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TRANSMISSION OF ARBOVIRUSES TO HAMSTERS BY THE BITE OF NATURALLY INFECTED *CULEX* (*MELANOCONION*) MOSQUITOES*

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A number of authors have called attention to the probable role of *Culex* mosquitoes of the subgenus *Melanoconion* in the natural transmission of Venezuelan equine encephalitis (VEE), Group C, and Guamá viruses.[1-4] However, the evidence presented has been for the most part circumstantial, based mainly on the frequency with which *Melanoconion* mosquitoes are found harboring these viruses in nature, and on the close association that exists between these culicines and the vertebrate hosts of the viral agents. The exception is a report[5] of single successful transmissions of Oriboca and Guamá viruses to suckling white Swiss mice by naturally infected *Culex* (*Melanoconion*) *portesi* Sennevet and Abonnenc (= *Culex* sp. No. 9 of Trinidad) and *Culex* (*Melanoconion*) *taeniopus* Dyar and Knab, respectively.

Recent investigations[4] have demonstrated that a number of species of *Culex* (*Melanoconion*) readily engorge on golden hamsters (*Mesocricetus auratus*) when these rodents are exposed in the field as sentinel animals. It was further shown that the golden hamster is very susceptible to VEE and some group C arboviruses, usually developing rapidly fatal infections.

The present report deals with results of experiments set up to determine if *Culex* (*Melanoconion*) mosquitoes, and *C. vomerifer* in particular, are important natural vectors of arboviruses in the Almirante study area, by obtaining a convincing number of laboratory transmissions from naturally infected mosquitoes to golden hamsters.

MATERIALS AND METHODS

Collection of Mosquitoes

Mosquitoes for this work were collected in the vicinity of Almirante, Panamá, where intensive studies on the ecology of arboviruses have been in progress since 1959.[1] Human beings were used as bait and were exposed both in the forest canopy and on the ground. *Culex* (*Melanoconion*) mosquitoes that approached to bite the collectors were captured alive by means of a suction tube and transferred to 6- or 8-oz jars lined with a layer of plaster of Paris, which was moistened just before collecting started. Mosquito collecting was carried out 5 nights a week from 1800 to 2100 hours, in the vicinity of stations where activity of group C, VEE, and Guamá viruses had been intense in the past, as judged by isolations from sentinel animals, wild rodents, and mosquitoes.[1, 4] Captured insects were supplied with freshly prepared sugar solution nightly by means of a cotton wick and were kept at room temperature in the field until ready for shipment to the central laboratory. Three times a week, jars containing insects were flown to Panamá City in special thermos containers with just enough ice to maintain females at rest within the jars. Collections left Almirante at 0700 and arrived at the laboratory between 1300 and 1500 on the same day.

Experimental Procedures

As soon as collections were received in the insectary, all cotton wicks were removed from the jars and replaced by dry cotton plugs. The layer of plaster of Paris in each jar was moistened again by means of a capillary pipette, and jars were set aside for 24 hours in a room at a constant temperature of 76°F and 90 to 94% relative humidity. After this period, designed to permit at least partial digestion of ingested sugar, the mosquitoes were removed and separated according to species into 1-cubic-foot bobbinet cages, no more than 70 specimens per cage being allowed. Each cage was then given a number and covered with a pair of wet towels.

Every afternoon, Monday through Friday, at 4 p.m., a number of golden hamsters bred in a tightly screened mosquito-free laboratory room were placed in wide-meshed wire cages 7½ × 2½ × 2½ inches. Each caged hamster was immediately hung inside a bobbinet cage containing wild-caught mosquitoes and left there overnight. At 0730 the following morning ham-

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sters were transferred to stainless-steel holding cages with screened tops. These cages were kept in a tightly screened air-conditioned room where possibilities were remote for mosquitoes to enter. Engorged mosquitoes in the bobbinet cages were then removed and placed in oviposition cages for colonization attempts. Twice during the day all cages containing unengorged mosquitoes were examined for dead or moribund insects, which were removed, checked for correct identification and frozen at dry-ice temperatures, to be used later for attempts at virus isolation. Each lot of mosquitoes was given an opportunity to feed on the same hamster during 3 or 4 consecutive nights, after which the unengorged insects left were killed, and a fresh lot of 70 mosquitoes of the same species was introduced into the bobbinet cage.

