LABORATORY TRANSMISSION OF OROPOUCHE VIRUS BY *CULEX QUINQUEFASCIATUS* SAY[1]

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INTRODUCTION

Oropouche (ORO) virus is presently the leading cause of urban epidemics of arboviral disease in the Amazon Basin. Results from detailed epidemiologic and entomologic studies during several such epidemics, as well as laboratory transmission studies, provide clear evidence that the biting midge *Culicoides (C.) paraensis* (Goeldi) is the principal vector of Oropouche fever in urban areas *(1-6)*. As such, it is the first arbovirus of known public health importance vectored by *Culicoides*. However, the isolation of ORO virus from three pools of *Culex (Cx.) quinquefasciatus* Say mosquitos and the occurrence of human cases in urban areas with dense pop-

ulations of *Cx. quinquefasciatus*, as well as *C. paraensis*, raised the possibility that this common urban mosquito could be a vector of ORO virus *(1, 3, 6)*. This article reports our attempts to demonstrate and quantify the efficiency of biological and mechanical transmission of ORO virus with *Cx. quinquefasciatus* in the laboratory.

MATERIALS AND METHODS

Mosquitoes

Adult *Cx. quinquefasciatus* females used in the transmission studies were collected from various sites in Belém, Pará, Brazil, during 1975 and 1978. The first two biological transmission experiments were conducted with colonized *Cx. quinquefasciatus* from laboratory colonies begun with wild-caught specimens collected in 1975. The third biological transmission experiment was conducted with laboratory populations from the F_1 generation of wild-caught *Cx. quinquefasciatus* females collected in 1978. All the mosquitoes were maintained in the laboratory at 26.5°C with 95% relative humidity and 12 hours of light alternating with 12 hours of darkness. *Cx. quinquefasciatus* adults were provided with a 10% sucrose solution.

Recently emerged adult mosquitoes were held three to four days before being allowed to feed on a viremic hamster. The sucrose solution was removed from the mosquito holding cages 24 hours before each attempt at blood feeding. Oviposition containers also were removed during blood feeding attempts, but were available to the test populations at all other times.

Virus Strains and Assays

The methods used for virus isolation, virus identification, and serologic testing have been described previously *(1, 4, 7)*. The Belém prototype of ORO virus (Be An 19991) was used in all the tests. Information about this strain, including its passage history, has been provided elsewhere *(5)*.

Biological Transmission

Adult *Cx. quinquefasciatus* females were offered an infectious blood meal by placing a restrained viremic hamster in a one-cubic-foot mosquito cage containing 200 to 350 adult mosquitoes. Following 90 minutes of exposure the viremic hamster was removed, and all the blood-engorged mosquitoes were collected and placed in a separate holding cage. All of the blood feeding attempts were conducted during the dark phase of the photoperiod. Groups of mosquitoes that fed on different viremic hamsters were kept separate from one another, and all blood-engorged mosquitoes were considered potentially infected.

Young Syrian golden hamsters (21–23 days old) were used to demonstrate virus transmission. These hamsters were exposed individually to one or more mosquitoes on days 3 to 21 following the mosquitoes' infectious blood meal. The mosquitoes used in each of these transmission trials were cryopreserved at −70°C and assayed for virus content at a later date.

Each test hamster was utilized only once during the series of transmission trials. The hamsters fed on by potentially infected mosquitoes were isolated after their exposure and were observed during the next 21 days for signs of illness. Sera from individual hamsters that survived the observation period were tested for antibodies to ORO virus by plaque reduction neutralization.

Mechanical Transmission

Using the interrupted blood meal method, two test runs were conducted to demonstrate mechanical transmission of ORO virus by *Cx. quinquefasciatus*. To begin with, a large number of mosquitoes (over 50 in each run) were aspirated from a viremic hamster before completing their blood meals and were transferred to a holding cage. Next, a virus-susceptible hamster was exposed to that population for about one hour. This hamster was then removed and a second hamster was placed in the cage, where it remained until the next morning. The viremic hamster used in one test run was circulating $10^{8.7}$ suckling mouse 50% lethal doses of ORO virus per ml ($10^{8.7}$ $SMLD_{50}$/ml), while the hamster used in the other test run was circulating $10^{9.2}$ $SMLD_{50}$/ml of ORO virus. Observations were made with the aid of a flashlight to verify that the potentially infectious mosquitoes probed or fed on each of the four recipient hamsters used in the two runs. The hamsters were then observed for 21 days and blood samples were assayed for neutralizing antibodies to ORO virus.

