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VECTORS AND NATURAL RESERVOIRS OF OROPOUCHE VIRUS IN THE AMAZON REGION

Final Report

December 1978

by

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FINAL REPORT

December, 1978

INTRODUCTION AND SUMMARY OF ACCOMPLISHMENTS

The PAHO Project BRA 4311 is located in Belém, Brazil and works in close conjuction with and has laboratory space at the Institute Evandro Chagas, a research center administered by the Brazilian Public Health Service (FSESP). The sole area of investigation for the USAMRU-Belém is the ecology of arbovirus diseases. Current efforts attempt to elucidate the endemic and epidemic cycles of Oropouche (ORO) virus. This virus cause disease in man generally of about 7 days duration, with some patients severely ill, occasionally to the point of prostration. This disease has been reported to cause large scale epidemics in urban areas of northern Brazil.

Accomplishments included in this report with regard to ORO virus have been divided into two major categories; studies on the epidemic cycle, and studies on the endemic cycle. Under the category studies on the epidemic cycle, conclusive data are presented which establish the midge, Culicoides paraensis, as the primary epidemic vector of ORO virus. Subsequent sections present results of investigations of the basic biology of this midge, all of which will be of immense value should effective control measures be desired, such as would be necessary to combat an epidemic of ORO virus disease.

A second section of studies on the epidemic cycle of ORO virus deals with man as the principal vertebrate host in the epidemic cycle. Here evidence is presented which indicates that, when infected, man circulates ORO virus in sufficient titer to infect feeding C. paraensis.

Finally, results of an epidemic of ORO virus disease which occurred in and around Tomé, Acú, Pará, Brazil are reported. These results provide striking verification of the theories proposed in the previous two sections. Clearly, C. paraensis is the predominant vector in this outbreak, and in all likelihood man is the only amplifying host.

Investigations of the endemic cycle of ORO virus are also reported. Three separate sites were sampled during 1977-1978, Curuá-Una, Mojui dos Campos and Cachoeira Porteira, all located in Pará, Brazil. Vertebrates were sampled from all 3 localities, and insects were sampled at 2 of the 3 sites. Oropouche virus was not found among any insects tested; however, results of the serological surveys indicate that primates and certain groups of birds had been previously exposed to the virus.

In early 1978 the PAHO team was invited to participate in investigations of an epidemic of unknown etiology which was in progress in Belterra, Pará, Brazil. It was soon determined that Yellow Fever (YF) and Mayaro (MAY) viruses were both responsible for this epidemic. The occurrence of epidemic MAY virus provided a unique opportunity to study a virus known to cause disease in man in the Amazon Basin, but previously reported only incidentally.

Studies of MAY virus are organized in the same format as those for ORO virus; studies on the epidemic cycle, and studies on the endemic cycle. Studies on the epidemic cycle describe in detail the epidemic of Belterra.

Following a brief synopsis of the literature pertainint to MAY virus and a detailed description of Belterra, a summary of the clinical manifestations of MAY virus infection in man is presented. These results indicate that infection with this virus results in severe, debilitating disease of approximately one week's duration. No deaths were attributed to infection with MAY virus. It was especially fortunate that a complete census of Belterra had just been completed in December, 1977, consequently accurate denominator information was available for accurate estimates of attack rates. A clinically apparent attack rate of between 89 and 96% was estimated for those infected among all age groups in Belterra, and from 94 to 100% among that segment of the population over 10 years old. Approximately 20% of the 4000 residents of Belterra were infected during this epidemic.

Subsequent sections deal with vectors and vertebrate involved in the epidemic cycle of MAY virus. Entomological investigations indicate that the principal, if not only vector of MAY virus in ths epidemic was Haemagogus janthinomys. Noteworthy, this was also the only epidemic vector of YF virus recognized as well. Studies of the vertebrate population indicate that a small marmoset, Callithrix argentata, was most likely the primary vertebrate amplifying host of MAY virus in the outbreak. This was supported by the isolation of MAY virus from a feral marmoset, and subsequent laboratory demonstration of a considerable viremia produced following experimental infection with Mayaro virus.

Studies on the endemic cycle of MAY virus, also conducted at Curua-Una and Cachoeira Porteira, failed to isolate the virus. Serological results of the vertebrates sampled indicate that primates have a high antibody prevalence rate to MAY virus, but few other groups of vertebrates were found with antibody.

The epidemic of MAY virus investigated at Belterra indicates that the epidemiology of this virus is different from that proposed for ORO virus. Apparently MAY virus is restricted to a sylvatic cycle similar to jungle YF virus. An urban vector capable of maintaining massive outbreaks as occurs with C. paraensis and ORO virus is apparently lacking.

I. ECOLOGY OF OROPOUCHE VIRUS

A. Studies on the Epidemic Cycle

1. Vector biology

a. Laboratory transmission studies

OBJECTIVE: Objective of the Oropouche virus transmission studies was to further substantiate preliminary results with Culicoides paraensis (Goeldi) and Culex pipiens quinquefasciatus (Say).

BACKGROUND: Oropouche (ORO) virus was first discovered in Brazil in 1960, and subsequently it has become recognized as an important public health problem due to its occurrence in several urban epidemics in the Amazon Basin. Epidemiological and entomological investigations conducted during these epidemics have strongly indicated that ORO virus was vector-borne. Culex pipiens quinquefasciatus and Culicoides paraensis were considered to be the most probable vectors of the virus due to their common occurrence and superior numerical abundance in all epidemics investigated.

In order to determine the vector potential of Cx. p. quinquefasciatus and C. paraensis, laboratory transmission studies were conducted under controlled conditions using hamsters as the model host. Biological transmission of ORO virus was demonstrated for both species during the preliminary transmission trials; however, C. paraensis appeared to be the better vector of the two. The extrinsic incubation period for ORO virus in C. paraensis appears to be 5 to 7 days, but was not determined for Cx. p. quinquefasciatus. The threshold viremia level required to infect feeding C. paraensis and Cx. p. quinquefasciatus was also not established.

DESCRIPTION: Culicoides used in the transmission studies were collected at a field station where the midge populations remain active throughout the year. Oropouche virus is not known to exist within this collecting area. Culex p. quinquefasciatus used in the test were first generation mosquitoes reared from wild caught populations obtained from the Belém urban area.

Virus strains: Oropouche virus, strain Br An 19991, was used to inoculate the experimental animals. Young hamsters (21-23 days of age) were used as the donor and recipient vertebrate hosts in all transmission experiments.

Virus Titration: Hamsters were inoculated intracerebrally with 0.1 ml of undiluted hamster serum containing ORO virus. Twenty to 24 hrs. following inoculation, 0.1 ml of blood was obtained from the hamster by cardiac puncture and immediately added to 0.9 ml of phosphate buffered saline (PBS) containing 0.4% bovine albumin. This 1:10 dilution was further diluted and titrated in Vero cells or suckling mice to determine the titer of ORO virus in the donor hamster. After bleeding, the hamsters were immediately exposed to insects included in the transmission tests.

Virus Isolation and Identification: Individual insects were homogenized and suspended in PBS containing bovine albumin and antibiotics. A 1.5 ml aliquot of diluent was used for Culex mosquitoes and 1.0 ml was used for Culicoides.

After centrifugation of the insect homogenate at 1500 RPM, the supernatant fluid was aspirated and 0.1 ml was inoculated into each of 3 tubes of Vero cells. The tubes were observed every 2 days to detect viral cytopathic effect (CPE). Tubes demonstrating a 3 to 4 + CPE were harvested and frozen at -60°C. To identify the virus, a 1:100 dilution of the infected fluid was mixed with equal amounts of ORO virus hyperimmune mouse ascitic fluid, incubated for 1 hour at 37°C, then assayed for infectivity. These tests were performed in microtiter plates to which Vero cells were added after incubation of the virus and virus-serum mixtures. The test control series consisted of infected fluids without additions of the ORO virus hyperimmune mouse ascitic fluids. The tests were routinely read 3-4 days post-inoculation, or when the virus controls showed a 3-4 + CPE.

PROGRESS: Tables 1 and 2 present the results of attempts to transmit ORO virus to susceptible hamsters by Cx. p. quinquefasciatus which fed on infected hamsters circulating ORO virus. As shown in Table 1, Cx. p. quinquefasciatus were fed on a hamster with a viremia of $10^{4.6}$ $SMLD_{50}$/0.02 ml of ORO virus. Repeat feeding of "infected" Culex on susceptible hamsters were conducted on days 7, 14, 21 post exposure. After the final feeding, hamsters were observed for an additional 3 week period for clinical signs of ORO virus. If clinical signs of ORO virus were not observed, then the hamster was sacrificed and serologically tested for antibody to ORO virus. As the data indicate, none of the 30 susceptible hamsters in this experiment showed clinical signs of ORO virus, and all serological tests were negative for antibody to ORO virus.

