.

virus, *A. quadrimaculatus* and *A. albimanus* mosquitoes were infected on a comparable titered virus suspension and subsequent samples taken and titrated at two-day intervals; *A. maculatus,* infected on a lower virus concentration, were likewise examined. The results of these titrations are presented in Table 3. It can be seen that in all species there was a drop in titer by day 2 followed by a subsequent rise. It is apparent that the median titers in the *A. quadrimaculatus* climbed to levels by day 10 which were considerably higher than those found in the *A. albimanus.* The *A. maculatus* failed to produce high titers of virus even though 100 percent of the mosquitoes were infected after 6 days of incubation.

Attempts were made to transmit Tensaw virus by *A. quadrimaculatus* after 14 days of extrinsic incubation, and by *A. albimanus* after 10 to 13 days of incubation. The results of these attempts are shown in Table 4. A total of 12 of 69 *A. quadrimaculatus* transmitted the virus. These mosquitoes contained 3.3 to 5.0 mouse $1/Log_{10}$ IC LD_{50s} of virus. The amount of virus initially ingested did not appear to affect the transmission rate. A total of 5 of 48 *A. albimanus* transmitted the virus. These mosquitoes contained 4.3 to 5.3 mouse $1/Log_{10}$ IC LD_{50s} of virus.

DISCUSSION. The infection studies indicated that all three anophelines tested were readily susceptible to infection, and that the *A. quadrimaculatus* appeared to develop the highest virus titers. This is in contrast to the findings with eastern encephalitis virus in which the *A. albimanus* developed higher titers than did the *A. quadrimaculatus* (Collins, *et al.,* 1965).

Of considerable interest was the apparent lack of relationship in the *A. quadrimaculatus* between the amount of virus ingested and the subsequent virus titer. Apparently, once infected, this mosquito is able to support Tensaw virus production to a certain maximum level. Thus, the amount of virus being circulated in the donor animal is critical only in that it must be of a minimum infection threshold level. Beyond this, higher titers would appear to have little or no effect on virus titers in the infected mosquitoes.

The transmission of Tensaw virus by *A. quadrimaculatus* confirms the findings of Sudia, *et al.* (1966). The experimental transmission by *A. albimanus* appears to be a new observation.

SUMMARY. *Anopheles quadrimaculatus, A. albimanus* and *A. maculatus* mosquitoes were infected with Tensaw virus using the membrane feeding technique. Higher mean titers were seen in *A.*

TABLE 3.—Infectivity of Tensaw virus to *Anopheles quadrimaculatus*, *A. albimanus* and *A. maculatus*.

Days Post-Inf.	*A. quadrimaculatus*			*A. albimanus*			*A. maculatus*		
	Pos. Mosq./ No. Titrated	Pos. Virus Titers*		Pos. Mosq./ No. Titrated	Pos. Virus Titers		Pos. Mosq./ No. Titrated	Pos. Virus Titers	
		Median	Range		Median	Range		Median	Range
** Initial	5/5	5.0	4.7–5.2	5/5	4.9	4.7–5.0	5/5	4.0	3.9–4.2
2	4/5	3.0	2.1–3.3	5/5	3.2	2.5–4.0	3/5	2.1	2.1
4	5/5	2.9	2.3–3.7	5/5	3.7	3.3–4.3	4/5	3.3	3.3–3.7
6	5/5	4.1	2.3–5.3	5/5	4.3	4.0–4.3	5/5	3.0	2.5–4.3
8	5/5	4.1	3.7–4.5	5/5	4.0	3.2–4.7	5/5	3.1	2.8–3.8
10	5/5	5.0	4.3–5.7	5/5	3.9	3.3–4.2	5/5	3.3	3.0–3.7
12	5/5	5.0	4.1–5.7	5/5	4.2	3.1–4.8	5/5	2.9	2.3–3.0
13	...	...	...	7/7	4.0	2.9–4.7	...	...	...
14	3/5	5.3	4.7–5.5	...	...	...	...	...	...
16	1/2	4.1	4.1	...	...	...	5/5	2.7	2.2–4.2

* All titers expressed as the mouse $1/Log_{10}$ IC LD_{50}.

** Immediately after feeding.

TABLE 4.—Transmission of Tensaw virus by *Anopheles quadrimaculatus* and *A. albimanus* mosquitoes.

Median initial titer*	Day	Trans./ attempts	Tensaw virus titers: Median pos.**	Transmitting mosquitoes
Anopheles quadrimaculatus				
4.8	14	3/15	4.0	4.1, 3.7, 3.3
4.3	14	1/8	3.6	4.7
4.1	14	3/15	4.0	4.8, 4.8, 4.1
3.8	14	1/16	4.2	4.2
2.7	14	4/15	4.0	5.0, 4.3, 4.0, 3.7
Anopheles albimanus				
4.1	13	1/15	3.3	4.3
3.9	10	0/20	4.1	
3.9	12	4/13	4.5	5.3, 5.0, 4.8, 4.7

* All titers expressed as the mouse $1/Log_{10}$ IC LD_{50}.

** Median positive Tensaw virus titer for all mosquitoes allowed to feed.

quadrimaculatus than in the other two mosquitoes and there appeared to be little or no relationship between the amount of virus ingested by this species and the resultant virus titers. Very low quantities of virus were sufficient to produce infection in the *A. quadrimaculatus* and *A. albimanus* mosquitoes, and infection thresholds were similar for these two species.

Transmission of Tensaw virus was obtained by the feeding of *A. quadrimaculatus* and *A. albimanus* on suckling mice after 12 to 14 days of extrinsic incubation. The amount of virus initially ingested did not appear to affect the transmission rate.

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AN IMPROVED PORTABLE RESTING STATION FOR *ANOPHELES QUADRIMACULATUS* SAY

S. G. BREELAND AND J. W. GLASGOW

Reservoir Ecology Branch, Division of Health and Safety, Tennessee Valley Authority, Muscle Shoals, Alabama

Malaria control has always been an integral part of the TVA resource development program. The extensive mosquito control measures against the vector, *Anopheles quadrimaculatus,* require intimate knowledge of the extent of anophelism in the various reservoirs involving approximately 10,000 miles of shoreline in parts of seven states. For this purpose, an annual mosquito inspection service is conducted whereby inspectors report weekly counts of adult mosquitoes from more than 250 diurnal resting shelters throughout the Valley. In connection with this program, TVA, since 1935, has been interested in devices that would give dependable measurements of anophelism in the vicinity of its reservoir shorelines. Such devices must be portable, economical, and effective.

The idea that *Anopheles* mosquitoes entered diurnal resting places primarily