RESULTS

Biological Transmission

Results of the first of the three biological transmission experiments, involving two groups of *Cx. quinquefasciatus* mosquitoes, are presented in Table 1. The first and second mosquito groups fed on hamsters that were circulating $10^{9.9}$ and $10^{9.7}$ $SMLD_{50}$/ml of ORO virus, respectively. However, none of the recipient hamsters fed upon by the mosquitoes became ill, and only one of 16 animals developed neutralizing antibodies. The single animal that experienced seroconversion had been fed upon by 21 mosquitoes on day 8 following the mosquitoes' infectious blood meals.

In the second experiment (see Table 2), a group of four-day-old *Cx. quinquefasciatus* fed on a hamster circulating $10^{9.5}$ $SMLD_{50}$/ml of virus and another group of three-day-old mosquitoes fed on a viremic hamster with a titer of $10^{9.7}$ $SMLD_{50}$/ml. Either one or both groups were then allowed to feed on normal hamsters 15, 16, and 21 days after feeding on the viremic hamsters. Oropouche virus was recovered from one hamster that received bites from 33 mosquitoes on day 21. No other hamsters exposed to either group of mosquitoes became infected. Immediately after the second animal exposure, 16 days after the mosquitoes' infectious blood meal, 30 potentially infected mosquitoes were collected and assayed individually for the presence of virus. Oropouche virus was recovered from a single specimen. On day 22 another 15 mosquitoes were assayed, but no Oropouche isolates were obtained.

Summary data from the third experiment appear in Table 3. Mosquitoes used in this experiment were obtained from the F_1 generation of wild-caught *Cx. quinquefasciatus* so as to preclude any genetic changes in susceptibility that might have resulted from prolonged colonization. Three groups of mosquitoes engorged themselves on hamsters circulating $10^{6.3}$, $10^{7.0}$, and $10^{7.1}$ $SMLD_{50}$/ml of ORO virus. These potentially infected mosquitoes were then exposed to a series of normal hamsters on days 7, 14, and 21 after their infectious blood meals. In all, 29 normal

TABLE 1. Results of the first experiment attempting to transmit Oropouche (ORO) virus from infected to normal hamsters with *Culex quinquefasciatus*. Normal hamsters were exposed simultaneously to two groups of mosquitoes on scheduled days after the mosquitoes had engorged themselves on viremic hamsters.

| Days elapsed between the mosquitoes' infectious blood meals and exposure | Virus titer of infectious blood meal | | | |
| | $10^{9.9}$ SMLD$_{50}$/ml | | $10^{9.7}$ SMLD$_{50}$/ml | |
	No. of mosquitoes fed	Transmission results (+ or −)[a]	No. of mosquitoes fed	Transmission results (+ or −)[a]
3	14	—	17	—
4	24	—	30	—
6	28	—	33	—
8	21	+	19	—
10	4	—	8	—
12	5	—	14	—
14	17	—	23	—
16	9	—	13	—

[a] Transmission results were determined by virus isolation attempts from sick animals or by neutralization tests conducted with serum samples collected from the hamsters 21 days after exposure to the potentially infected mosquitoes The single positive result was obtained by the neutralization test.

TABLE 2. Results of the second experiment attempting to transmit Oropouche (ORO) virus from infected to normal hamsters with *Culex quinquefasciatus*.[a] Normal hamsters were exposed to one or two groups of mosquitoes 15, 16, or 21 days after the mosquitoes had fed on viremic hamsters.

| Days elapsed between the mosquitoes' infectious blood meals and exposure | Virus titer of infectious blood meal | | | |
| | $10^{9.7}$ SMLD$_{50}$/ml | | $10^{9.5}$ SMLD$_{50}$/ml | |
	No. of bites	Transmission results[b]	No. of bites	Transmission results[b]
15	49	—	—	—
16	—	—	50	—
21	10	—	33	+[c]

[a] The mosquitoes were three or four days old when exposed to the viremic hamsters.
[b] Transmission results were determined by virus isolation attempts from sick animals or by neutralization tests on serum samples collected 21 days after exposure to the potentially infected mosquitoes.
[c] Oropouche virus infection was confirmed by virus isolation and serologic identification.

hamsters were exposed to the group of mosquitoes that fed on the infected hamster with the lowest virus titer ($10^{6.3}$ SMLD$_{50}$/ml of ORO virus), and 59 normal hamsters were exposed to the other two groups of mosquitoes.

The number of bites received during the exposure period by any one normal hamster varied from one to 12, with most receiving more than five bites each. None of these animals demonstrated any subsequent signs of virus infection, nor were any seroconversions detected by plaque reduction neutralization tests.