Table 2 summarizes the results seen where 4 hamsters with viremias of 10^5, 10^5, $10^{5.4}$ and $10^{5.3}$ $SMLD_{50}$/0.02 ml were fed on by 4 separate lots totalling 210 Cx. p. quinquefasciatus. Susceptible hamsters were subsequently exposed to each lot in the same manner as described for hamsters in Table 1. None of the 60 susceptible hamsters used in these tests showed clinical signs of ORO virus infection or exhibited antibody to ORO virus.

Table 3 presents the results of attempts to transmit ORO virus to susceptible hamsters by C. paraensis which fed on infected hamsters circulating $10^{6.6}$ $SMLD_{50}$/0.02 ml of ORO virus, and results of virus isolation attempts from midges following feeding on susceptible hamsters. These results show that 4 of 5 (80%) Culicoides assayed following feeding on susceptible hamsters harbored ORO virus; however, only 1 (25%) of the 4 infected Culicoides actually transmitted ORO virus to a susceptible hamster.

Culicoides which did not feed on the susceptible hamsters on the 7th day of exposure were sacrified and individually tested for the presence of ORO virus. From twelve Culicoides tested, 3 (25%) ORO virus isolations were made.

Table 4 presents the results of attempts to transmit ORO virus susceptible hamsters by C. paraensis fed on infected hamsters circulating $10^{5.0}$ $SMLD_{50}$/0.02 ml of ORO virus, and virus isolations from midges following feeding on susceptible hamsters. These findings show that ORO virus was transmitted to 5 (83%) of the 6 susceptible hamsters exposed to "infected" Culicoides. Each infection resulted in the death of the hamster. Seven (54%) of the 13 Culicoides which fed on the hamsters on day 7 were confirmed to be infected with ORO virus.

Culicoides which did not feed on the susceptible hamsters on the 7th day of exposure were sacrificed and individually tested for the presence of ORO virus. Twelve Culicoides were tested and 5 isolates (42%) of ORO virus were made. One of these infected Culicoides probably fed undetected on the positive hamster whose associated Culicoides was negative in Table 4.

COMMENTS: In an effort to summarize the laboratory transmission results of ORO virus, a brief discussion of the preliminary results presented in the annual report year of 1976-77 will be included in the present comment section.

Initial biological transmission of ORO virus by Cx. p. quinquefasciatus was demonstrated on 2 occasions during 1976-77 investigations. The first transmission of ORO virus to a susceptible hamster was achieved when a test lot of Culex were fed on a hamster circulating $10^{8.2}$ $SMLD_{50}$/0.02 ml of virus. The ORO virus transmission occurred on the 8th day when the experimental hamster was fed upon by 21 "infected" mosquitoes. Oropouche virus transmission to this hamster was confirmed by serological test. Transmission of ORO virus was again demonstrated in another experiment in which a Culex lot had fed on a hamster circulating $10^{7.8}$ $SMLD_{50}$/0.02 ml of virus. Oropouche virus was isolated from a susceptible hamster fed upon by a group of 33 Culex, 21 days post exposure. Three later transmission trials were attempted with Culex feeding on viremic hamsters circulating $10^{7.0}$, $10^{8.2}$, and $10^{8.0}$ $SMLD_{50}$/0.02 ml of virus; however, all the 15 susceptible hamsters exposed to refeeding by "infected" Culex were not infected.

Based on these results, it was concluded that Cx. p. quinquefasciatus demonstrated a high infectivity threshold and was apparently an inefficient vector of ORO virus.

Due to the extended colonization of the test Culex material within the laboratory environment, it was felt that the vector competence of the laboratory colony may have been modified, and was no longer representative of the wild urban Culex population. Therefore, the transmission experiments were modified and performed with 1st generation Culex adults obtained from urban areas of Belém. These results are presented in Tables 1 and 2. The data in these tables show that a composite total of 283 Culex were fed upon 90 susceptible hamsters. Serological tests of all exposed hamsters were negative for antibody to ORO

virus. The Culex pools which fed on these hamsters are being tested for the presence of ORO virus.

A review of ORO virus transmission experiments with C. paraensis presented earlier shows that ORO virus was transmitted to 8 susceptible hamsters by Culicoides which had fed previously on viremic hamsters which titered $10^{6.0}$, $10^{6.5}$, $10^{7.2}$ and $10^{8.2}$ $SMLD_{50}$/0.02 ml of ORO virus. The earliest recorded successful transmission was on day 4 post-exposure, with a majority of the seccessful transmissions occurring between days 5 and 8. Of the 8 hamsters infected, 4 (50%) were fed upon by 2 or fewer Culicoides. In addition, ORO virus was isolated from 13% (28/208) of the individually tested Culicoides which were sampled from both fed and unfed individuals. The 13% positive for ORO virus is thus not an infectivity rate, since many of those tested had not received an initial infective blood meal.

In the transmission trials reported here the methods of separating the "infected" midge population from the "non-infected" population were modified and greater standardization in the population of "infected" Culicoides was obtained. Restrained viremic hamsters were exposed to C. paraensis for a period of approximately 45 minutes. Following the exposure period, midges were subjected to cool temperatures to reduce their movement. With the use of a stereomicroscope, blood engorged midges could be separated from the non-engorged. Therefore, only blood engorged midges were used in the subsequent transmission trials.

Results presented in Table 3 indicate that ORO virus was transmitted to susceptible hamsters by one (25%) of 4 infected Culicoides which fed singly. In a second experiment presented in Table 4, where as many as 3 individual Culicoides were allowed to feed on a susceptible hamster, ORO virus was transmitted by 4 (80%) of 5 infected groups of Culicoides. Among those groups tested, 3 (75%) of the 4 positive transmissions were accomplished by single infected Culicoides. Taken together, these results indicate a range of transmission rates among infected Culicoides from 25 to 80%. These results suggest that Culicoides are fairly efficient vectors, and are certainly better vectors than Cx. p. quinquefasciatus.

It is difficult to explain why the transmission rates were higher for those Culicoides which fed on the lower titered infectious blood meals. One possible explanation is a difference in age among individuals tested in these experiments. Since all Culicoides tested were wild caught as adults, it is impossible to know the age of individuals tested. A second possibility is that these results reflect the normal range of susceptibility among the wild population, and that too few replicates have been conducted to be more specific.

In summary, laboratory transmission of ORO virus has been demonstrated for both Cx. p. quinquefasciatus and C. paraensis. These species represent the dominant nocturnal endophilic and the dominant diurnal endo/exophilic species respectively, and both have been reported to feed on man in all epidemics of ORO virus. Based on the experimental transmission studies reported here, it

appears that C. paraensis is the predominant epidemic vector of ORO virus in the urban environment. Subsequent sections of this report attempt to define the basic biology of this vector species.

TABLE 1. SUMMARY OF ATTEMPTS TO TRANSMIT OROPOUCHE VIRUS TO SUSCEPTIBLE HAMSTERS BY CULEX PIPIENS QUINQUEFASCIATUS FED ON INFECTED HAMSTERS CIRCULATING $10^{4.6}$ SMLD50/0.02 ml OF OROPOUCHE VIRUS

Days post-infectious blood meal	Number of susceptible hamsters	Nº Mosquitoes Fed	Transmission* results
7	7	7	Neg.
14	9	9	Neg.
15	4	33	Neg.
21	10	24	Neg.
TOTAL	30	73	

* Serological tests were performed 21 days after last exposure to experimental insect population. Serological confirmation was determined by Neutralization test.

TABLE 2. SUMMARY OF ATTEMPTS TO TRANSMIT OROPOUCHE VIRUS TO SUSCEPTIBLE HAMSTERS BY CULEX PIPIENS QUINQUEFASCIATUS FED ON INFECTED HAMSTERS CIRCULATING 10^5 TO 5.4 $SMLD_{50}$/0.02 ml OF OROPOUCHE VIRUS

Days post-infectious blood meal	Number of susceptible hamsters	Nº mosquitoes fed	Transmission* results
7	5	17	Neg.
8	15	61	Neg.
14	20	35	Neg.
21	20	97	Neg.
TOTAL	60	210	

* Serological tests were determined by Neutralization test 21 days after last exposure to experimantal insect population

TABLE 3. SUMMARY OF RESULTS OF ATTEMPTS TO TRANSMIT OROPOUCHE VIRUS TO SUSCEPTIBLE HAMSTERS BY CULICOIDES PARAENSIS FED ON INFECTED HAMSTERS CIRCULATING $10^{6.6}$ $SMLD_{50}$/0.02 ml OF OROPOUCHE VIRUS, AND ASSOCIATED VIRUS ISOLATIONS FROM MIDGES FOLLOWING FEEDING ON SUSCEPTIBLE HAMSTERS

Days post-infectious blood meal	Nº Fed hamster	Transmission Results	Virus isolated from midges following feeding
7	1	Neg.	Pos. **
7	1	Neg.	Neg.
7	1	Neg.	Pos.
7	1	Neg.	Pos.
7	1	Pos.*	Pos.

