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URBAN EPIDEMICS OF OROPOUCHE ORO FEVER IN THREE MUNICIPALITIES IN PARA, BRAZIL WERE STUDIED IN 1975. CULICOIDES PARAENSIS GOELDI WERE COLLECTED DURING EACH OF THE EPIDEMICS AND THERE WAS A POSITIVE CORRELATION, BY STUDY AREAS WITHIN THE CITY OF SANTAREM, BETWEEN HUMAN SEROPOSITIVITY TO ORO VIRUS AND POPULATION DENSITIES OF C. PARAENSIS AND CULEX QUINQUERASCIATUS SAY. THE BEST NUMERICAL CORRELATION WAS WITH POPULATIONS OF C. PARAENSIS. THE RELATIVE ABSENCE OF OTHER SPECIES IN THE AREAS OF HIGH DISEASE ATTACK RATES WAS FURTHER EVIDENCE C. PARAENSIS WERE THE PROBABLE VECTORS OF ORO VIRUS. THESE BITING

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MIDGES WERE FOUND TO BITE READILY INSIDE OF HOUSES, WITH AN INDOOR/OUTDOOR RATIO OF 29 PERCENT, AND WERE MOST ACTIVE AROUND 1700 1800 HOURS. OTHER BIOLOGICAL OBSERVATIONS ON C. PARAENSIS ARE PRESENTED.

Am J Trop Med Hyg, 30(1), 1981, pp 165–171

OROPOUCHE VIRUS

III. ENTOMOLOGICAL OBSERVATIONS FROM THREE EPIDEMICS IN PARÁ, BRAZIL, 1975*

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Abstract. Urban epidemics of Oropouche (ORO) fever in three municipalities in Pará, Brazil were studied in 1975. *Culicoides paraensis* (Goeldi) were collected during each of the epidemics and there was a positive correlation, by study areas within the city of Santarém, between human seropositivity to ORO virus and population densities of *C. paraensis* and *Culex quinquefasciatus* Say. The best numerical correlation was with populations of *C. paraensis*. The relative absence of other species in the areas of high disease attack rates was further evidence *C. paraensis* were the probable vectors of ORO virus. These biting midges were found to bite readily inside of houses, with an indoor/outdoor ratio of 29%, and were most active around 1700–1800 hours. Other biological observations on *C. paraensis* are presented.

Oropouche (ORO) virus is a cause of urban outbreaks of febrile disease in the Amazon basin and has been the subject of several investigations since 1960.[1] These investigations have resulted in relatively few isolations of ORO virus from invertebrates. The first isolation was reported from *Coquillettidia venezuelensis* (Theobald) in Trinidad, W. I.[2] It was subsequently isolated from *Culex quinquefasciatus* Say during epidemics in Belém, Brazil in 1961 and 1968, and from *Aedes serratus* (Theobald) captured along the Belém-Brasilia Highway.[3,4] More recently, collections of *Culicoides* midges resulted in two isolations during an epidemic at Mojuí dos Campos, Pará, Brazil in 1975.[1]

Two species from which isolates have been obtained, viz., *Cx. quinquefasciatus* and *Culicoides paraensis* (Goeldi), are prevalent in the urban environments in the Amazon region. *Culex quinquefasciatus* are the urban vectors of bancroftian filariasis in Brazil. These mosquitoes are found in most urban areas, are nocturnal, anthropophilic and endophilic; with a peak in biting activity around 2400 hours.[5] *Culicoides paraensis* are serious pests in some urban areas in Brazil and their bites may result in allergic reactions.[6] These biting midges are diurnal and are reported to have early morning and early and late afternoon peaks in biting activity.[7]

Since isolation of ORO virus from insects were rare, a joint program of epidemiological and systematic entomological studies was undertaken in an attempt to incriminate the urban vector. The purpose of this report is to relate observations from the program of entomological surveillance included in these joint studies. Results from the epidemiological studies are reported separately.[8]

MATERIALS AND METHODS

An epidemic of ORO virus was investigated in Mojuí dos Campos, Pará, Brazil in March 1975.[1] A second epidemic was studied at Itupiranga, Pará, Brazil in June 1975. This is a village of approximately 1,500 inhabitants located on the

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Tocantins River, 45 km northwest of Marabá, Pará, Brazil. A third epidemic was in the city of Santarém, Pará, Brazil in July 1975.[8] In addition, systematic collections of insects were conducted in Belém, Brazil, since this city had been the site of two previous epidemics of ORO fever. A literature review, along with procedures for isolation of ORO virus and serological testing, is presented separately with the epidemiological study results on the above ORO virus epidemics.[1]

Collecting procedures and methods were designed to obtain particular types of information during each epidemic. The main emphasis was on standardized human bait collections to select for the more anthropophilic species. Landing captures were made from the exposed legs and arms of the collectors by means of oral aspirators. After the specimens were aspirated they were blown into pint-sized ice cream carton holding containers. Specimens were subsequently killed and preserved in liquid nitrogen for later processing in the laboratory. Approximately 3 days were required for training of collectors and standardization of collecting procedures at each investigation site.

