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DETECTION OF ARBOVIRUSES BY SENTINEL HAMSTERS DURING THE LOW PERIOD OF TRANSMISSION*

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INTRODUCTION

Previous investigations conducted at Almirante, an area of tropical rain forest in northwestern Panamá, indicated that there were definite seasonal fluctuations in arbovirus activity. The months from February to April of any given year proved to be the period of lowest transmission of arboviruses, as detected by isolations from insect vectors, from wild animals, and from sentinel mice.[1] Of 33 virus strains recovered from the latter in 1965–66, none was isolated during the months of February and March.[2]

Young adult golden hamsters (*Mesocricetus auratus*) were successfully used as sentinels to detect Venezuelan equine encephalitis (VEE) virus in Veracruz, Mexico, in July and August 1963.[3] Laboratory experiments have shown that this animal is especially susceptible to VEE and to many group C arboviruses[4] that are known to be present in the Almirante study area.[5, 6] This report presents results of a field experiment designed to determine whether adult hamsters, which require little care during exposure, could be used as sentinels at Almirante to detect arboviruses during the unfavorable transmission season when virus activity was found to be so low that it could not be detected by more conventional methods.

MATERIALS AND METHODS

Exposure of sentinel animals. Detailed description of the Almirante study area has been reported earlier.[1] The exposure of hamster sentinels was initiated in February 1966 as a 1-year experimental program. The present report describes the first month of activities.

The five localities selected in the vicinity of Almirante for exposure of hamsters were as follows:

Patoistown stations. Established on the periphery of human habitations in the slum sector of Patoistown. One station was located by a small swamp covered with aroids and heliconias about 100 yards from the nearest dwelling, while a second was placed in the immediate vicinity of a house.

Campamento station. At the edge of a sedge swamp directly behind our field headquarters 2 miles north of Almirante.

Weatherborn station. Located on a low forested hill overlooking a narrow and long open swamp which was bordered on each side by "silica" palms (*Raphia taedigera*).

Bamboo station. In a swampy forest overlooking a large grassy bog surrounded by a thick grove of bamboo.

Toucan station. Deep within an extensive, forested swamp that was dominated by "sangrillo" trees (*Pterocarpus officinalis*) and "silica" palms. This swamp was located about 1½ miles north of Almirante.

Adult golden hamsters 2 to 3 months of age were placed individually in ½-inch mesh galvanized wire cages and hung under small tin roofs prepared by cutting 5-lb lard cans diagonally. The sentinels in Patoistown were placed at ground level, while in four other stations hamsters were exposed both at ground level and in the canopy of the forest. Animals were left until they either sickened, died, or survived the pre-established 1-month period of exposure. Food consisted of pellets of Purina, carrots, and fresh cabbage. No water was made available. Inspectors in charge of caring for the animals visited them twice daily and added food as needed. When a hamster was found sick or dead, it was immediately brought to the field station where half of the brain and small sections of heart and liver were removed by aseptic techniques. The material was immediately refrigerated and shipped by air within 3 days to the laboratory for attempts at virus isolation.

Sentinel mice were also exposed at ground level once a week at these same stations by the use of a technique previously described.[1]

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Mosquitoes Collected from Sentinel Hamsters

During the first days of exposure of each group of hamsters, inspectors visited the animals exposed on the ground every half hour from hours 1800 to 2200 and collected mosquitoes that were feeding on them. Instructions were given to capture only those insects that were actually feeding and that were either partly or fully engorged. Such collections were not intended to measure the population of mosquitoes attracted to the hamsters, but rather to determine the species that were known to have fed on them during the period of exposure.

Laboratory Methods

Initial attempts at virus isolation were carried out in 3- to 5-day-old white Swiss mice, by intracerebral (i.c.) route of injection, 0.02-ml volume of inoculum per mouse being used. Tissue extracts were prepared, 10% w/v, in 0.75% bovine albumin in phosphate-buffered saline solution, pH 7.2, and further diluted 1:5 in the same diluent before inoculation. Each of the tissue extracts was tested for the presence of bacteria. Heart extracts were first inoculated into suckling mice. If negative results were obtained, extracts of liver and brain tissue were then inoculated. Concurrently exposed sentinel mice, as well as those injected with hamster-tissue extracts were kept under observation for a period of 15 days. Mice exhibiting signs of illness were killed for passage into additional litters of suckling mice by the i.c. route, brain tissue as source of passage material being used. Inoculated mice appearing sick within 3 days were exsanguinated, and hemagglutinating (HA) antigens were prepared from serum according to a technique previously described.[7, 8]