* All pos. results confirmed by Complement Fixation test.
** All pos. results confirmed by Neutralization test.

TABLE 4. SUMMARY OF RESULTS OF ATTEMPTS TO TRANSMIT OROPOUCHE VIRUS TO SUSCEPTIBLE HAMSTERS BY *CULICOIDES PARAENSIS* FED ON INFECTED HAMSTERS CIRCULATING $10^{5.0}$ $SMLD_{50}$/0.02 ml OF OROPOUCHE VIRUS, AND ASSOCIATED VIRUS ISOLATIONS FROM MIDGES FOLLOWING FEEDING ON SUSCEPTIBLE HAMSTERS.

Days post-infectious blood meal	Nº fed/ hamster	Transmission results	Virus isolated from midges following feeding
7	2	Pos.*	2 Pos.**
7	3	Pos.	1 Pos.-2 Neg.
7	2	Neg.	2 Pos.
7	1	Pos.	1 Neg.+
7	3	Pos.	1 Pos.-2 Neg.
7	2	Pos.	1 Pos.-1 Neg.

* All pos. results confirmed by Complement Fixation test.
** All pos. results confirmed by Neutralization test.
\+ Likely that this hamster was fed upon by an undetected infected *C. paraensis*

I. ECOLOGY OF OROPOUCH VIRUS

A. Studies on the Epidemic Cycle

1. Vector biology

b. Urban seasonal abundance of Culicoides paraensis

OBJECTIVES: The objectives of this section are to:

a. determine the seasonal activity patter for Culicoides paraensis within the urban environs of Belém

b. demonstrate a relationship between seasonal rainfall and midge population activity.

BACKGROUND: Epidemics of Oropouche (ORO) virus have been documented in several urban areas within the state of Pará, Brazil since 1961: Belém, Bragança, Itupiranga, Mojui dos Campos, Santarém, Belterra and Tomé Açú. In all epidemics investigated, two insect species have been the most common human feeders: Culex pipiens quinquefasciatus nocturnally and Culicoides paraensis diurnally. Laboratory transmission studies of ORO virus with both species have previously demonstrated that C. paraensis is the more efficient of the two species, and these results have formed the basis for our working hypothesis that this species is the principal epidemic vector of ORO virus. In this and subsequent sections, discussions of the basic biology of C. paraensis are presented. This information is essential in the development of effective disease prevention and/or control strategies.

DESCRIPTION: Seasonal population studies for C. paraensis were conducted in a peridomiciliary environment near (10-20 meters) houses in 2 separate city zones of Belém, Pará, Brazil. Landing captures were performed by a team of two collectors who captured Culicoides attempting to feed on the exposed portion of the lower leg (knee to ankle). Captures were conducted for four consecutive 30 min. intervals from 14:00-16:00 hrs, at each collection site for two consecutive days on alternate weeks. The study was initiated in July, 1977 and was continued through July 1978. Rainfall data was obtained from an agriculture experimental research station (CEPLAC) located on the periphery of Belém, Pará, Brazil.

PROGRESS: Figure 1 presents the mean hourly numbers of C. paraensis collected per month, and total monthly rainfall. It is apparent from the rainfall data that the municipality of Belém receives a voluminous quantity of rainfall annually. In only one month (November) was the total rainfall measured below 75 mm. It is also apparent that the monthly rainfall shows a definite seasonal change, with those months of December to March recording the greatest quantity.

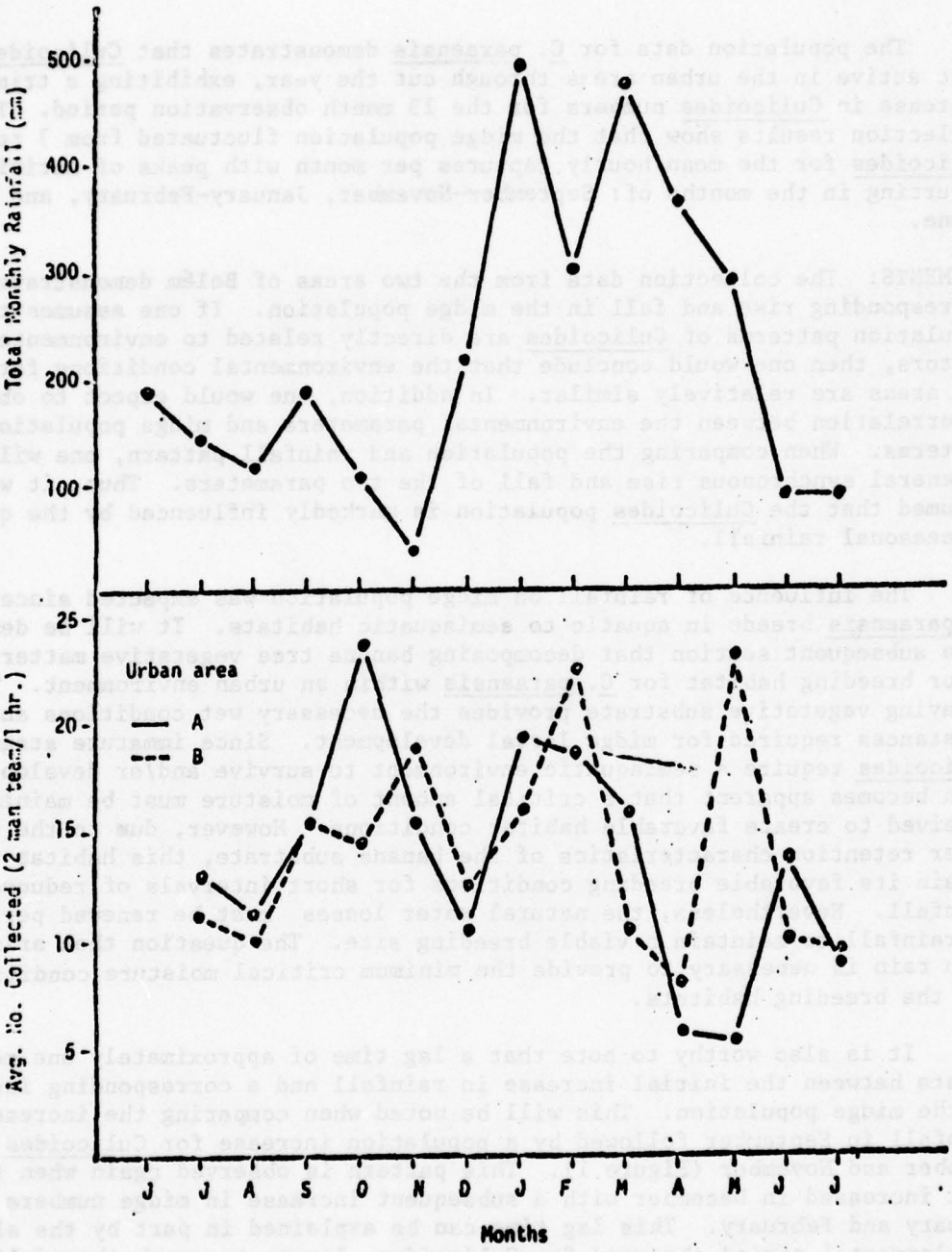

FIG. 1. Peridomestic population patterns of Culicoides paraensis (Goeldi) collected at two separate urban locations in the city of Belém, Pará, Brazil, 1978

The population data for C. paraensis demonstrates that Culicoides were active in the urban areas through out the year, exhibiting a trimodal increase in Culicoides numbers for the 13 month observation period. The collection results show that the midge population fluctuated from 7 to 25 Culicoides for the mean hourly captures per month with peaks of activity occurring in the months of: September-November, January-February, and May--June.

COMMENTS: The collection data from the two areas of Belém demonstrate a corresponding rise and fall in the midge population. If one assumes that the population patterns of Culicoides are directly related to environmental factors, then one would conclude that the environmental conditions for the two areas are relatively similar. In addition, one would expect to observe a correlation between the environmental parameters and midge population patterns. When comparing the population and rainfall pattern, one will note a general synchronous rise and fall of the two parameters. Thus, it was assumed that the Culicoides population is markedly influenced by the quantity of seasonal rainfall.

