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EPIDEMIC OROPOUCHE VIRUS DISEASE IN NORTHERN BRAZIL[1]

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An epidemic of Oropouche virus occurred in a rural part of northern Brazil in 1978. Findings related to that event suggest that approximately 40 per cent of the local residents were infected. The probable vector was a biting midge, Culicoides paraensis, *from which the virus was recovered.*

Introduction

In recent years Oropouche (ORO) virus has become one of the most significant causes of arboviral disease in Brazil *(1)*. First isolated in Trinidad in 1955, ORO virus had initially been considered a clinical novelty of little public health importance *(2,3)*. Within the past several years, however, large epidemics of ORO virus have been recorded in northern Brazil, where several thousand people have become infected *(4-7)*. While the virus is not known to cause death, morbidity is often substantial, and the resulting loss in productivity may be considerable. In addition, it has been well documented that antigenically similar viruses of the Simbu serogroup, family Bunyaviridae, of which ORO virus is a member, can cause abortion and teratogeny among domestic animals *(8,9)*. Neither abortion nor teratogeny have yet been attributed to human ORO virus infections, but such conditions may become apparent as increased urban transmission occurs.

This article gives a detailed account of an ORO virus epidemic that occurred in a remote agricultural community of northern Brazil. Our primary objectives relating to that event were (1) to describe the apparent nidus of this most recent ORO virus epidemic, an epidemic that subsequently spread northward through a number of rural communities and reached Belém in epidemic proportions in 1979-1980; (2) to demonstrate the impact of the epidemic on the community affected; and (3) to provide additional evidence supporting the idea that *Culicoides paraensis* (Goeldi) is the epidemic vector of ORO virus *(10,11)*.

Documentation of this outbreak is especially relevant because ORO virus epidemics have recently occurred with greater frequency, and each outbreak has infected increasing numbers of people. Despite this increased ORO virus activity, however, our knowledge of the mechanisms of ORO virus maintenance and transmission remains rudimentary; and it is only through full understanding of the ecology and epidemiology of this virus that effec-

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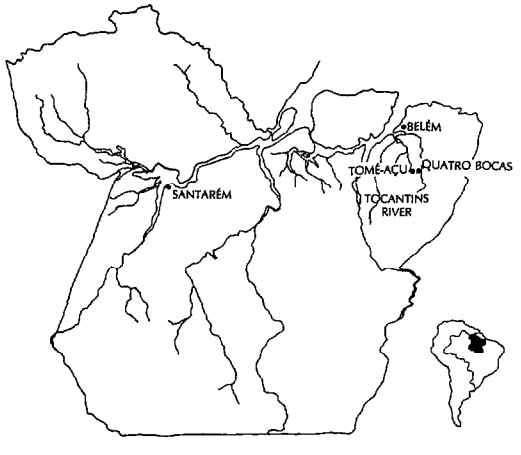
tive prevention and control measures can be developed. The purpose of this report is to contribute to that understanding.

Materials and Methods

Quatro Bocas is a small agricultural village of approximately 2,280 inhabitants located south of the city of Belém in the northern Brazilian state of Pará. The village serves as a commercial center for the surrounding agricultural community, whose principal products are cacao and pepper. Many of the farms are run by Japanese immigrants who migrated to the region within the last 50 years. As Figure 1 shows, the nearest city, Tomé Açu, is approximately 13 km to the west; Tomé Açu has a population of about 4,340. The cleared croplands that surround both Quatro Bocas and Tomé Açu are bordered by secondary scrub undergrowth and undisturbed primary forest.

The first cases of ORO virus, diagnosed in early July 1978, were reported from Quatro Bocas, and it soon became apparent that the disease focus was located there. Consequently, investigations of the outbreak focused upon Quatro Bocas and the immediately surrounding farms.

Figure 1. A map of the State of Pará, Brazil, showing the locations of Quatro Bocas and Tomé Açu, sites of 1978 Oropouche virus outbreaks. The locations of Belém and Santarém, sites of previous Oropouche virus outbreaks, are also shown.