Complement-fixing (CF) antigens of the virus isolates were prepared from infected suckling-mouse brains or livers by overnight extraction in borate-buffered saline solution, pH 9.0. Reference virus strains used for comparison of new isolates included Ossa prototype strain,[6] at the 46th mouse passage level, kindly supplied by Dr. Enid de Rodaniche, of the University of Panamá; 10 other previously characterized virus types isolated in Panamá, and 25 additional prototype strains were obtained from The Rockefeller Foundation Virus Laboratories in New York and Belém, and from the Communicable Disease Center in Atlanta. HA antigens for group C arboviruses were prepared from infected suckling-mouse serum by double acetone extraction, whereas HA antigens, other than group C as well as CF antigens of all viruses, were made from sucrose-acetone extracted brain or liver material.[7]

Immune serum for each of the group C type strains and suspected group C isolates was prepared by the intraperitoneal injection of adult mice with two doses of virus suspensions prepared from infected suckling-mouse livers, administered 10 days apart. Formalin-inactivated virus was used for the first injection in some cases. Bleeding for immune serum was performed 10 days after the second injection. Immune sera for viruses other than group C and for some virus isolates were prepared essentially as above, except that brain instead of liver material was used.

Hemagglutination and hemagglutination-inhibition (HI) tests were carried out according to the technique described by Clarke and Casals.[7] Titration of HA antigens was performed at room temperature (22°C to 24°C). In the HI technique, immune sera were treated with kaolin for removal of nonspecific inhibitors and tested with 4 units of antigen. Mixtures of serum-antigen were held at 4°C overnight before the addition of goose erythrocytes. CF tests were performed in plastic plates with a modification of the Fulton and Dumbell microtechnique.[9]

RESULTS

Result of Hamster Exposures

From 15 February to 15 March 1966 21 golden hamsters were exposed in five localities as described above. Encouraging results were obtained as early as a few days after the first group of hamsters was exposed. Table 1 shows the duration of exposure of individual hamsters before illness or death was detected. Twenty of 21 exposed hamsters were found either dead or ill before the exposure period of 1 month was completed, and 65% of them became sick within 10 days after exposure. At the Campamento Station, all five hamsters were either sick or dead in 7 days. Although inspection of the exposed animals was carried out twice a day, 30% of them were found dead.

Isolations of Viruses

Of the 20 hamsters harvested for attempts at virus isolation, 16 were found to be positive. Fourteen isolations were made from heart, one

TABLE 1

Duration of exposure of sentinel hamsters before illness was detected

Station	Hamster no.	Illness	Exposure	
		Sick (S) or Dead (D)	No. of days	Ground or Canopy
Patois-town	BTSH 1	S	10	G
	BTSH 2	D	9	G
	BTSH 5B	No illness	23	G
	BTSH 14	D	26	G
Campa-mento	BTSH 3	S	4	G
	BTSH 6A	S	4	G
	BTSH 10	S	7	C
	BTSH 15	S	4	G
	BTSH 16	S	4	C
Weather-born	BTSH 4	S	3	G
	BTSH 7	S	4	G
	BTSH 11	D	11	C
	BTSH 17	S	4	G
	BTSH 18	D	12	C
Bamboo	BTSH 8	S	23	G
	BTSH 9	S	4	C
	BTSH 21	D	12	C
Toucan	BTSH 12A	S	4	G
	BTSH 13	S	4	C
	BTSH 19	S	4	G
	BTSH 20	D	10	C

from liver, and another from brain tissue. Most of the mice inoculated with tissue suspensions that yielded viruses appeared ill within 2 to 5 days after injection. Isolates were subsequently obtained without any difficulties after the first or second passage.

Preliminary identification was performed by HI tests with infected suckling-mouse serum as the HA antigen. The identity of these isolates was confirmed by CF and cross HI tests. Isolates that possessed no hemagglutinins were tested by the CF technique against known immune sera.