The influence of rainfall on midge population was expected since C. paraensis breeds in aquatic to semiaquatic habitats. It will be demonstrated in a subsequent section that decomposing banana tree vegetative matter is a major breeding habitat for C. paraensis within an urban environment. The decaying vegetative substrate provides the necessary wet conditions and food substances required for midge larval development. Since immature stages of Culicoides require a semiaquatic environment to survive and/or develop, it then becomes apparent that a critical amount of moisture must be maintained/or received to create favorable habitat conditions. However, due to the good water retention characteristics of the banana substrate, this habitat will retain its favorable breeding conditions for short intervals of reduced rainfall. Nevertheless, the natural water losses must be renewed periodically by rainfall to maintain a viable breeding site. The question then arises, how much rain is necessary to provide the minimum critical moisture conditions for the breeding habitats.

It is also worthy to note that a lag time of approximately one month exists between the initial increase in rainfall and a corresponding increase in the midge population. This will be noted when comparing the increase in rainfall in September followed by a population increase for Culicoides in October and November (Figure 1). This pattern is observed again when the rainfall increased in December with a subsequent increase in midge numbers in January and February. This lag time can be explained in part by the slow developmental period observed for Culicoides larvae to reach the adult phase. Controlled laboratory studies indicate that the developmental cycle (egg to adult) for Culicoides is approximately one month.

It is difficult to directly associate the quantity of rainfall with the variations noted in the population of Culicoides. For example, during the rainy season (January to May) it was observed that the midge population fluctuated markedly during a part of the season which was receiving more than 300 mm of rainfall per month. It would be reasonable to assume that sufficient rainfall was being received to provide favorable habitat conditions and to maintain a high population of adult Culicoides. Nevertheless, a marked reduction in the population numbers was noted. At this time, data are not available to explain the noted population reduction occurring during the rainy season. However, it is conceivable that heavy rainfall may result in adult midge mortality caused by drowning.

In summary, the population activity for C. paraensis was found to be continuous throughout the year in the urban study areas of Belém. Collection data tend to support the association of Culicoides activity with seasonal rainfall.

I. ECOLOGY OF OROPOUCHE VIRUS

A. Studies on the Epidemic Cycle

1. Vector biology

c. Daily activity patterns of Culicoides paraensis

OBJECTIVE: The objectives of the following investigations are to quantify the daily activity patter of Culicoides paraensis in 3 principal domestic environs, and to monitor designated environmental parameters in each environ.

BACKGROUND: Epidemiological and entomological observations of hematophagous insects affecting man in his urban environment indicate that C. paraensis is frequently the most common and dominate species. Information collected previously concerning the daily activity of this species indicates that they are strictly diurnal blood feeders, with apparent regulated behavioral activity patterns. It has also been noted that the biting pressure observed within a particular domestic environment is significantly variable. Therefore, it was hoped that observations of certain environmental factors within a particular domestic environment is significantly variable. Therefore, it was hoped that observations of certain environmental factors within a particular domestic setting might help to explain the differences observed in C. paraensis activity pattern.

DESCRIPTION: The domestic environment evaluated in this investigation is located on the property of an agriculture research station near experimental cacao plots. This location was chosen due to the high population of C. paraensis that existed in the area and the absence of adjacent housing units. Known breeding sites for C. paraensis within this area were those of decaying vegetative matter of banana trees and cacao pods. Fortunately the diurnal midge population of this area was essentially a monoculture of C. paraensis.

The house used in the study was a simple 5 room house constructed of stucco walls and a tile roof. During the day, as is custom in tropical regions, the doors and windows were left open for natural ventilation. The family unit consisted of 8 members. Domestic animals consisted of a dog and a few caged chickens. No large domestic animals such as cattle, sheep or pigs were known to exist within a one kilometer radius of the study site; therefore, man was the dominate host for blood-feeding midges.

A few shade trees of various sizes and heights were maintained in the yard. A group of larger trees flanked one side of the house at a distance of 7 to 10 meters. These trees provided a continuously shaded area throughout the day.

The domestic environs evaluated in this investigation were intradomiciliary and peridomiciliary. Two peridomiciliary sites were located at approximately 10 to 15 meters from the house. One site was under a group of shade trees while the other site was in the open a few meters (10 meters ca.) from both the shade trees and house.

A team of two collectors was stationed at each of the 3 collecting sites, and each team performed biting collections for a period of 45 minutes per hour. Each collecting team was rotated hourly among the 3 collecting sites to prevent differences caused by collector efficiency. Continuous hourly captures were conducted between 06:00 to 18:45 hours. Observations were made 3 times a week for approximately one months duration. Collecting containers were gathered periodically to enumerate the number of Culicoides captured in each area and to determine the species present. Hydrothermographs were used to monitor temperature and relative humidity.

PROGRESS: A comparative summary of the results obtained during this study is presented in Figure 2. With little variation, midge biting activity was noted to begin at approximately 06:00-06:15 hrs each day during the month of observation. The activity data show that there was an early morning peak in biting activity in the house, followed by a steady decline in activity until 10:00 hrs., subsequently, a moderate midday increase in activity was observed for the shaded peridomiciliary site; however, a general increase in biting activity was recorded until midday, after which a marked decrease in biting activity occurred until 16:00 hrs. At 16:00 hrs all sites showed a synchronous increase in biting activity. The late evening activity period lasts for approximately one hour before a sharp decline in numbers is noted. Shortly after 18:30, all biting activity for C. paraensis stops.

The temperature data show that the early morning (06:00-09:00 hrs) temperatures and late evening temperatures (17:00-18:00 hrs) for the three sites vary only a few degrees of each other, while the mid-afternoon temperatures (13:00-16:00) show the largest range of temperature reading. The site recording the highest temperatures was the exposed site, while the inhouse readings were intermediate between the two peridomiciliary temperature readings.

Relative humidity readings were near 100% at the beginning of the collections and fell the below 90% between the hours 08:00 and 17:00 hours. The exposed peridomiciliary site showed the lowest level of humidity.

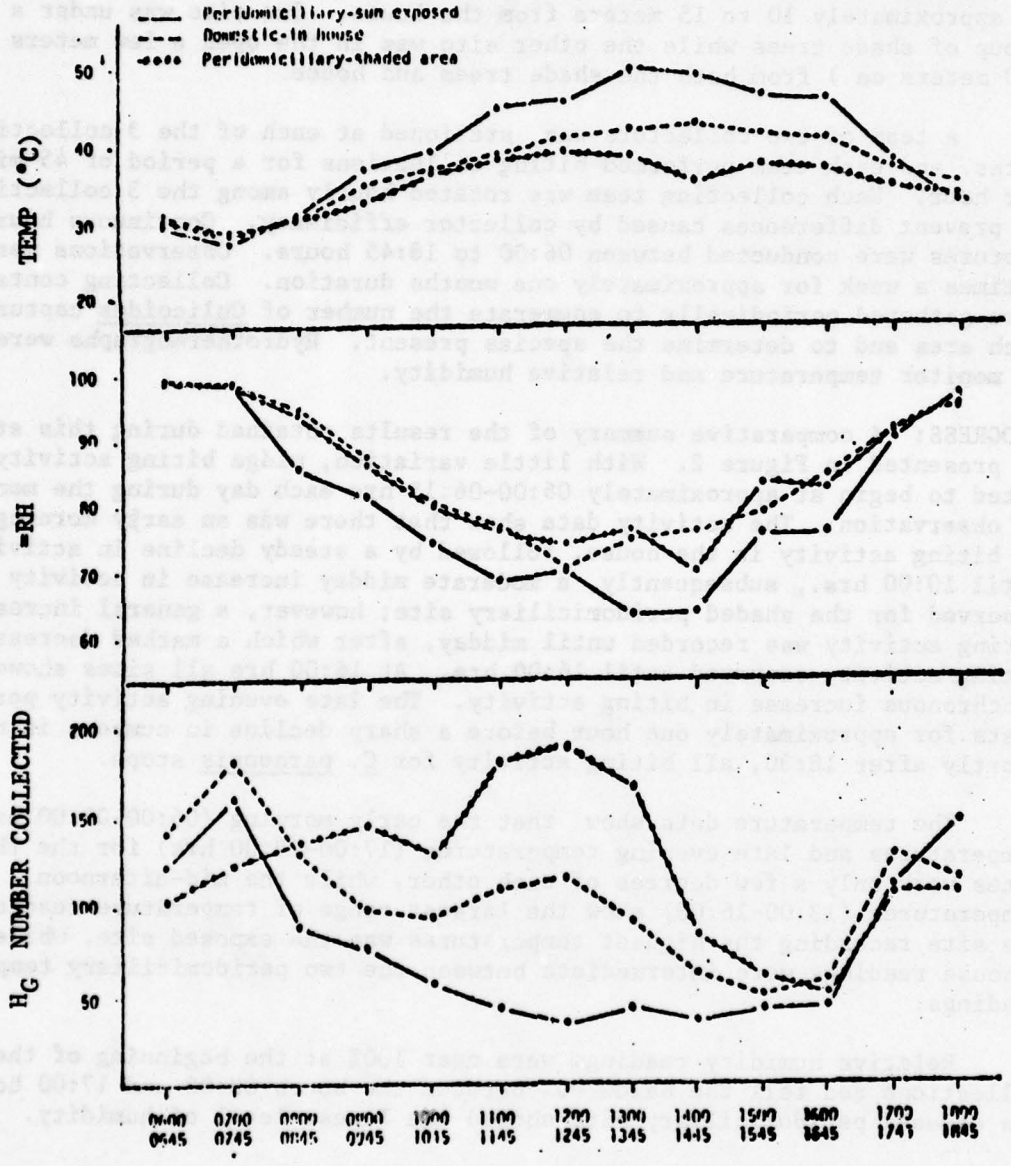

Fig. 2 Diurnal activity pattern of Culicoides paraensis (Goeldi) determined in 3 different environmental settings: Domestic (1) in house; Peridemoestic (2) area shades; (3) areas exposed in the sun, Belém, Pará, Brazil, 1978