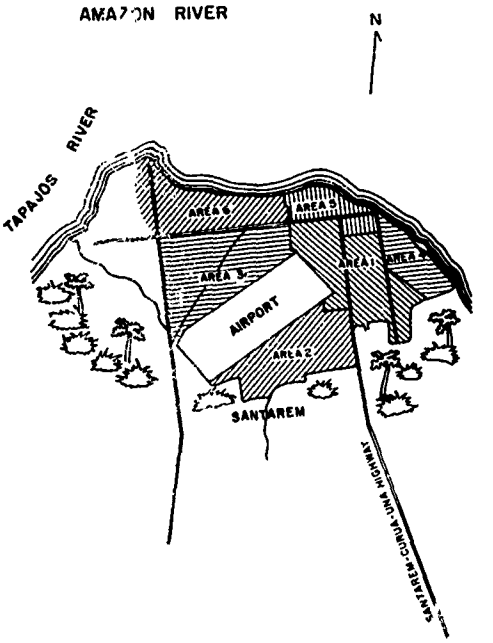

FIGURE 1. Division of Santarém, Pará, Brazil into six study areas during an epidemic of Oropouche virus in June–July 1975.

Mojuí dos Campos

Hourly collections were made in the early morning and late afternoon at 3 houses each day. These collections were conducted simultaneously inside and outside houses from 3–14 March 1975. The objective of these efforts was to evaluate the house-frequenting tendencies of *C. paraensis* and to collect material for virus isolation attempts.

Itupiranga

Continuous landing captures were conducted in the backyards of selected houses within this city from 0600 to 2000 hours. Collections were made by two-man teams that rotated in 3-hour shifts. Landing captures were also carried out at the margin of the Tocantins River at sunset. Shannon and CDC light trap collections were made within the city. The collecting program at Itupiranga was designed to quantify the abundant man-biting species and study the diurnal activity cycle of *C. paraensis*.

Santarém

This city was divided into six sampling areas and collections were made at six randomly selected houses in each area (Fig. 1). The six areas were identical to areas employed in the epidemiological investigations,[8] and the entomological effort was designed to obtain information for correlations between the spatial distributions of insect populations and human ORO seropositivity. Collections were made in the backyards and were conducted continuously from 1400–2000 hours for 3 days at each house. This time interval was selected to sample both diurnal and nocturnal insects. Twelve teams of collectors were employed (two men per team) and two areas were covered simultaneously. Each team was visited hourly by trained entomology technicians. During the visits, the hourly collections were gathered and new collection containers were left with the teams. The collections were returned to the laboratory where the *C. paraensis* were killed, identified, enumerated, and grouped for virus isolation. Teams were rotated between houses each day. Questionnaries were also completed for each house on environmental variables e.g., types of trees, domestic water source, sanitary facilities, etc., to correlate with population densities of insects captured.

Speculation that *C. paraensis* was limited in distribution to human population centers resulted

TABLE 1

Numbers of insects collected in hourly landing captures during an epidemic of Oropouche virus in Itupiranga, Pará, Brazil, June 1975. Each value is based on 25 man-hours of collection time, and collections were continuous from 0600–2050 hours