As soon as a hamster showed obvious signs of illness it was bled to death, and pieces of heart, liver, and brain were removed for attempts at virus isolation. A complete autopsy was then performed, and samples of most organs were fixed for pathological studies. All hamsters that survived at least 1 month were bled to death, and the serum was used to determine whether antibodies against certain arboviruses had developed, thus demonstrating probable transmission of these viruses by the bites of naturally infected mosquitoes. Transmission experiments were begun during February 1966, and the results herein reported cover work until 1 October 1966.

For attempts at virus isolation from suspensions of mosquitoes, insects were grouped by species in pools of 30 or less and ground in a mortar with sterile alundum and 2 cc of diluent consisting of 0.75% bovine albumin in phosphate-buffered saline solution pH 7.2, 500 units of penicillin, and 500 μg of streptomycin per ml. The resulting suspension was centrifuged in the cold at 2,500 rpm for 20 minutes, and the supernatant fluid (0.02ml) was inoculated both intracerebrally (i.c.) and intraperitoneally (i.p.) into each of a group of seven 2- to 5-day-old white Swiss mice, which were kept under observation for 15 days.

The techniques utilized for virus isolation from exposed hamsters, for virus identification, and for serologic tests were essentially the same as previously described.[4] The following antigens were used for testing the sera of hamsters that survived at least 1 month:

TABLE 1

Transmission to hamsters of naturally acquired arboviruses by Culex (Melanoconion) *mosquitoes (February to October 1966)*

Species of wild-caught mosquito	No. of hamsters exposed to mosquito bites	No. of mosquitoes given access to hamsters	No. of mosquitoes that engorged on hamsters	No. of hamsters that sickened or died after mosquito bites	Transmissions confirmed by virus isolations from dead or sick hamsters		Virus transmissions confirmed by serologic tests on surviving hamsters					Transmission rate: viruses per engorged mosquitoes
					Ossa virus	Madrid virus	Ossa virus	Madrid virus	Patois virus	Ghana virus	Mayaro virus	
C. nemerifer	46	11,598	3,987	15	5	4	1	1	1	5	1	1:222
C. taeniopus	3	37	9	2	1	—	—	—	—	—	—	1:37
C. epithelopus	2	81	42	0	—	—	—	—	—	—	—	—
C. crybda	2	108	23	1	—	—	—	—	—	—	—	—
C. menytes	1	5	0	0	—	—	—	—	—	—	—	—
Totals	54	11,820	4,061	18	6	4	1	1	1	5	1	

TABLE 2

Results of positive serologic tests in hamsters confirming transmissions by the bites of wild-caught C. vomerifer

Specimen no. of positive hamsters*	Reciprocal of HI titer†				CF titers‡		
	Ossa antigen	Madrid antigen	Guamá group antigen	Mayaro antigen	Guamá antigen	Mayaro antigen	Patois antigen
LSH-13					32+		
LSH-15				20		32+	
LSH-18		40	40		32+		16
LSH-29	20		80		32+		
LSH-42			20		32+		
LSH-45					32+		

* Total number of hamsters tested: 36.

† Other antigens included in tests with negative results: VEE, EEE, Mucambo, Pixuna, Una, Ilhéus, Bussuquara, YF, Dengue II, Powassan, Nepuyo, Itaqui, Caraparu, Oriboca, Marituba, Maguari, Chagres, Icoaraci, and Turlock.

‡ Other antigens included in tests with negative results: VEE, Una, Ossa, Cache Valley, and California.

1. Hemagglutination-inhibition (HI) test. Group A: VEE, Eastern equine encephalitis (EEE), Mucambo, Pixuna, Mayaro, Una. *Group B:* Ilhéus, Bussuquara, yellow fever (YF), Dengue II, Powassan. *Group C:* Ossa, Madrid, Nepuyo, Itaqui, Caraparu, Oriboca, Marituba. *Group Guamá:* Catú. *Others:* Maguari, Chagres, Icoaraci, and Turlock.

2. Complement-fixation (CF) test. VEE, Una, Ossa, Patois, Cache Valley, Guamá, and California.

RESULTS

A total of 11,829 wild-caught mosquitoes, of which 11,598 (98%) were *Culex vomerifer*, were given a chance to feed on 54 hamsters (Table 1). Of the *C. vomerifer* females, 3,987 became engorged with blood and 15 of the 46 hamsters exposed to the bites of these mosquitoes either sickened or died. Two of the hamsters exposed to the bites of *C. taeniopus*, and one fed on by *C. crybda*, also perished.