I. ECOLOGY OF OROPOUCHE VIRUS

A. Studies on the Epidemic Cycle

1. Vector biology

d. Breeding sites of Culicoides paraensis

OBJECTIVES: The principal objective of the following investigation was to define the breeding sites of Culicoides paraensis. Additional objectives were to:

a. determine the relationship of seasonal rainfall and midge abundance.

b. determine the association of preferred breeding sites and cultural practices.

c. elucidate reasonable control methods for C. paraensis.

BACKGROUND: Epidemiological investigations conducted during epidemics of Oropouche (ORO) virus indicate that C. paraensis was the most common and dominate diurnal insect species collected in all epidemic environments. Review of the epidemiological findings tend to support the hypothesis that C. paraensis is the main urban vector of ORO virus, and laboratory transmission studies have demonstrated that this species is capable of transmitting this virus. However, before the present investigation was initiated, very little was known about the preferred breeding sites of C. paraensis in the urban environs. Since vector biology and ecology must be understood to establish effective and efficient control programs, it was felt that a surveillance program should be conducted to find and describe the natural breeding sites for this biting midge.

DESCRIPTION: The present investigation was conducted at an agriculture experimental research station (CEPLAC) located on the periphery of Belém, Pará, Brazil. This site was selected due to the high population of C. paraensis and security provided for collecting traps. In addition, it was apparent from the high density of C. paraensis, that the breeding sites for this midge must be within the confines of the research station. The spatial and ecological associations of the research station are graphically represented in Figure 3. The graphed area represents only a small portion of the actual area involved in cacao production; however, it was not felt that those areas outside the immediate collecting sites were influencing the midge population in the study area.

Ecologically, the study site would be described as a mixed cacao and banana tree plantation. The planting of banana trees in cacao plots is a common cultural practice and is done so that the banana trees will provide protective shade for young cacao trees. Adjacent areas of secondary forest and lowland marsh bordered the area of investigation.

Emergent traps were used to survey different ecological habitats for breeding of C. paraensis. The type of emergent trap utilized was dependent upon the ecological habitats being evaluated. Description of the habitat types and emergent trap designs were included in the 1976--1977 Annual Report. Therefore, only the breeding sites will be briefly described in this section.

Since the breeding habitats in the study environment were unknown, it was felt that several habitats would have to be investigated to determine the preferred breeding sites. The habitats evaluated were: (1) processed cacao hulls discarded in open piles to decompose; (2) copious layers of cacao leaf litter lying below the cacao trees; (3) cut banana stumps, that portion remaining after the main trunk had been removed; (4) decomposing banana stalks laying on the gound within the cacao and banana plots, and (5) the marsh area composed of a rich organic composite of decaying vegetative matter and stagnant water.

Insect collecting containers employed with each emergent trap were constructed with an alcohol reservoir for preserving the small bodied Culicoides. These reservoir cups were routinely collected to process the captured specimens. Since the purpose of the study was to define the breeding sites of C. paraensis, this species was enumerated, while counting and volumetric techniques were used to quantify the other midge species. Emergent traps utilized in the different habitats were relocated periodically to allow continuous observations of the particular study habitats.

Monthly rainfall data were collected within the CEPLAC research station.

The adult population of C. paraensis was also monitored daily at the research station by a team of two collectors making biting counts on the lower exposed portion of the legs for two sequential 15 minute biting captures.

PROGRESS: Data obtained from emergent traps indicate that C. paraensis were breeding in three of the five habitat types investigated (Table 5). Two of these habitat types were associated with decaying banana vegetative substrate and the third breeding site was decaying cacao hulls. Of the two banana tree substrate habitats, banana stalks were shown to be the preferred breeding site by greater numbers of emerging midges.

The third breeding site for C. paraensis was shown to be decomposing cacao hulls. Emergent trap data for the cacao leaf litter and marshland habitats were negative for C. paraensis and thusly, collections in these habitats were discontinued in February and March, respectively. However, other species of Culicoides were collected in the marshland habitat.

Table 6 presents the quantitative composition of the Culicoides fauna in the two principal breeding habitats represented. Culicoides paraensis is the principal species of Culicoides breeding in the decaying banana stalk habitat however, the cacao habitat contributed considerably less to the total Culicoides species present. Some of the species associated with the cacao and banana habitats are also presented in Table 6.

Figure 4 presents the total monthly rainfall and the number of C. paraensis recorded per trap-day collected from the cacao hull and banana stalk habitats. It is apparent from the data available that there is considerable variation in emergent pattern between the two breeding habitats. The emergent population from the cacao habitats appears to be more directly related to the rainfall pattern than the results obtained from the banana stalk breeding site.

Figure 5 illustrates the monthly population activity pattern for C. paraensis within the study area. It would appear from these data that the breeding pattern is associated with the quantity of rainfall. Moreover, the data tend to demonstrate that a high quantity of rainfall is required to sustain a larger population of C. paraensis at a relatively stable population level.

Figure 6 shows the effects of a dust insecticide (BHC 1.5%) on the population of C. paraensis when the experimental cacao trees were being treated for insect pests. The population showed a temporary decline following the application of insecticide; however, it is noted that by the 3rd and 4th day post-treatment, the population had returned to numbers equivalent to the pre-treatment levels.

COMMENTS: Data obtained in this breeding site surveillance program show that rotting banana vegetative materials and decomposing cacao hulls are the preferred breeding habitats in the study area.

At first it would appear that the banana stumps and stalks should be considered the same habitat; however, after noting the quantity of water associated with the two banana substrates, it was felt that a habitat distinction was warranted. The upright banana stumps function as an artificial reservoir for rain water, thus creating an aquatic environment, while the decaying banana stalk on the ground creates a semiaquatic environment due to its moisture retention properties. The separation of these two habitats is also supported by the emergence data presented in Table 5. It is apparent from the available information that decaying banana stalks are the preferred breeding habitat when compared to banana stump habitat. Unfortunately, no behavioral data for C. paraensis are available to explain the distinction of breeding site selection. However, the most important fundamental fact is that C. paraensis readily breeds in the decaying banana tree substrate.

Decaying cacao hulls were also demonstrated to be a preferred breeding site for several species of midges and C. paraensis. When the cacao pod is processed manually, the pod is divided into two parts to facilitate the removal of the cacao seeds. Thus the divided hulls form artificial containers for breeding sites. However, it has been observed that some organic decomposition must occur before breeding in the highly organic substrate is possible.

It has been a cultural practice for many Brazilians in the Amazon region to plant banana trees near their residences. Within the urban environment it is quite common to observe numerous small groups of banana trees associated with the residences. Banana trees are maintained near the residences for

various reasons. Normally, banana trees when planted in small groups near the houses provide a source of fruit which supplements the family diet; however, it has also been observed in numerous cases that banana trees are maintained to provide shade or to prevent soil erosion.

When a banana tree yields fruit, it is common practice to harvest the banana cluster by cutting the entire trunk on which the fruit was borne, since this portion of the tree will not bear fruit again. Normally, the tree trunk is cut into several smaller sections (stalks) and left at the base of the remaining tree. It is important to note that sectioning the banana tree trunk increases the available breeding material, since egg oviposition and larval development occurs primarily on the exposed ends of the stalks and the decaying material within. Banana stalks form a decaying substrate which is utilized as an organic fertilizer. The length of time required for decomposition of the banana substrate is variable depending on environmental conditions; however, the substrate will normally serve as a breeding site for midges for 2 to 4 months.