Virus isolates obtained in different stations were identified as follows:

1. *Patoistown Station.* A single isolate (BTSH 1H), identified as Patois virus,[6] was obtained from a hamster placed by the swamp. Animals exposed in the immediate vicinity of a human dwelling yielded two viruses, VEE (BTSH 14H) and Ossa (BTSH 2H), both agents being well-known pathogens to human beings.

2. *Campamento Station.* Three strains of Ossa virus, two from the ground (BTSH 6AH and BTSH 15H) and one from the canopy (BTSH 16H) and a single Madrid virus[5] from the canopy (BTSH 10H) were isolated.

3. *Weatherborn Station.* The following isolates were obtained: one strain of Ossa virus each from the ground (BTSH 4H) and the canopy (BTSH 11L), one VEE virus (BTSH 7H) and one Madrid virus (BTSH 17H) from the ground, and one isolate of Guamá group virus (BTSH 18B) from the canopy.

4. *Bamboo Station.* Two Ossa strains (BTSH 9H, BTSH 21H) were isolated, both from the canopy.

5. *Toucan Station.* Two Ossa strains were recovered, one each from the ground (BTSH 19H) and the canopy (BTSH 20H).

Table 2 shows a comparison of viruses isolated from hamsters exposed on the ground and in the canopy. All sentinels at the first station, Patoistown, were exposed on the ground, where three virus strains were isolated. Of the 13 isolates obtained at the other four stations, six were isolated from hamsters exposed on the ground and seven from canopy-exposed sentinels, showing that virus activity appears to be equally prevalent at the levels of the ground and the forest canopy in the study area.

Detection of Mixed Infections

Since all sentinel hamsters were left in the field until they either sickened or died, the occurrence of multiple infections in individual hamsters was

TABLE 2

Arboviruses detected from sentinel hamsters exposed at different forest levels

Virus isolates	No. of isolates		
	Total	Ground	Canopy
Group A			
VEE	2	2	0
Group C			
Ossa	10	5	5
Madrid	2	1	1
Patois	1	1	0
Group Guamá			
Guamá	1	0	1

TABLE 3

Prevalence of mosquitoes collected from sentinel hamsters

Mosquito species	No. collected
Culex (Melanoconion) vomerifer Komp	391
Mansonia (Rhynchotaenia) venezuelensis (Theobald)	363
Culex (Melanoconion) opisthopus Komp	68
Mansonia (Rhynchotaenia) arribalzagae (Theobald)	37
Culex (Melanoconion) crybda Dyar	24
Culex (Culex) nigripalpus Theobald	22
Culex (Melanoconion) taeniopus Dyar and Knab	19
Culex (Melanoconion) new species	18
Mansonia (Rhynchotaenia) nigricans (Coquillett)	14
Aedes (Ochlerotatus) angustivittatus Dyar and Knab	11
Psorophora (Grabhamia) cingulata (Fabricius)	8
Psorophora (Janthinosoma) ferox (Humboldt)	4
Culex (Culex) declarator Dyar and Knab	4
Culex (Culex) coronator Dyar and Knab	2
Culex (Melanoconion) menytes Dyar	1
Mansonia (Mansonia) indubitans Dyar and Shannon	1
Aedes (Ochlerotatus) serratus nubilus Theobald	1

quite possible. Newly isolated strains from these hamsters were, therefore, tested for the presence of two or more viruses as follows:

1. Both liver and brain antigens of each of the isolates were tested by the CF technique with immune sera from 18 arboviruses previously encountered in the Almirante area.

2. Immune sera prepared from each of the isolates were checked by HI tests with 25 arbovirus antigens belonging to different groups and by CF with six other antigens.

Results of these tests revealed that a mixed infection did occur in at least one instance. The only Guamá isolate obtained (BTSH 18B) was shown to be mixed with Ossa virus. Preliminary attempts to separate these two viruses were, however, unsuccessful.

Results of exposure of sentinel mice. A total of 48 litters of suckling mice exposed for 12-hour periods once a week in the same stations as the hamsters yielded no arboviruses from January through March 1966.

Results of mosquito collections. A total of 991 mosquitoes were collected (Table 3). *Culex (Melanoconion) vomerifer* and *Mansonia (Rhynchotaenia) venezuelensis* were among the most prevalent species found feeding on hamsters.