Viruses isolated from sick or dead hamsters. Ten virus isolates were obtained from tissues of the 18 hamsters that sickened or died after being fed on by mosquitoes. Eight of these strains were isolated from either serum or heart extract, the other two from both liver and brain extracts. All of the isolates had a short incubation period of 2 to 3 days after intracerebral inoculation into suckling mice. Serologic identification showed that six of these isolates were Ossa virus, and the other 4 were Madrid virus, both of which belong to the group C of arboviruses and are pathogenic to man. Of the six hamsters that yielded Ossa virus, five had been bitten by *C. vomerifer* and one by *C. taeniopus*. The four Madrid isolates came from hamsters fed on by *C. vomerifer*. No attempt was made to determine if any of the infections were the result of a mixture of two or more viruses.

Demonstration of postinfection antibodies. The sera of 36 hamsters that survived were tested for HI and CF antibodies. As seen in Table 1, nine of these rodents bitten by *C. vomerifer* produced antibodies to certain arboviruses, five to Guamá and one each to Ossa, Madrid, Patois, and Mayaro. Details of the positive reactions are given in Table 2.

Rate of transmission. As may be noted in Table 1, *C. vomerifer* was the only species used in sufficient numbers to permit conclusions regarding its potential as a vector for a number of arboviruses. If viruses isolated from hamsters are added to infections demonstrated by presence of antibodies, there were at least 18 arboviral infections transmitted to hamsters through the bite of *C. vomerifer*. This gives a rate of one arbovirus transmitted for every 222 mosquitoes that engorged on the hamsters, which is a much higher infection rate than has been obtained in the Almirante area by the standard techniques of

TABLE 3

Transmissions by C. vomerifer *of naturally acquired arboviruses by month*

Month	Ossa virus	Madrid virus	Patois virus	Guamá virus	Mayaro virus	Total viruses transmitted	Engorged mosquitoes	Transmission rate: virus per engorged mosquitoes
February	1	—	—	—	—	1	60	1:60
March	—	1	—	—	—	1	173	1:173
April	—	—	—	—	—	—	—	—
May	1	—	—	—	—	1	298	1:298
June	2	2	1	1	1	7	1,377	1:195
July	3	1	—	2	—	6	988	1:164
August	—	1	—	1	—	2	666	1:333
September	—	—	—	—	—	—	425	0:425
Totals	7	5	1	4	1	18	3,987	1:222

virus isolation through inoculation of wild-caught mosquitoes into suckling mice.[1, 6]

Monthly transmission rates. Table 3 presents the monthly rates of transmission of the different arboviruses. The transmissions were well distributed from February through July, but a definite drop appearing in August became accentuated in September, the driest month of the year.[1] Whereas group C viruses appeared during all months in which transmissions were obtained, Guamá virus transmissions were restricted to June, July, and August.

Results of mosquito inoculations into mice. More than 4,000 unengorged, dead, or moribund *C. vomerifer* females (the great majority dead) removed from the transmission cages were inoculated into mice in 153 different pools, with negative results. All engorged mosquitoes in the cages were used to obtain eggs needed for attempts at colonization of the species.

DISCUSSION

The virus isolates (five Ossa and four Madrid) obtained from sick or dead hamsters bitten by wild-caught *Culex vomerifer* females were undoubtedly the result of transmission through the bite of these particular mosquitoes. The hamsters, when not exposed to the mosquito bites in the bobbinet cages, were held in individual stainless-steel cages with screened tops and maintained in a mosquito-proof room, different from that in which caged mosquitoes were held, so that there was no opportunity for insects to bite them.

The positive serologic tests obtained in hamsters bitten by *C. vomerifer* also appear to be the result of transmissions by bite from these mosquitoes, although no base-line serum was obtained before the experiments to demonstrate actual serologic conversions. Reasons for this conclusion follow:

1. All hamsters used in these experiments were bred in our laboratory in Panamá City, in an air-conditioned, tightly screened room, where opportunities for mosquitoes to enter are very remote.

2. None of the arboviruses whose antigens gave positive serologic results have ever been demonstrated in Panamá City or within a radius of 20 miles from the laboratory, so it is difficult to imagine that such infections were the result of mosquito bites, other than those of insects used in the experiments.

3. During the experiments, hamsters were handled in the manner described above, leaving no opportunity for any mosquito, other than the experimental ones, to feed on the hamsters.

4. All five viruses whose antigens gave positive reactions have been isolated from the Almirante study area where the wild-caught mosquitoes that fed on the hamsters were captured, and four of the five are commonly encountered there.