The second preferred breeding habitat associated with cultural practices in the Amazon basin is that of cacao production. Normally, cacao plantations are not closely associated with larger urban areas; however, large plantations of cacao have been observed to be closely associated with smaller agriculturally oriented urban areas. Cacao is normally harvested once a year during the months of January-March. Normally, after the cacao pods are removed from the tree they are taken to a central area, usually near the owners residence, and processed for the seed from which cocoa is derived. This process is performed by manual labor which requires the cacao pods to be opened and the seeds extracted. The problem that is then derived from the processing is proper disposal of large volumes of cacao hulls which have no commercial value. Normally, the cacao hulls are pilled in large mounds and left to descompose naturally. As the cacao substrate is decomposing it provides an excellent breeding habitat for C. paraensis and other midge species. Depending on the quantity of material present, the hulls may produce thousands of man-biting midges.

Since available data indicate that the breeding sites for C. paraensis are closely associated with cultural practices of man, then the problem arises, what are the best methods for reducing or controlling the midge population. Since decomposing banana vegetative matter has been found to be a preferred midge breeding site, it appears that urban populations of C. paraensis could be significantly reduced if the decomposing banana material could be removed from the residential areas. This could be accomplished by simple burial of the banana breeding materials within the area where banana trees are grown or by an organized municipal program in which the material is disposed of in a sanitary landfill. Either method would isolate the material from serving as breeding site. However, cultural modification within the urban environment cannot be accomplished unless a majority of the residences participate in the control program. It is conceivable that by simple cultural methods that the urban population of C. paraensis could be reduced to a population level below that required for transmission of ORO virus.

Cultural control efforts in areas where there exist large cacao plantations share similar problems to those noted in the urban areas, namely the removal or destruction of the midge breeding sites. Studies conducted in a cacao plantation environment indicate that the decomposing cacao pods are the only substrate producing large numbers of C. paraensis. Therefore, if the cacao pods were disposed in a proper manner this breeding habitat could be significantly reduced. Unfortunately, it is common practice to leave piles of this material near the residence, thereby creating a favorable association of human blood meal and midge breeding sites.

In the event that cultural modification programs are ineffective, then temporary control programs with the use of insecticide would have to be initiated. Diurnal activity patterns for C. paraensis described previously indicate that the biting midge population is most active during the later evening hours (16:00-18:00). Therefore, it is only reasonable to conclude that a cost-effective pesticide control program would take advantage of this information and concentrate control efforts during this period.

In summary, it would appear that basic modifications in the cultural practices associated with the proper management of potential breeding materials for C. paraensis could significantly reduce the potential for occurrence of ORO epidemics in the Amazon Basin.

COMMENTS: When reviewing population activity of C. paraensis in the domestic environs, it is apparant that the behavioral bitting patterns were markedly different between sites, and that monitored physical parameters were also variable between environs. With the available data it is difficult to correlate biting activity with the physical parameters, since several factors affecting biting activity were not evaluated (ex. physiolocal status of the biting midge, influence of light intensity and duration). However, some beneficial information can be derived from the data.

It is apparent from the large number of Culicoides collected in the domestic environs, that this species would be a good suspect for disease transmission. It is strongly endophilic and readily feeds on man. The data show that biting activity in the house and shaded collecting sites remained fairly high throughout the day. This behavioral activity is important since it is also human and animal behavior to seek protected areas from the sun and higher temperatures during midday. In addition, this activity pattern suggests that the physical parameters and humidity may be involved in modifying the temporal activity pattern of C. paraensis.

A third parameter, which was not monitored but which is believed to have a major effect on midge population activity, is light intensity. It was noted that the early morning peak in biting activity began with the rapid change in light intensity; however, during this transition phase the physical parameters of temperature and humidity were stable.

The higher number of midges collected in areas showing lower temperatures and higher humidities during midday tends to suggest that the Culicoides are leaving those areas of higher temperatures and concentrating in more protected areas. However, when environmental conditions become more favorable late in the afternoon, between 16:00-18:00 hours, the midge activity markedly increases and disperses.

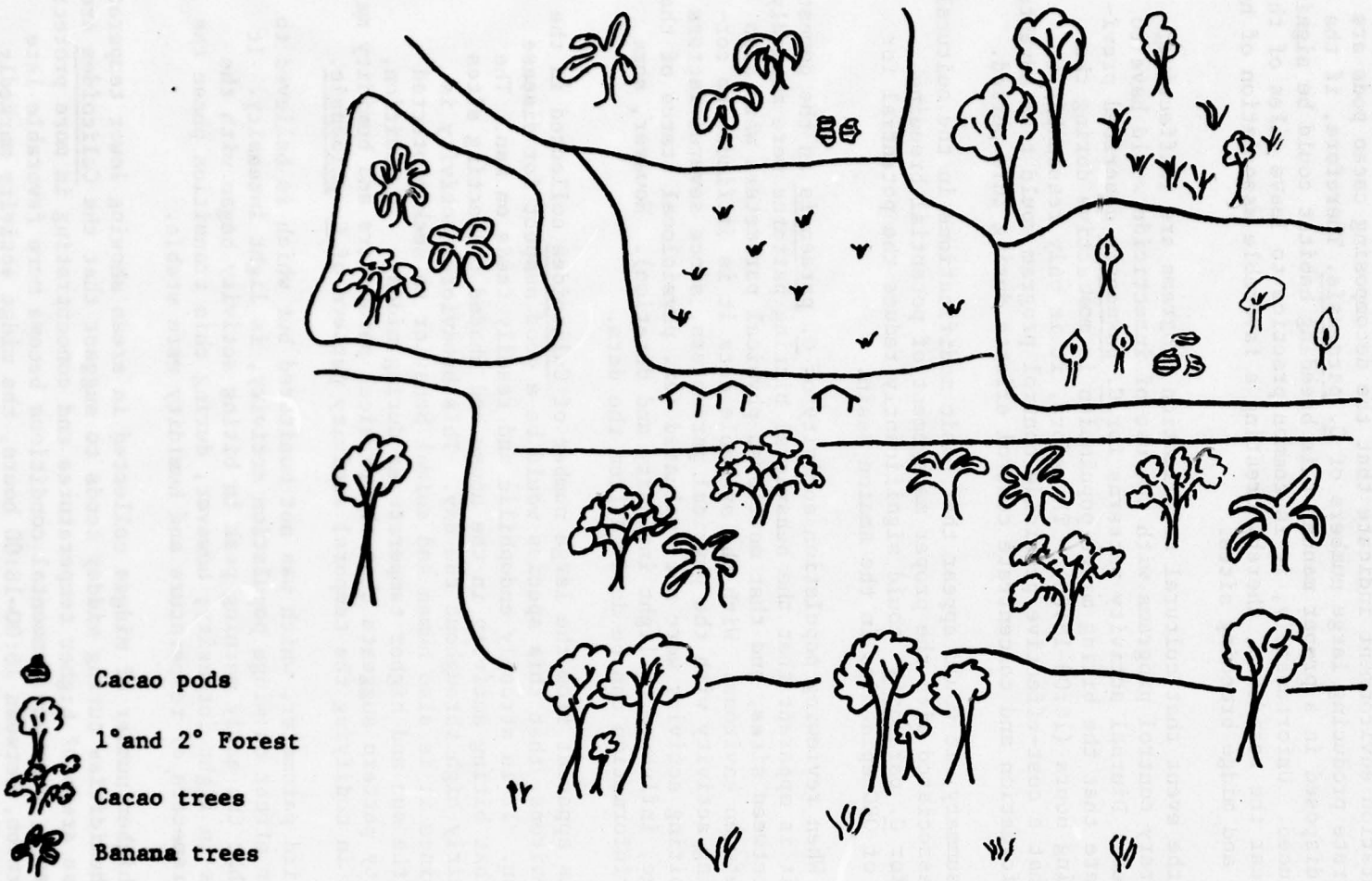

FIGURE 3. Spatial and ecological association of breeding site habitats at a cacao research station Belém, Pará, Brazil, 1977-1978.