Species	Number collected/hr														
	0600–0650	0700–0750	0800–0850	0900–0950	1000–1050	1100–1150	1200–1250	1300–1350	1400–1450	1500–1550	1600–1650	1700–1750	1800–1850	1900–1950	2000–2050
An. (*Nys.*) *nuneztovari*	17	0	0	0	0	0	0	0	0	0	0	0	55	4	0
An. (*Nys.*) *triannulatus*	9	0	0	0	0	0	0	0	0	0	0	0	52	30	2
Cx. (*Cux.*) *quinquefasciatus*	2	0	0	0	0	0	0	0	0	0	0	0	67	103	307
Ma. humeralis	0	0	0	0	0	0	0	0	0	0	1	0	28	12	7
Ma. titillans	29	1	2	1	1	1	0	0	1	0	2	7	382	203	89
Culicoides paraensis	23	26	34	64	62	57	55	64	88	139	106	225	82	0	0
Simuliidae	528	1,097	2,015	1,106	652	367	597	1,219	1,194	1,066	1,454	1,728	21	0	0
Total number of Culicidae collected	57	27	2	1	1	1	0	0	1	0	4	7	669	399	428

in efforts to define the population distribution of this biting midge at various distances from the city. Four houses within the city and eight houses along 21 km of the Santarém-Curua-Una Highway were randomly selected as collection sites. The collecting program was identical to that employed in the city.

Belém

Belém has been the site of two epidemics of ORO virus since 1961. Six barrios (districts) in Belém were selected for study in the manner employed in Santarém. This study was undertaken to see if *C. paraensis* occurred in urban habitats similar to those in Santarém and for future reference should ORO fever again occur in the city.

RESULTS

Mojuí dos Campos

Culex quinquefasciatus and *C. paraensis* were abundant during the epidemic at Mojuí dos Campos. In 36 paired indoor-outdoor collections 29% of the total number of *C. paraensis* collected were captured inside the houses.

Itupiranga

Several hematophagous insects were abundant during the epidemic at Itupiranga. These included *C. paraensis*, *Cx. quinquefasciatus*, *Mansonia titillans* (Walker) and black flies (Simuliidae) (Table 1). The black flies and *C. paraensis* were the dominant manbiters during the day. The Simuliidae were most active in the early morning and late afternoon, with a maximum attack rate of 80.6 bites per hour. The *C. paraensis* had a peak attack rate of nine bites per man per hour and demonstrated a pronounced peak in biting activity between 1700 and 1800 hours (Table 1). The most abundant nocturnal species were *Ma. titillans* and *Cx. quinquefasciatus*. Night collections were discontinued at 2050 hours so no comments on periods of peak activity of nocturnal species are possible.

Santarém

Several species were collected in Santarém, but only *C. paraensis* and *Cx. quinquefasciatus* were present in dense populations (Tables 2, 3). Other

TABLE 2

Average numbers of the most abundant species of mosquitoes in landing collections conducted in Santarém, Pará, Brazil, July 1975. Collections were made from 1400–2000 hours daily at six sites in each area for 3 consecutive days

Species	Average number collected/site by area					
	1	2	3	4	5	6
Culicidae						
An. (Nys.) albitarsis	0	0	1.0	0	0	0.3
An. (Nys.) triannulatus	2.2	6.0	0	0	0.3	0
Cx. (Cux.) coronator	0.5	2.5	0.7	1.0	0.3	0
Cx. (Cux.) quinquefasciatus	4.2	0.7	26.7	7.2	24.5	31.3
Ma. amazonensis	8.2	6.0	0	0	0.3	0
Ma. titillans	1.3	1.0	1.5	0.5	0	6.8

anthropophilic species were generally more abundant in rural localities away from the city (Table 3). The populations of *C. paraensis* were dense in some areas of the city and were also abundant in rural areas along the Santarém-Curua-Una Highway.

Population densities of various insect species were calculated for each of the six study areas within the city for comparisons with the distribution of human seropositivity to ORO virus. The serological data were obtained from epidemiological surveillance.[8] A high prevalence of seropositives to ORO virus occurred in areas with the highest densities of *C. paraensis* and *Cx. quinquefasciatus* (Figs. 2, 3). The best numerical correlation was obtained with the populations of *C. paraensis*. Data for *C. paraensis* for the 1700 to 1800 hour collections were normalized with a sqare root transformation and employed in a 3-way analysis of variance test for areas, days and houses. Main factor analysis revealed significance ($P < 0.001$) between areas and between houses within areas. Collections between days by areas were not significantly different.