A house-to-house survey of Quatro Bocas was begun in August 1978 to identify those people having clinical signs consistent with ORO virus infection. In the course of this survey, case histories were recorded and blood samples were drawn from all febrile patients. The blood samples were stored in liquid nitrogen and were later transferred to Belém laboratory facilities for virus isolation. Upon arrival in Belém, blood from febrile patients was inoculated into either suckling mice or adult hamsters in an attempt to isolate the responsible virus. Hamsters were chosen as a laboratory host because they are highly susceptible to ORO virus and usually succumb to the infection within 36-48 hours after intracranial inoculation *(11)*. All the virus isolations were titrated in suckling mice to allow a comparison with previous laboratory transmission experiments and titrations.

Several serological surveys to determine the incidence of infection and the prevalence of antibody to ORO virus were made in the area before, during, and after the epidemic. The first survey was made in 1974 as part of investigations of a yellow fever outbreak. At that time sera were collected from residents of Quatro Bocas, Tomé Açu, and surrounding areas. Four years later, a small sample of Quatro Bocas residents was selected; serum samples were collected from them in July 1978 and again in May 1979. Then, in May and June 1979, additional sera were again collected from Quatro Bocas, Tomé Açu, and surrounding areas.

All the subjects bled in each of the surveys were selected so that each geographic area, all age groups, and both sexes were represented. The collected sera were assayed for the presence of antibody to ORO virus (Belém prototype BeAn 19991) by hemagglutination-inhibition (HI) tests, using a reference strain of ORO virus and following a standardized procedure described previously *(12,13)*.

Entomological surveys for hematophagous insects were conducted in several areas of Quatro Bocas and at two peripheral farms. The choice of collection sites was based on

epidemiologic evidence of ORO virus activity. Both day and night man-biting captures at each collection site were made by two-man teams. In addition, CDC miniature light traps baited with dry ice were operated at night. Captured insects were gathered several times a day and were transferred to a field laboratory, where they were separated into general taxonomic groups and divided into blood-fed and unfed. The collections were preserved in liquid nitrogen and transported to the Belém laboratory for virus isolation. Only unfed insects were tested for viruses.

In Belém, collected insects were identified and pooled for virus isolation attempts. The initial pool size for the biting midge *Culicoides paraensis* was 100 individuals. When it later became apparent that several thousand *Culicoides* would be collected, the pool size was increased to 200. Mosquitoes were tested in groups of 50 or less.

The insects collected were assayed for virus with tube-grown Vero cells. Pools of insects were triturated with tissue grinders in 1.0 ml of 0.75 per cent bovine albumin in phosphate-buffered saline with antibiotics. Triturated pools were centrifuged at low speed for 15 minutes, and 0.1 ml portions of the supernatant fluids were inoculated into duplicate drained tubes of Vero cells. The tubes were then incubated for one hour at 37°C, rinsed, and supplemented with 1.0 ml of fresh medium. After this they were observed daily over a fifteen-day period for evidence of viral cytopathic effects. The viruses isolated were identified by neutralization tests following previously described standardized procedures *(14)*.

Birds and some bats were captured with mist nets at several collecting sites in secondary scrub forests on the periphery of Quatro Bocas. These nets were operated for three to four hours per day, beginning at dawn, for 11 days. Bats were netted in the evening (7:00-9:00 p.m.) for two evenings. The captured birds and bats were bled by means of syringes previously moistened with a dilute heparin solution. Blood samples were inoculated intracerebrally into suckling mice for virus isolation attempts. The remaining plasma samples were examined by HI tests for the presence of antibody to ORO virus; positive reactors were confirmed by neutralization tests with Vero cell cultures.