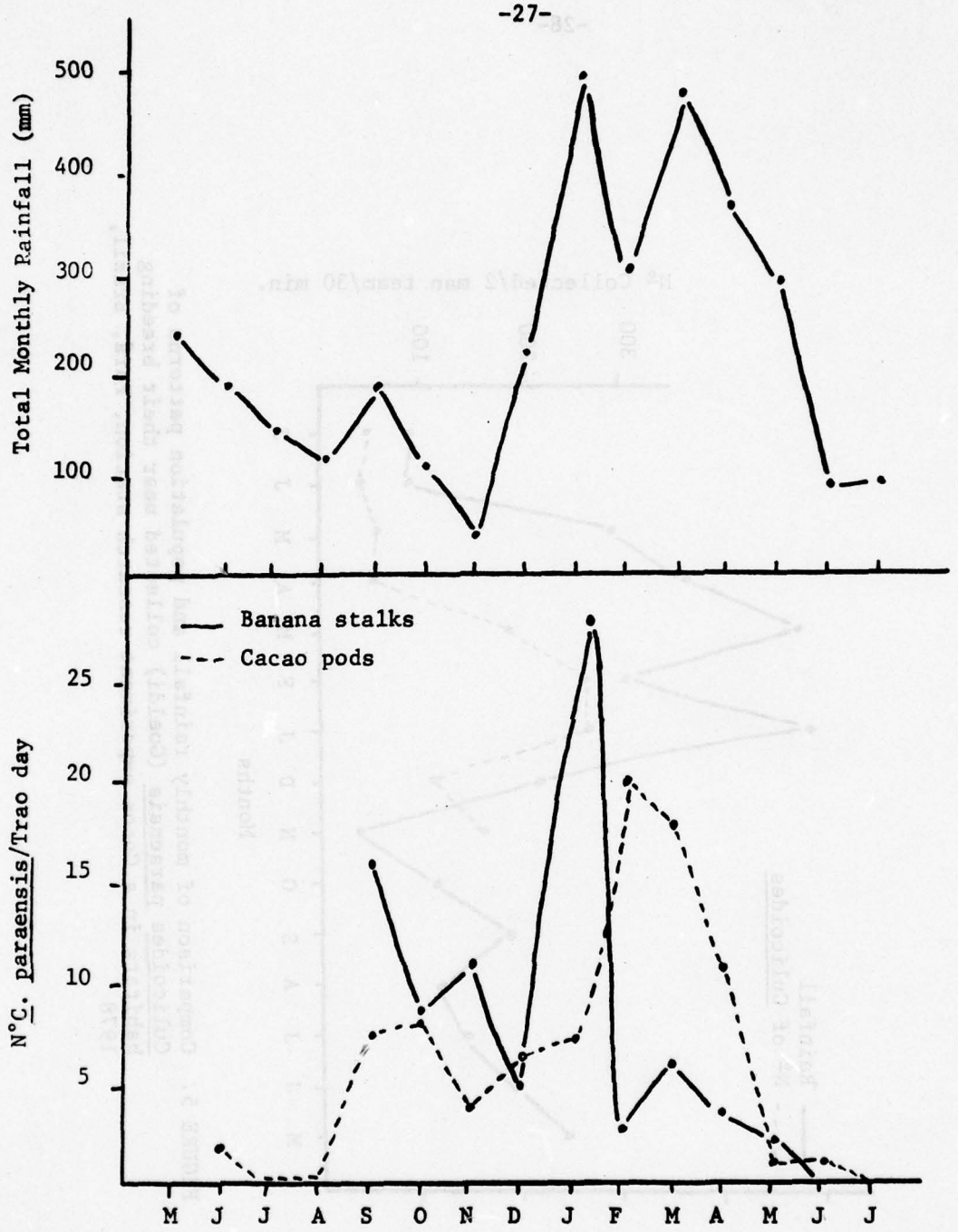

FIGURE 4. Cumulative monthly rain fall and pattern of Culicoides paraensis (Goeldi) emerging from breeding substrates of decaying cocoa pods and banana stalks. Belém, Pará, Brazil, 1977-1978

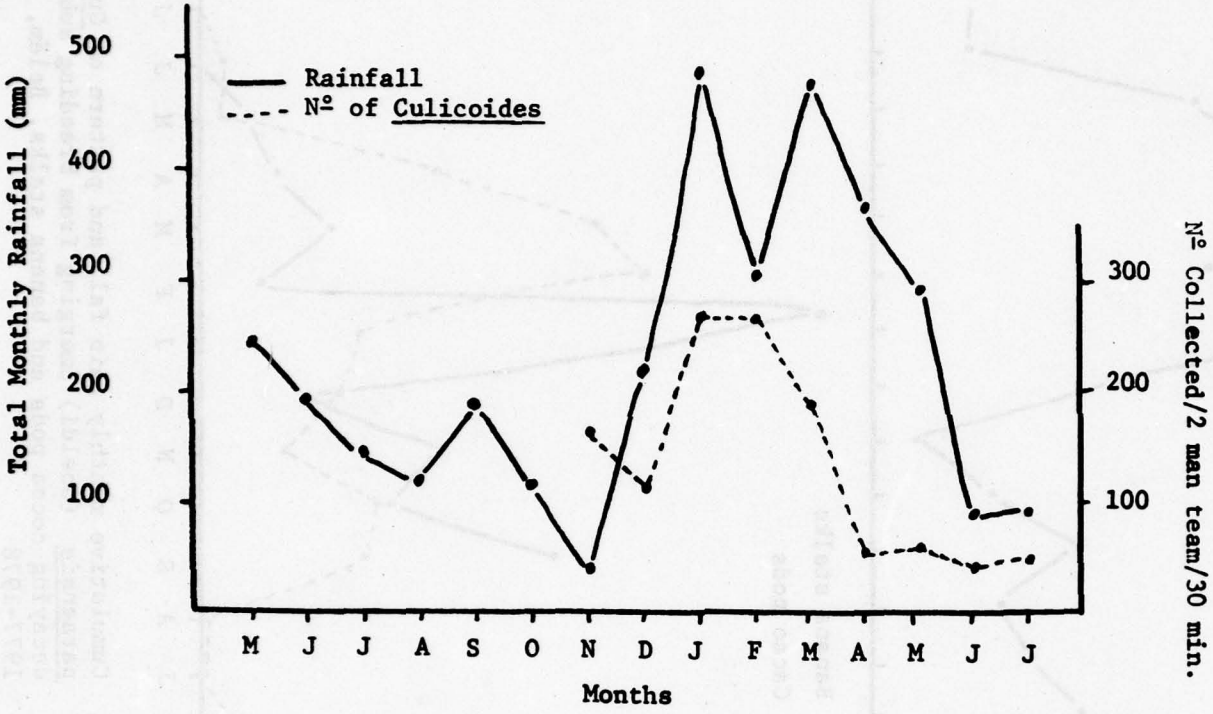

FIGURE 5. Comparison of monthly rainfall and population patterns of Culicoides paraensis (Goeldi) collected near their breeding habitats in a Cocoa experiment research station, Pará, Brazil, 1978

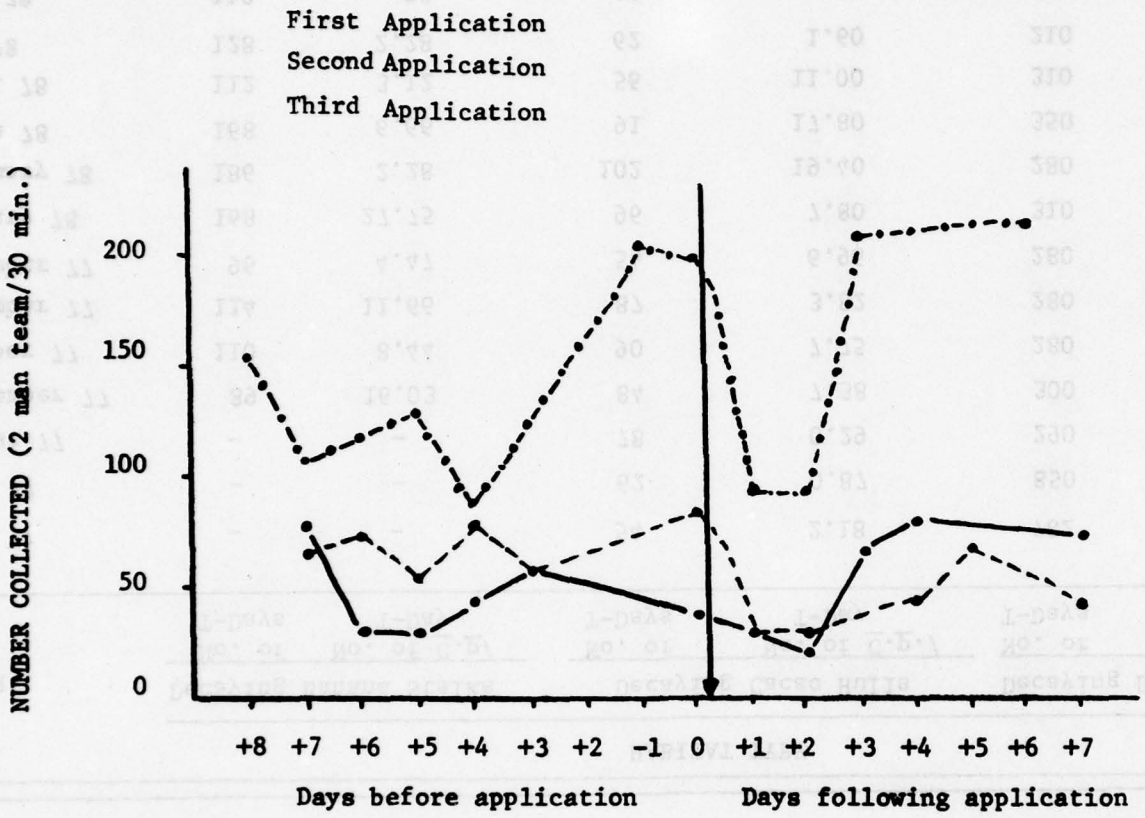

FIGURE 6. Effects of insecticide (BHC, 1.5%, dust) applications on populations of Culicoides paraensis (Goeldi) in Cocoa tree experimental plots being treated for Cocoa insect pest.

