

STUDIES ON YELLOW FEVER IN SOUTH AMERICA

V. TRANSMISSION EXPERIMENTS WITH CERTAIN SPECIES OF CULEX
AND AËDES

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Bauer (1) has reported the transmission of yellow fever virus by several species of African mosquitoes, all but one of which belong to the genus *Aedes*. The Western Hemisphere contains no close relatives of the "yellow fever mosquito," *Aedes (Stegomyia) aegypti*, but we have recently conducted experiments with a number of species belonging to other groups, which are common in the American tropics, in order to determine whether any of these were capable of transmitting the virus.

The common house mosquito of the tropics and subtropics, *Culex quinquefasciatus* (*C. fatigans*), has engaged the attention of yellow fever workers as a possible transmitter, but no positive evidence has ever been adduced against it. Both The American Yellow Fever Commission (2) in Cuba and the French Commission (3) in Rio de Janeiro obtained negative results in experiments with this species. The French Commission also carried out one experiment with *Aedes (Ochlerotatus) scapularis* (called by them *Culex confirmatus*) and one with a *Culex* of uncertain species, both without success. The experiments of these two commissions were all upon human volunteers.

RESULTS OF EXPERIMENTS

Utilizing as an experimental animal *Macacus rhesus*, we have carried out a number of feeding and inoculation experiments with South American mosquitoes. The accompanying table summarizes the results with *Culex quinquefasciatus*, *Aedes (Ochlerotatus) scapularis*, *Aedes (Ochlerotatus) serratus* and *Aedes (Taeniorhynchus) taeniorhynchus*.

Summary of Transmission Experiments

Animal No.	Experiments with mosquitoes						Immunity test				Control to immunity test								
	Mosquito Lot No.	Species of mosquito	Fed or injected	Number of mos- quitoes	Virus strain	Fever	Duration of fever (days)	Maximum tem- perature	Death or recovery	Test strain	Fever	Duration of fever (days)	Maximum tem- perature	Death or recovery	Animal No.	Fever	Duration of fever (days)	Maximum tem- perature	Death or recovery
A1	106	<i>C. quinquefasciatus</i>	Fed	5	Asibi	-	1	103.5	R	Asibi	+	3	104.7	R	B1	+	2	105.0	D
A2	106	<i>C. quinquefasciatus</i>	Injected	13	Asibi	+	4	104.1	R	Asibi	+	4	104.7	R	B2	+	2	105.1	D
A3	132 & 146	<i>C. quinquefasciatus</i>	Injected	4	1 B. B. 3 Asibi	+	4	104.6	R	Asibi	+	4	105.8	R	B3**	+	3	105.6	R
A4	166	<i>C. quinquefasciatus</i>	Fed	4	Asibi	-	-	103.4	R	Asibi	+	3	105.0	D	B4*	+	3	106.1	D
A5	166	<i>C. quinquefasciatus</i>	Injected	9	Asibi	-	-	103.5	R	Asibi	+	2	105.9	D	B4*	+	3	106.1	D
A6	193 & 194	<i>C. quinquefasciatus</i>	Injected	16	Asibi	-	-	103.6	R	Asibi	+	7	104.5	R	B5***	+	2	104.4	D
A7	83	<i>Aedes scapularis</i>	Fed	20	B. B.	-	-	103.8	R	Asibi	-	-	103.6	R	B6	+	9	105.4	R
A8	83	<i>Aedes scapularis</i>	Fed	6	B. B.	+	3	106.4	D	Asibi	-	-	103.6	R	B7****	-	-	103.6	D
A9	129	<i>Aedes scapularis</i>	Injected	62	B. B.	-	-	103.5	R	Asibi	-	-	-	-	-	-	-	-	-
A10	129	<i>Aedes scapularis</i>	Fed	10	B. B.	+	1	106.0	R	Asibi	+	2	105.2	D	-	-	-	-	-
A11	130	<i>Aedes scapularis</i>	Injected	4	B. B.	+	1	106.0	R	Asibi	+	2	104.5	R	B4*	+	3	106.1	D
A12	172	<i>Aedes scapularis</i>	Fed	12	Asibi	+	2	105.2	D	Asibi	+	3	105.2	D	B4*	+	3	106.1	D
A13	174	<i>Aedes serratus</i>	Fed	2	Asibi	-	-	103.5	R	Asibi	+	2	104.5	R	-	-	-	-	-
A14	174	<i>Aedes serratus</i>	Fed	8	Asibi	-	-	103.5	R	Asibi	+	3	105.4	D	-	-	-	-	-
A15	183	<i>Aedes taeniorhynchus</i>	Injected	13	Asibi	+	2	104.2	R	Asibi	+	3	105.8	D	B8	+	3	105.8	D
A16	192	<i>Aedes taeniorhynchus</i>	Fed	7	Asibi	+	2	104.2	R	Asibi	+	7	104.7	R	B9	+	3	104.4	D
A17	183 & 192	<i>Aedes taeniorhynchus</i>	Injected	6	Asibi	+	3	104.2	R	Asibi	-	-	103.6	R	B5***	+	2	104.4	D

*M. rhesus No. B 4 served as control in four of the immunity tests. The same dosage of virus killed eight other animals.
 **The same dosage of virus killed six animals, but the control, No. B 3, survived. No. B 3 had previously been fed upon by normal *Aedes aegypti*.