DISCUSSION

The efficiency of the method of field exposure of adult hamsters for the detection of VEE and group C arboviruses has been amply demonstrated by the preliminary results presented above. The fact that in a 1-month period, between 15 February and 15 March, a total of 16 arboviruses was isolated from the sentinel hamsters, while none had been obtained over a 6-year period during the same season, either from sentinel mice exposed on a weekly basis, from wild rodents captured alive, or from mosquitoes inoculated into baby mice, points out that the sentinel-hamster technique was highly successful for the detection of VEE and group C arboviruses during low periods of transmission. This technique appears to be more practical and less expensive than the use of sentinel mice. In addition, it offers excellent possibilities for certain epidemiologic investigations of these viruses, such as comparative measurements of the intensity of virus activity in different ecologic niches or sectors of a town. It would also serve as an efficient tool to gauge quickly the existing dangers to man of acquiring infections with these pathogenic agents in areas where no previous knowledge exists about the activity of these viruses.

Results obtained during the first month of operation of the hamster-exposure program have helped fill a gap in our knowledge of the ecology of VEE in Almirante. While it has been suspected that the Venezuelan virus was enzootic and ever present in the area, the lack of isolations from sentinel mice, mosquitoes, and wild mammals during February and March in previous years posed the question as to the virus activity during this period. This question has been partly answered by the results.

The intense activity of group C arboviruses, and of Ossa virus in particular, revealed by the sentinel-hamster program, was indeed surprising, as these agents were previously known in Almirante only by occasional isolations from wild rodents, man, sentinel mice, and only rarely from

mosquitoes. Whether this intense activity is the result of unusual circumstances or actually represents a normal occurrence not detected by other methods will have to be determined.

After the initial exposures reported here, it was realized that feed-back of virus from sentinel hamsters to wild mosquitoes could be of such magnitude as to obscure the picture of the natural dynamics of virus transmission. In order to avoid the establishment of such artificial local foci of virus activity, successive exposures of sentinels were spaced at 2-month intervals and alternated between two exposure sites separated by at least 800 ft, at each of the sentinel stations described above. In this manner, no hamster was exposed in a place previously used as a sentinel site for at least 4 months.

The masking of a virus that is in a period of marginal transmission by a virus undergoing intense activity and producing rapidly fatal infections in the hamsters is a possibility that should be contemplated in the use of this method. In our study area such a problem arose with the possible masking of VEE by Ossa virus. Experiments are now in progress in which hamsters immunized against Ossa virus are exposed together with normal hamsters, in order to detect Ossa virus in the latter animals and other agents in the immunized sentinels.

Insect collections on the hamsters would seem to point to *Culex* mosquitoes of the subgenus *Melanoconion*, and in particular to *C. vomerifer*, as the vectors of the bulk of the viruses isolated from hamsters, as the only mosquito not belonging to this group that was captured in appreciable numbers was *Mansonia venezuelensis*, a species that has been shown to be a poor carrier of arboviruses in the study area.[1] This conclusion may be questioned since collections on the hamsters were limited to the period between hours 1800 and 2200. However, previous work has demonstrated that sentinel mice acquire virus infections in the area almost exclusively at night.[1] It has also been found that about 80% of the mosquitoes that feed at night in Almirante do so during the evening and early nocturnal period.[10] The fact that investigations in the study area[1] and elsewhere[11, 12] have demonstrated that *Melanoconion* mosquitoes were frequently found harboring these viral agents would seem to offer support to the theory that they are the natural vectors of these viruses. The vertical distribution of virus activity in the forest, as gauged by sentinel hamsters, is also consonant with the vertical stratification in the forest of the population of the suspected vectors reported earlier.[1]

SUMMARY

Sixteen arbovirus isolates were obtained from 21 golden hamsters exposed in five different locations in an area of tropical rain forest of Panamá during February and March 1966. This animal proved to be a successful sentinel for the detection of Venezuelan equine encephalitis, Ossa, Madrid, Patois, and Guamá viruses during the low transmission period of 1966. Mosquito collections on the exposed hamsters seemed to point to *Culex* mosquitoes of the subgenus *Melanoconion*, and to *C. vomerifer* in particular, as the vectors of the bulk of the viruses isolated from hamsters. Solutions are suggested for problems, such as feed-back of virus from the sentinel hamsters to wild mosquitoes, which may develop from the use of this method.

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