Results

Oropouche virus was isolated from 23 of 68 febrile patients examined (34 per cent) who resided in Quatro Bocas and nearby localities. An additional seven isolates (33 per cent) were obtained from 21 members of field teams who contracted the disease while investigating the epidemic. The clinical manifestations were similar to those observed in previous epidemics—namely fever, chills, headache, myalgia, arthralgia, and dizziness. Exanthemas were not noted. Cases were diagnosed from early July until late September 1978. No reports of similar illness before or after the epidemic were received. The cases were generally mild, although a few patients became severely ill and were hospitalized. Both sexes were infected, and the ages of those involved ranged from two to 50 years. Viremia titers in suckling mice inoculated intracranially ranged from 2.5 to 5 $\log_{10}$ LD_{50}/0.02 ml during the first three days of illness, then dropped to 1-2 $\log_{10}$ on day four; the patients generally became aviremic by day five. Approximately 8 per cent (two out of 25) of the viremias titrated yielded results above 5.0 $\log_{10}$.

Two measurements were made of the prevalence of antibody, as revealed by HI tests, to ORO virus among residents of the greater Tomé Açu area. The first measurement was based on a sample collected in 1974 that included 462 residents of Quatro Bocas, Tomé Açu, and surrounding areas. In that survey, seven (1.5 per cent) of the sera were found to contain antibody to ORO virus. This rate may serve as a preepidemic estimate of the prevalence of HI antibody to ORO virus.

The second measurement was made during May and June 1979, after the outbreak had subsided. At that time persons residing in

either Tomé Açu or Quatro Bocas were bled, and their sera were tested by HI for antibody to ORO virus. The overall antibody prevalences found were 19.5 per cent (57 positive of 292 tested) and 43 per cent (84 positive of 195 tested) for residents of Tomé Açu and Quatro Bocas, respectively. The antibody prevalence rates increased with age in both villages sampled, and antibody was equally distributed between both sexes. At that time the incidence of infection with ORO virus was approximately 18 per cent and 41.5 per cent in Tomé Açu and Quatro Bocas, respectively.

A more precise estimate of the incidence of infection among residents in the general area has been provided by sequential sera collected from the same individuals both before and after the outbreak. In July 1978, 80 sera were collected from residents of five villages outside Quatro Bocas. These same residents were then bled in May 1979, after the epidemic had subsided. Of the sera collected initially, only 1 (1.3 per cent) contained HI antibody to ORO virus. Of those collected after the outbreak, 29 (36.5 per cent) were positive, indicating an incidence of approximately 35 per cent in this sample.

Thus, two separate measurements of the incidence of ORO virus infection are available, these suggesting an incidence of 35 per cent for the entire community and 41.5 per cent for Quatro Bocas proper. The population of Quatro Bocas having been 2,280 people at the time of the outbreak, it can be concluded that between 798 and 946 people were most probably infected in that village alone; and it is clearly possible that several thousand people were infected in the general area.

Summary data on insects collected and processed for virus isolation are presented in Table 1. These data include all insects captured between August and October 1978 in both human bait collections and light traps from all the sites sampled. Clearly, *C. paraensis* was the most abundant insect species collected. Eleven species of mosquitoes were found, but their numbers were small in comparison to those of *C. paraensis*. Oropouche virus was isolated from four pools of *C. paraensis*, all of which were captured at a cacao plantation in Quatro Bocas. Several human cases of ORO virus disease were documented at this plantation as well. Superficial investigations to determine the breeding sites of *C. paraensis* found substantial breeding in discarded cacao husks there.

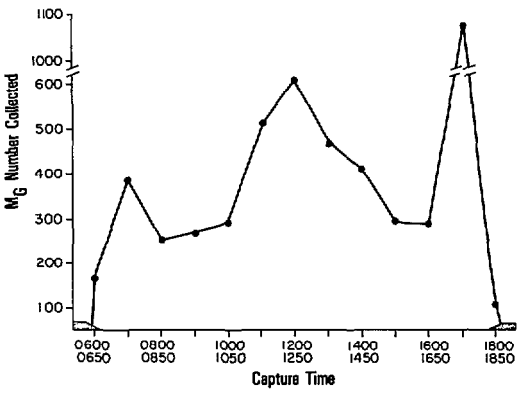
Table 1. Summary data on insects captured and Oropouche virus isolations made during investigation of an outbreak of Oropouche virus disease in the villages of Quatro Bocas and Tomé Açu (Pará, Brazil); all the insects were collected in August-October 1978.