The distribution of specific insect populations could not be correlated with environmental variables included on the housing questionnaires, except for a correlation between population densities of *C. paraensis* and age of houses. In this case, a

TABLE 3

Relative densities of seven species in the urban and rural areas of Santarém, Para, Brazil. The landing collections were conducted from 1400–2000 hours for 3 consecutive days at each of four houses in July 1975

Species	Average number collected per house	
	Within the city of Santarém*	Rural area along the Santarém-Curua-Una Highway†
Culicidae		
An (Nys.) albitarsis	0.25	6.6
An (Nys.) triannulatus	1.5	29 1
Cx. (Cux.) coronator	0	2.25
Cx. (Cux.) quinquefasciatus	10.0	1.0
Ma. amazonensis	0	0.5
Ma titillans	1 25	10.5
Ceratopogonidae		
Culicoides paraensis	18 2	35 9

* Represents 36 man-hours of collections at each of four houses
† Represents 36 man-hours of collections at each of eight houses

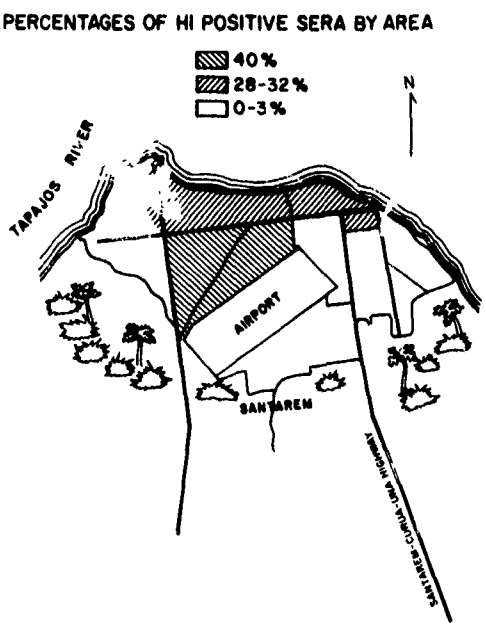

FIGURE 2. Distribution, by study area, of percentage seropositivity to Oropouche virus in Santarém, Pará, Brazil

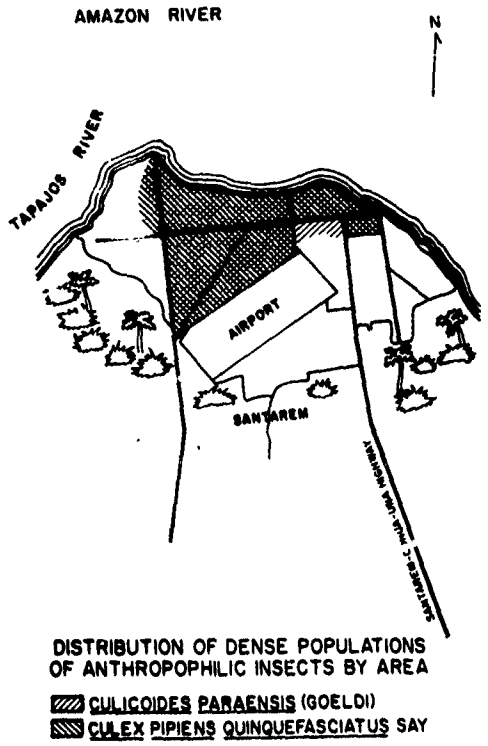

FIGURE 3. Distribution, by study area, of dense populations of anthropophilic insects in Santarém, Pará, Brazil.

one-way analysis of variance test did show a significantly ($P < 0.05$) greater number of *C. paraensis* associated with houses that were 5 or more years old.

Belém

Mosquito species collected during the survey of six districts in Belém in January 1976 are listed in Table 4. The most abundant species were *C. paraensis* and *Cx. quinquefasciatus*. Although there was no epidemic at the time this survey was conducted, we did find a reasonable correlation in the distribution of population densities of *C. paraensis* and the attack rates of ORO fever documented for different districts of Belém in the 1961 and 1968 epidemics.[1] The maximum number of cases of ORO fever was detected in the district of Marco in 1968 and in Marco, Pedreira and Sacramento districts during the 1961 epidemic. The least number of cases was found in the old city and commercial area. We found highest densities of *C. paraensis* in Marco district and lowest densities in the old city, commercial area and Jurunas district (Table 5). Moderate densities were documented for the Nazaré, Umarzal and Guamá districts. The Umarzal district was near the three districts of high attack rates during the 1961 epidemic. The seropositive rates for Guamá and Nazaré after the 1968 epidemic were 14% and 3%, respectively. Again, there was a correlation between population densities of *Cx. quinquefasciatus* and *C. paraensis*. However, tests conducted on data from both Santarém and Belém revealed no correlation of population densities between these species by houses within individual districts.