The lack of isolations from dead *C. vomerifer* removed from the exposure cages is understandable if we consider that insects were held at the very high relative humidities that favor quick decomposition of dead mosquitoes. That such decomposition took place is indicated by the unusual number of contaminated mosquito suspensions encountered despite the use of antibiotics in the diluent. However, from 1 July until 30 September, live *C. vomerifer* mosquitoes obtained from the same collecting pools as those that

transmitted the viruses to hamsters were triturated and inoculated into suckling mice in connection with a different project,[6] and 10 isolates were obtained. Of these, five proved to be Guamá, four were identified as Ossa or Madrid viruses, and one was VEE. These results show conclusively that at least Guamá, Ossa, and Madrid viruses, which represented 16 of the 18 transmissions obtained, were active in the *C. vomerifer* population during the transmission experiments.

From the data presented the conclusion has been reached that *Culex* (*Melanoconion*) *vomerifer* Komp is a proved and important vector of Ossa, Madrid, and Guamá viruses, the first two of which are well-known pathogens to human beings in the Almirante area.

The lack of transmissions of VEE virus came as a definite surprise, since *C. vomerifer* has been found frequently harboring this viral agent in the past,[1, 7] and the virus was known to be active in the area, as witnessed by several isolations obtained from sentinel hamsters[4] and two isolations yielded by *Melanoconion* mosquitoes.[6] It is difficult to believe that if *C. vomerifer* is an important vector of VEE in the Almirante area, the bites of at least 4,000 specimens of this species would fail to produce infections in such a susceptible host as the golden hamster, especially at a time when the virus was being transmitted in the same areas where the mosquitoes were captured.[4] The answer to this problem will have to await results of carefully controlled transmission experiments using laboratory-reared mosquitoes.

The role of other species of *Culex* (*Melanoconion*) mosquitoes in the transmission of these arboviruses cannot yet be assessed, as too few specimens were available for the transmission experiments. *C. taeniopus*, which in previous years reached very high population levels and was found infected with VEE,[1, 7] maintained extremely low densities throughout the period of the experiments, so that only 37 specimens could be used to bite the hamsters. These mosquitoes, however, were responsible for a single transmission of Ossa virus. *C. taeniopus*, therefore, should be looked upon with suspicion as a vector of Ossa and perhaps of other arboviruses in years when high population peaks are attained.

The single transmission of Mayaro virus by *C. vomerifer* was unexpected, as this virus appears to be rare in Almirante. Proof of its previous presence there was a single isolation from *Psorophora ferox* mosquitoes.[1]

The high rates of infection demonstrated in wild populations of *Culex* (*Melanoconion*) mosquitoes by the transmission method described above points to this method as a feasible routine procedure to be used instead of direct inoculation of mosquitoes into mice. This is of particular significance considering the fact that at least some of the isolations obtained from inoculation of mosquitoes may be dead-end infections. Unless carefully weighed, results obtained from such isolations could lead to erroneous conclusions regarding the vector potential of the species of mosquito found infected. In the transmission method all the virus isolations obtained are the result of direct transmission by bite, thus offering indisputable evidence of the ability of a species of mosquito to pick up arboviral infections in nature and to transmit them by its bite to a susceptible vertebrate host.

SUMMARY

A total of 11,598 wild-caught females of *Culex vomerifer* and 229 specimens of other species of *Culex* (*Melanoconion*) mosquitoes captured in Almirante, Panamá, were allowed to feed on 54 laboratory-bred hamsters. Of the *C. vomerifer* given access to hamsters, 3,987 were observed to engorge on blood. A total of 18 transmissions of arboviruses were demonstrated in hamsters bitten by *C. vomerifer* mosquitoes, and one strain of Ossa virus was obtained from a hamster fed on by *C. taeniopus*. Nine of the transmissions by *C. vomerifer*, five of Ossa virus, and four of Madrid virus, were identified by isolation of the agents from the tissues of hamsters bitten by these mosquitoes. The other nine transmissions, five of Guamá, and one each of Ossa, Madrid, Patois, and Mayaro viruses, were demonstrated and identified by subjecting the serum of surviving hamsters to HI and CF tests against antigens of 27 different arboviruses. All five viruses transmitted were previously known to occur in the Almirante area, and four of them are commonly encountered there. It is concluded that *C. vomerifer* is a proved important vector of Ossa, Madrid, and Guamá viruses in Almirante, the first two of which are important pathogens to human beings in the area. It, however, does not seem to be an efficient vector of VEE in the study area even though it is frequently found

harboring this viral agent. The species *C. taeniopus* is suspected to be a vector of Ossa and perhaps of other arboviruses when high population peaks are attained.

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