Insect species	No. tested	No. of pools	Oropouche virus isolations
Culicoides paraensis	60,453	343	4
Aedes oligopistus	4	1	
Ae. scapularis	28	2	
Ae. serratus	53	3	
Anopheles albitarsis	3	1	
Culex coronator	219	10	
Cx. corniger	73	5	
Cx. declarator	16	1	
Cx. fatigans	377	16	
Psorophora cingulata	50	5	
Ps ferox	93	5	
Trichoprosopon digitatum	5	1	
Total	61,374	393	4

The activity pattern of *C. paraensis* was measured in terms of the numbers of insects attracted to human bait at the cacao plantation. This pattern is depicted in Figure 2, which shows the mean numbers of *C. paraensis* captured per fifty-minute collection period at different times of day. These observations are based on collections made over a period of 11 days. The results clearly show the daily activity pattern of this species. Biting activity was trimodal, with the greatest activity being recorded between 4:00 p.m. and 6:00 p.m., when an average of over 1,000 specimens were captured per collection period.

Bloods from wild and domestic birds and mammals collected in Quatro Bocas and surrounding forests were tested for virus and, where volumes permitted, sera were tested for HI antibody to ORO virus. No virus was re-

Figure 2. The average number of *Culicoides paraensis* (Goeldi) captured in fifty-minute human bait collections at Quatro Bocas, Pará, Brazil, in August-October, 1978, by time of capture.



covered from the blood of 460 wild birds, 139 domestic birds, 24 bats, or one cat. A total of 138 domestic chickens and 290 sylvatic birds representing 14 families were tested for HI antibody to ORO virus. Antibody was found in only six birds, all of which were sylvatic species. One of 46 Tyrannidae was positive, as were 2 of 60 Fringillidae and 3 of 101 Thraupidae. When attempts to confirm positive sera by N tests were made, only a single serum of the Fringillidae and one of the Thraupidae remained positive. Plasma from 17 bats were negative for antibody to ORO virus in HI tests.

Discussion

It seems evident that over a third of the entire population of Quatro Bocas was infected with ORO virus during this outbreak. With the typical course of illness lasting several days, the epidemic may have had a substantial impact on the productivity of the community. While the number of actual man-days lost due to ORO virus infection was not calculated, it is obvious that widespread illness among agricultural workers at crucial harvest or planting times could well have serious implications for the entire community and could result in considerable economic loss. The magnitude of the outbreak described here adds to the growing body of information indicating that ORO virus is a very significant human health problem in northern Brazil.

Pinheiro et al. *(1)* recently presented a discussion of the epidemiology of ORO virus in northern Brazil and suggested that two basic transmission cycles exist: a silent jungle cycle and an overt urban cycle. *C. paraensis* has been proposed as the primary vector in the urban cycle, with man serving as the amplifying vertebrate host. The results presented here support this conception of that cycle. Clearly, *C. paraensis* was the most abundant insect captured and was also the sole source of ORO virus isolated from captured insects. While virus recovery rates were low, as compared to rates at which viruses causing mosquito-borne diseases are typically recovered from those insects, they were consistent with the rates previously reported for ORO virus elsewhere *(1)*; and even with low rates of infection, the exceptional abundance of *C. paraensis* was probably sufficient to account for the high incidence of infection that was found.

The outbreak in the Quatro Bocas and Tomé Açu area provided the first evidence of ORO virus activity since the epidemic that involved Santarém and surrounding villages in 1975 *(5)*. Following the Quatro Bocas-Tomé Açu event, the virus spread northward through several rural villages until it finally reached Belém in 1979 (Pinheiro, unpublished observations). This pattern of spread conforms to that suggested by Pinheiro et al. *(1)*, with man being initially infected in a sylvatic setting by an as yet unidentified vector, and then returning to his village where he becomes the source of infection for feeding *C. paraensis*. The virus is then disseminated between villages through travel by infected persons. Quatro Bocas, with its surrounding undisturbed forests and high population densities of *C. paraensis* within the village, ideally fits the requirements of an index locality at the start of the urban ORO virus cycle.