TABLE 3. Results of the third experiment attempting to transmit Oropouche (ORO) virus to suscepti-
ble hamsters with *Culex quinquefasciatus* fed on infected hamsters circulating $10^{6.3}$ to $10^{7.1}$
$SMLD_{50}/ml$ of Oropouche virus.

Days elapsed between the mosquitoes' infectious blood meals and exposure	No. of susceptible hamsters exposed	No. of mosquitoes feeding on susceptible hamsters	ORO virus transmission results (+ or −)[a]
7	27	86	−
14	33	55	−
21	28	141	−

[a] Transmission results were determined as in the previously described biological transmission experiments (see Tables 1 and 2).

In addition, 282 mosquitoes that fed on the uninfected hamsters during this experiment were processed for isolation of ORO virus. One isolate was obtained from a pool of eight mosquitoes that had fed on the hamster circulating $10^{6.3}$ $SMLD_{50}/ml$ of virus.

Mechanical Transmission

As already described, mechanical transmission of ORO virus from viremic to normal hamsters was attempted during two separate test runs. However, none of the four normal hamsters exposed to the test mosquitoes exhibited symptoms of virus infection, nor were any antibodies to ORO virus detected in blood samples collected from these hamsters 21 days after exposure to the potentially infected mosquitoes.

DISCUSSION AND CONCLUSIONS

The possible role of *Cx. quinquefasciatus* as a vector of ORO virus has been a subject of considerable speculation, based mainly on observations made during investigation of epidemics. The transmission studies reported here were conducted to evaluate the vector poten-

tial of *Cx. quinquefasciatus* under laboratory conditions. These results provide the first demonstration that *Cx. quinquefasciatus* is capable of animal-to-animal transmission of ORO virus. They also indicate (because of the low percentage of successful transmissions and the paucity of mosquito infections under optimized conditions) that *Cx. quinquefasciatus* is not an efficient vector in the laboratory.

Successful ORO virus transmission was found to have occurred in two of 108 attempts, and virus was recovered from two of 327 mosquitoes that fed on viremic hamsters. The latter data indicate an ORO virus infection ratio of 1:163 for our test populations of *Cx. quinquefasciatus*.

Since the titers of ORO virus in our donor hamsters were usually much higher than those reported for humans with Oropouche fever ($10^{5.2}$ to $10^{7.3}$ $SMLD_{50}/ml$), it is reasonable to assume that the infection rates for field populations of *Cx. quinquefasciatus* would

generally be much lower than our experimental rates (8). In fact, the field infection rate during epidemics, calculated from unpublished surveillance data, has been estimated at one in 18,037.

High human infection rates have been documented during epidemics of Oropouche fever (1, 2). Since person-to-person transmission does not occur, a high attack rate implies the occurrence of (1) efficient biological transmission, (2) efficient mechanical transmission, or (3) a combination of the two. Our experimental data indicate that *Cx. quinquefasciatus* is unlikely to be the principal ORO virus vector in urban epidemics. In addition, our failure to show mechanical transmission in two separate laboratory tests provides evidence that *Cx. quinquefasciatus* is probably not a mechanical vector of this virus in the field. (Negative mechanical transmission results were obtained from similar tests with *Culicoides paraensis* (5). Thus, we believe that mechanical transmission is not an explanation for high attack rates of Oropouche fever during epidemics.)

All in all, the work reported here has demonstrated that *Cx. quinquefasciatus* is a notably inefficient vector of ORO virus. This finding is consistent with the hypothesis that *C. paraensis* is the principal vector of ORO virus in urban areas of the Amazon Basin.

SUMMARY

Experimental studies were conducted to determine the vector potential of *Culex quinquefasciatus* Say for Oropouche (ORO) virus. Mosquitoes from a laboratory colony and mosquito offspring of field-collected adults were allowed to feed on viremic hamsters. The mosquitoes were held for various intervals of time following the infectious blood meal and were then fed on normal hamsters.

The efficiency of virus transmission by this method was low. Despite the fact that 108 normal hamsters were exposed to potentially infected mosquitoes, ORO virus was recovered from only one hamster; another hamster experienced seroconversion to ORO virus. The minimal infection rate of *Cx. quinquefasciatus* mosquitoes that fed on hamsters circulating $10^{6.3}$ to $10^{9.9}$ suckling mouse 50% lethal doses of ORO virus per ml was found to be 1:163 (2/327). Tests using the interrupted blood meal method failed to demonstrate mechanical transmission of ORO virus from infected to normal hamsters.

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