***M. rhesus No. B 5 served as control in two of the immunity tests.

****Control, No. B 7, died from yellow fever on the fourth day, although he had shown no fever at the hours of taking temperature.

No certain transmission was secured either by the bites or by the inoculation of *Culex*, although two of the experimental monkeys showed slight fever. It would appear, however, that in three of the experiments (rhesus Nos. A 1, A 2 and A 6) a low grade of immunity was produced. This was more noticeable in the cases of rhesus Nos. A 1 and A 2, in which *Culex* batch No. 106 was used. One can only conjecture whether attenuated virus actually survived in this species.

With *Aedes scapularis* the results were different. *M. rhesus* No. A 8 died after being bitten by, and inoculated with, the ground up bodies of this species.

The lot used was a combination of lots fed on four different infected rhesus monkeys between December 10 and 14, 1928. On January 2 and 3, 1929, the mosquitoes were allowed to feed on rhesus No. A 7. On January 7 six of the batch engorged on rhesus No. A 8. On January 9 the sixty-two remaining mosquitoes of the lot were ground up in salt solution and inoculated subcutaneously into the same animal. On January 13 the monkey's temperature rose to 105.0°F. and continued high for three days; on January 16 the animal died and the gross and microscopic lesions were typical of yellow fever.

Bites of *scapularis* lot No. 130 were sufficient to produce fatal yellow fever in rhesus No. A 11. The lot received its infective blood meal from rhesus No. B 10 on February 14, 1929. On February 27 about twelve of the mosquitoes engorged on rhesus No. A 11. On the fourth day following, March 3, the animal had a temperature of 105.2°F. On March 5, the temperature dropped to 98° and the monkey was killed when moribund. The gross and microscopic lesions were typical of yellow fever.

M. rhesus No. A 10 had a definite febrile attack following the inoculation of *scapularis* of batch No. 129.

Animals Nos. A 7, A 9 and A 12 developed a high degree of immunity from the bites of *Aedes scapularis*, without having shown any preceding fever. Such immunity without manifest disease has been shown to follow frequently the bites of infected *Aedes aegypti* (4).

Only one lot of *Aedes serratus* was used. Eight of these mosquitoes fed upon rhesus No. A 13 after an extrinsic incubation period of twenty-two days. No fever resulted and the animal later succumbed to the immunity test. The inoculation of thirteen of this lot on the thirty-first day after the infective blood meal produced a fatal attack of yellow fever in rhesus No. A 14. It is evident that in this particular lot the virus did not enter the salivary glands, although it remained alive at some place in the body of the insects.

Two lots of *Aedes taeniorhynchus* Nos. 183 and 192 were used. One of the two animals fed upon by these insects survived the immunity test. Probably virus remained alive in the body of this species of mosquito also.

Twenty-five *taeniorhynchus* were inoculated subcutaneously into rhesus No. A 17 on June 3, 1929. Twenty-four days had elapsed since the infective meal of batch No. 183, and twenty days since that of batch No. 192. At the time of inoculation the temperature of rhesus No. A 17 was 103.4°; on June 4 it rose to 104°; on June 5 it was 104.2°; and on June 6, 104° again; on June 7 it dropped by crisis to 101.8°. Thereafter it ranged between 102.6° to 103.6°. The immunity test caused no reaction; the highest temperature during the observation period was 103.5°. The control, rhesus No. B 5, which had been inoculated with the same virus as that used in the immunity test, had a typical attack of yellow fever and was killed on the fifth day when moribund. Autopsy findings were typical of the disease.

Lots of *Aedes (Ochlerotatus) hastatus* were on several occasions given an opportunity to feed, but none were ever observed to take blood.

Habits of the Species of Mosquitoes Used for Experimentation

Culex quinquefasciatus breeds in domestic and peridomestic locations, usually in artificial receptacles, and by preference in water containing much decomposing organic material. The females feed only at night, but during the daytime adults of both sexes may frequently be found resting in bedrooms, closets, toilets and lavatories. In spite of the prevalence of the species and the ease with which it is reared, we were able to use only a few specimens in each experiment because the females showed no great avidity for monkey blood. Our method was to anesthetize the monkeys with amytal, place them in the mosquito cages at about five o'clock in the afternoon and leave them for three to four hours exposed to mosquito bites. It is quite possible that if the animals had been left with the mosquitoes all night, better results might have been secured.