With this conceptual framework of the epidemiology of ORO virus in mind, we might

now address the question of whether ORO virus is a localized, regional problem restricted to Pará, Brazil, or whether it has potential importance elsewhere. All the evidence indicates that epidemic ORO virus is closely linked to the presence or absence of *C. paraensis*. The distribution of *C. paraensis* is widespread; but ample breeding sites, especially discarded cacao husks and rotting banana stalks, are required for significant numbers to exist. Both of these materials are abundant throughout northern South America and are likely to increase in abundance as rural development continues. The increased frequency of epidemic ORO virus seen in recent years parallels the development of rural Pará State and tends to support this interpretation. Thus, unless specific measures are taken to remove the breeding substrate and control *C. paraensis*, the distribution of ORO virus is likely to expand.

SUMMARY

A widespread epidemic of Oropouche (ORO) virus occurred in a remote agricultural community in northern Brazil in 1978. ORO virus was isolated from 23 of 68 febrile patients examined who resided in the village, and from 7 of 21 members of field teams infected while investigating the epidemic. The incidence of infection among the 2,280 residents of the village was estimated at between 35 and 41.5 per cent. Estimates of the prevalence of antibody to ORO virus following the epidemic ranged from 36.5 to 43 per cent.

Hematophagous insects were collected and assayed for the presence of virus. A biting midge, *Culicoides paraensis* (Goeldi), was by far the most abundant insect encountered, over 1,000 individuals being attracted to human bait per fifty-minute collection period during peak activity in the early evening. Four strains of ORO virus were recovered from over 60,000 *C. paraensis* assayed. The virus was not recovered from any other insect species collected.

The results presented support theoretical transmission cycles previously proposed for ORO virus and further incriminate *C. paraensis* as the principle epidemic vector. It is concluded, on the basis of current knowledge of the epidemiology of ORO virus, that this virus is likely to become even more important in developing areas of northern South America.

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BOTTLE-FEEDING CONTROL PROGRAM IN PAPUA NEW GUINEA

In July 1977, a Baby Feed Supplies (Control) Act restricting the sale of baby bottles and teats to registered pharmacists was passed in Papua New Guinea. Since then each sale has had to be authorized by a health worker, who has had to ensure that it was in the baby's best interest to be bottle-fed and who has also had to instruct the mother or guardian on how to clean the bottle and correctly prepare the appropriate dilution. Health workers are liable to a fine if they fail to follow the instructions laid down in the act, as are shopkeepers and others supplying baby bottles without a proper authorization. Under this act, advertising of milk for bottle-feeding is also banned.

The impact of the act has now been measured through surveys of infant feeding practices and analysis of hospital records. Two surveys, one conducted in 1975-1976 before the act and another conducted in 1979 twenty months after the act, have shown a definite increase in the incidence of breast-feeding. That is, of 127 children under 2 years old who were surveyed in 1975-1976, only 82 (65 per cent) were breast-fed; while of 144 children under 2 surveyed in 1979, a total of 127 (88 per cent) were breast-fed.

A substantial drop has also been observed in the number of gastroenteritis admissions and deaths among young infants (under 6 months old) entering the Port Moresby General Hospital. In 1975, 83 such infants were admitted for gastroenteritis and three died, while in 1976 the respective figures were 71 cases and two deaths. In 1977, the year the bottle-feeding act was introduced, gastroenteritis admissions for infants under 6 months dropped to 31, and only one gastroenteritis death was recorded. Similarly, gastroenteritis admissions in this age group totalled 29 in 1978, 28 in 1979, and 38 in 1980. Since 1977 the hospital has recorded no gastroenteritis deaths among infants in this age group. This suggests that the legislation introduced in 1977 has had a significant impact—not only on the incidence of breast-feeding but also upon gastroenteritis morbidity and mortality among infants less than 6 months of age.

Source: World Health Organization, *Weekly Epidemiological Record* 56:126, 1981.