TABLE 4

Numbers of different species of mosquitoes in landing collections conducted in January and February 1976 in Belém, Pará, Brazil. Collections were conducted for 2 days at each of 36 houses from 1400–2000 hours daily

Culicidae	Number collected
Ae. (Och.) oligopistus	1
Ae. (Och.) scapularis	15
Ae. (Och.) serratus	1
Ae. (Och.) taeniorhynchus	62
An. (Ano.) intermedius	1
An. (Nys.) aquasalis	98
An. (Nys.) nuneztovari	1
Cq. venezuelensis	168
Cx. (Cux.) corniger	2
Cx. (Cux.) coronator	345
Cx. (Cux.) quinquefasciatus	1,,117
Cx. (Mel.) spissipes	1
Cx. (Mel.) taeniopus	2
Ma. (Man.) amazonensis	108
Ps. (Gra.) cingulata	27
Ps. (Jan.) ferox	5
Li. durhami	10
Culex sp. *B §21*	1
Culex (Mel.) spp.	1
Coquillettidia spp.	4
Culicoides paraensis	1,486
Total	3,456

DISCUSSION

The positive correlation in distribution between population densities of *C. paraensis* and *Cx. quinquefasciatus* and ORO virus seropositivity in Santarém suggests that one of these two species is the urban vector of ORO virus to man. Absolute numerical correlation was best for *C. paraensis*, and epidemiological evidence has been collected that further incriminates this species as an urban vector of ORO virus. The absence of other anthro-

TABLE 5

Average number of Culex quinquefasciatus *and* Culicoides paraensis *collected in six districts in Belém, Pará, Brazil. Landing collections were conducted for 2 days at each of 36 sites (houses) from 1400–2000 hours daily, January–February 1976*

Species	Average number collected per house in each district*					
	Guamá	Jurumas	Marco	Umarzal	Old city	Nazaré
Culicidae						
Cx. (Cux.) quinquefasciatus	27.2	41 7	55	48.7	8.2	6.0
Ceratopogonidae						
Culicoides paraensis	22.7	2.7	121	32.5	7.7	61.7

* Average based on collections from six sites (houses) per district and includes 2 days collecting at each site.

pophilic insects in areas of Santarém with high ORO fever attack rates wi ; additional incriminatory evidence. The progra n of conducting systemat;- insect collections in Belém confirmed distribution of *C. paraensis* compatible with the distribution of ORO seropositivity documented during the Belém epidemic in 1968.[4]

With incrimination of *C. paraensis* as vectors of human disease it becomes important to elucidate their host-seeking behavior that results in virus transmission. Sherlock reported midmorning and late afternoon peaks in activity in these biting-midges in Salvador, Bahia.[7] In our studies we documented a late-afternoon peak in activity for populations in Itupiranga, but there was no pronounced peak at midmorning. The reason for these differences between populations is unknown and requires further investigation.

Culicoides paraensis were rare at colonists' lotes (farms) along 800 km of the Transamazon Highway but were generally present in villages (unpublished data). One major difference between the rural lotes along this highway and sites along the Curua-Una Highway, where dense populations were found, was the duration of habitation. The latter lotes had been inhabited for more than 20 years. Colonists along the Transamazon had been in the area for less than 5 years.[9] Interestingly, lowest population densities in the Santarém area were found near houses that had been occupied for less than 5 years. Based on these observations it seems likely that inhabitants produce optimum conditions, over time, for producing populations of *C. paraensis* by cultural or plant cultivation practices.

A correlation between population densities of *Cx. quinquefasciatus* and *C. paraensis* was found by areas within the cities, but not by houses (individual collection sites) within the areas. The habitat requirements for larval development are different for the two species. Since *C. paraensis* oviposit in containers or tree holes,[10] and *Cx. quinquefasciatus* preferentially oviposit in free water of high organic content, this correlation is perhaps biologically meaningless. It may result from basic socio-economic factors that result in the occurrence of the two different habitats in the same neighborhoods.

Results from this investigation and other studies indicate that *C. paraensis* are closely associated with human habitations.[6,7] In this paper and associated publications[1,8] data have been presented to incriminate these biting-midges as the vectors of ORO virus in urban areas. Laboratory studies on the transmission of ORO virus by *C. paraensis* have been conducted and will be reported separately.[11] Further studies are required to elucidate the biology of *C. paraensis* and define the sylvatic cycle of ORO virus. It is desirable to conduct more detailed studies on the dynamics of ORO virus transmission since this is the first virus of public health importance that seems to have a biting-midge vector. In addition, an explanation for the difficulty in isolating virus from *Culicoides* during epidemics of ORO virus should be sought.

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