Aedes scapularis is widely distributed from the West Indies through Columbia, Venezuela, the Guianas, Brazil, Bolivia and Northern Argentina. It breeds in temporary rainpools both in rural areas and in urban districts. In shaded localities it will attack man or animal at any hour of the day and will even pursue its victim into the sunlight. It attacks in greatest numbers at dusk. Although not truly domestic it freely invades yards and gardens and will occasionally enter houses to secure blood. Upon rare occasions it has been seen resting indoors during the daytime. It was found to feed readily on monkeys, but the adults did not survive as long in confinement as did specimens of *Aedes aegypti*.

Aedes serratus is found in Mexico, Central America, northern South America and

Brazil (5). It breeds in temporary rainpools. In this section of the country we have seen it only in rural and sylvan localities; it appears to be much less ubiquitous than *scapularis*. In nature it feeds by preference in the evening, but in the laboratory it engorged on monkeys at various hours of the day.

Specimens which resemble quite closely the Central American *Aedes hastatus* were bred out from rainpools occurring in the same neighborhood where *serratus* was found. The adults appear smaller and more delicate than either *scapularis* or *serratus*. In spite of repeated opportunities to feed during daylight hours they were never observed to take blood in the laboratory. It is possible that in nature they feed at night or upon some particular host only. We have not found them in collections made when using animal bait.

Aedes taeniorhynchus is the prevalent salt marsh mosquito of the South Atlantic and Gulf Coast states of the United States of America. It extends south through Mexico and Central America to Peru on the west coast and to Brazil on the east coast of South America. Dyar (5) in his recent book does not include Brazil in the distribution of the species. Peryassu (6) records it, under the name *Culicella taeniorhynchus* from Para, state of Rio de Janeiro, and the Federal District in Brazil. The French Commission (3) while in Rio de Janeiro kept specimens of "*Culex taeniorhynchus*" in captivity to observe the length of life and the feeding and egg-laying habits, but carried out no transmission experiments with them. During the month of April, 1929, the species was found breeding in crab-holes along the edge of the bay and also in rock-pools along the shore, in the city of Bahia. The crab-holes were above the level of high tide and appeared to contain only fresh water, probably largely rain-water. In the same month we received specimens of *taeniorhynchus* from Aracaju in the state of Sergipe and heard later that the species had been found in Alagoas. This mosquito is a vicious biter but fortunately has a rather limited breeding season.

DISCUSSION

Our experiments show that *Aedes scapularis* is capable of transmitting yellow fever by its bite. Whether it ever becomes a factor in the dissemination of the disease is not known. Its feeding habits do not preclude this possibility, because many yellow fever patients are not bedridden at first and may expose themselves to the bites of mosquitoes outdoors, and also because the insect at times enters houses in search of blood.

The tests have shown that yellow fever virus may survive for some time in the bodies of *Aedes serratus*, and probably also in *Aedes taeniorhynchus*; but whether these species ever become capable of transmitting the virus by their bites is not yet known. By mashing an infected mosquito on the skin a person might conceivably become

infected. The biting habits of *Aedes taeniorhynchus* are such as to bring it under suspicion during the season of maximum prevalence if yellow fever is present in the vicinity.

There is no evidence that *Culex quinquefasciatus* is able to transmit the disease in a recognizable form. However, in certain animals an apparent immunity to yellow fever was found to have developed subsequent to the bites of this species. We do not wish to over-emphasize this point, because the experiments were not as clear-cut as might be desired.

SUMMARY AND CONCLUSIONS

1. Yellow fever virus has been transmitted from monkey to monkey both by the bites of *Aedes (Ochlerotatus) scapularis* which had fed upon monkeys infected with yellow fever and by the injection of the ground up bodies of such mosquitoes.

2. A fatal infection has been obtained by the injection of the ground up bodies of *Aedes (Ochlerotatus) serratus*, which had previously fed on an infected monkey, and a mild infection has been secured by the similar injection of *Aedes (Taeniorhynchus) taeniorhynchus*.

3. No definite infection has been secured either by the bites or by the injection of *Culex quinquefasciatus (C. fatigans)*. However, some of the experimental animals bitten by this species have been relatively immune following inoculations of blood or tissues containing virus.

REFERENCES

1. Bauer, Johannes H., *Amer. Jour. Trop. Med.*, 1928, **8**, 261.
2. Reed, Walter, Carrol, James, and Agramonte, A., Paper read at Sixteenth Annual Meeting of the Association of American Physicians, Washington, 1901. "In Yellow Fever, a Compilation of Various Publications." Senate Document No. 822, 1911, 118.
3. Marchoux, E., and Simond, P. -L., *Ann. Inst. Pasteur*, 1906, **25**, 23.
4. Davis, Nelson C., and Shannon, Raymond C., *J. Exp. Med.*, 1929, **50**, 793.
5. Dyar, Harrison G., "The Mosquitoes of the Americas," *Carnegie Institution of Washington, Pub. No. 387*, 1928.
6. Peryassu, Antonio Goncalves, "Os Culicideos do Brazil," Rio de Janeiro, 1908.