TABLE 5. Observations on Habitat preference of *Culicoides paraensis* evaluated by emergence traps *Culicoides* species were collected by means of emergent traps, Belém, Pará, Brazil, 1977-1978

MONTH	HABITAT TYPE					
	Decaying Banana Stalks		Decaying Cacao Hulls		Decaying Banana Stumps	
	No. of T-Days	No. of *C.p.*/ T-Day	No. of T-Days	No. of *C.p.*/ T-Day	No. of T-Days	No. of *C.p.*/ T-Day
June 77	-	-	54	2.18	782	.01
July 77	-	-	62	0.87	850	.01
August 77	-	-	78	0.29	290	.01
September 77	89	16.03	84	7.38	300	.10
October 77	110	8.44	90	7.75	280	.06
November 77	114	11.66	87	3.82	280	.04
December 77	96	4.47	54	6.96	280	.08
January 78	168	27.75	96	7.80	310	.44
February 78	186	2.28	102	19.40	280	.00
March 78	168	6.66	91	17.80	350	.17
April 78	112	3.12	56	11.00	310	.00
May 78	128	2.28	62	1.60	210	.07
June 78	112	.05	66	1.20	320	.02
July 78	124	.83	62	.05	280	.02

TABLE 5. Observations of habitat preference of Culicoides paraensis evaluated by emergence trap. Culicoides species were collected by means of emergent traps. Belém, Pará, Brazil, 1977-1978 (Cont.)

MONTH	HABITAT TYPE			
	Decaying leaf-litter		Marsh land Area	
	No. of T-Days	No. of C.p./T-Day	No. of T-Days	No. of C.p./T-Day
June 77	1222	0	-	-
July 77	1316	0	-	-
August 77	1363	0	-	-
September 77	600	0	216	0
October 77	620	0	240	0
November 77	620	0	126	0
December 77	620	0	204	0
January 78	480	0	168	0
February 78	700	0	138	0
March 78	-	-	234	0

TABLE 6. Quantitative composition of the Culicoides fauna* represented by Culicoides paraensis determined in two principal breeding habits. Culicoides species were collected by means of emergent traps, Belém, Pará, Brazil 1977-1978

MONTH	HABITAT TYPE					
	Decaying Banana Stalks			Decaying Cocoa Hulls		
	No. of Culicoides species collected	No. C. p.	%C. p.	No. of Culicoides species collected	No. C.p.	%C. p.
June 77	-	-	-	7156	118	2.0
July 77	-	-	-	5158	54	1.0
August 77	-	-	-	3449	23	1.0
September 77	1473	1427	97.0	8541	620	8.0
October 77	933	928	99.0	9351	698	7.0
November 77	1788	1769	99.0	11079	332	3.0
December 77	474	333	70.0	4027	376	9.0
January 78	7121	4663	65.0	11524	746	6.5
February 78	880	424	48.2	18103	1977	10.9
March 78	2410	1120	46.5	16674	1625	9.7
April 78	1479	349	23.6	4967	618	12.4
May 78	829	292	35.2	2213	99	4.5
June 78	266	6	2.2	3838	79	2.1
July 78	1606	103	12.8	2567	3	< .1

* Species of Culicoides and Forcipomyia spp collected

C. paraensis	C. insinuatus	C. hylas
C. debilipalpis	C. tetrathynia	Forcipomyia spp.
C. fusipalpis	C. Foxi	

I. ECOLOGY OF OROPOUCHE VIRUS

A. Studies on the Epidemic Cycle

2. Man as the principal vertebrate host

OBJECTIVE: The objective of this section is to summarize available information regarding human infection with Oropouche (ORO) virus. Special areas of consideration will be the clinical syndrome and viremia produced following infection. The underlying question is: Can man serve as the principal vertebrate amplifying host in the epidemic cycle of ORO virus?

BACKGROUND: The previous sections have clearly established that Culicoides paraensis is in all probability the primary epidemic vector of ORO virus to man. The question remains though, where the infected vector acquires its infection. The sections on the basic biology of C. paraensis have demonstrated that this species is intimately linked to man though its utilization of breeding sites created by man, and in all probability by using man as its prime source of blood meals. The behavioral patterns described for this species indicate that it utilizes a diurnal activity period and that it is not adverse to entering houses in search of its hosts. Certainly the vector is adequately exposed to man.

The previous section which dealt with ORO virus transmission studies gives some idea as to the threshold of viremia needed to infect feeding C. paraensis. While a definite critical threshold was not established, many C. paraensis were infected when fed on a viremic hamster which titered $10^{5.0}$ $SMLD_{50}$/0.02 ml, and most of those infected went on to transmit ORO virus by bite to susceptible hamsters. Consequently, one might assume that a viremia titer of this magnitude would be sufficient to serve to infect feeding vectors.

DESCRIPTION: Information presented in this section has been taken from the published references cited. No new studies were conducted.

PROGRESS: Clinical disease in man due to infection with ORO virus was described in detailed by Pinheiro et al (1976). They reported on an epidemic which occurred in the village of Mojui dos Campos, Pará, Brazil during February, 1975. Clinical symptoms of 68 patients naturally infected with ORO virus during this outbreak are summarized in Table 7. Most frequently reported clinical manifestations were fever, headache, chills and myalgia. Illness was reported to last from 2 to 7 days, and several patients became severely ill, occasionally to the point of prostration.

Titrations of ORO virus isolated from naturally infected viremic patients have been reported on occasion. Pinheiro et al. (1962) presented titers of 15 patients bled during illness and showed a maximum titer of

$10^{4.8}$ $SMLD_{50}$/0.02 ml. Five (33.3%) of the fifteen sera tested, had titers equal to or greater than $10^{4.0}$ $SMLD_{50}$/0.02 ml. These results are reproduced in Table 8. In the Mojui dos Campos study, viremia was titrated in 6 patients, and results of comparative titration methods were also reported. In this study, 5 (83%) of the 6 sera tested titered equal to or greater than $10^{4.0}$$SMLD_{50}$/0.02 ml, and the highest titer recorder was $10^{5.2}$ $SMLD_{50}$/0.02 ml. These results are produced in Table 9. A seventh patient, not included in Table 9, was reported with a miximum titer of $10^{6.0}$ $SMLD_{50}$/0.02 ml. A patient in whom viremia was followed daily was viremic during the first 4 days of illness, but negative on the 5th day.

COMMENT: Results summarized here clearly indicate that man, when infected with ORO virus, produces a viremia of sufficient titer to infect at least a portion of the feeding C. paraensis. If $10^{5.0}$ $SMLD_{50}$/0.02 ml is used as a critical threshold to infect feeding midges then 2 (9%) of the 22 reported cases could serve as amplifying hosts. If $10^{4.0}$ $SMLD_{50}$/0.02 ml is considered the critical threshold, then 11 (50%) of the 22 patients tested could contribute. With a viremic period which spans 4 days, and apparently sufficient viremia titers to infect feeding vectors produced during at least part of that time, it seems probable that man is the principal vertebrate amplifying host in the epidemic cycle of ORO virus. The fact that man may become quite ill with ORO virus infection, to the point of remaining at home in bed, does not lessen the impact of this potential contribution, since it has already shown that C. paraensis will quite readily enter houses in search of human blood meals.

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2. Pinheiro, F.P., Travassos da Rosa, A.P.A., Travassos da Rosa, J.F. and Bensabath, G.: An outbreak of Oropouche virus disease in the vicinity of Santarém, Pará, Brazil. Tropenmed. Parasit. 27: 213-223, 1976.

TABLE 7. Clinical symptoms of 68 patients infected with Oropouche virus during an outbreak in Mojui dos Campos, Pará, Brazil, 1975. *

Signs or Symptoms	%
Fever	97
Headache	88
Chills	85
Myalgia	82
Arthralgia	67
Photophobia	66
Dizziness	63
Nausea	26
Conjunctival congestion	22
Vomiting	17
Diarrhea	13
Coryza	3
Cough	3

* From Pinheiro, et al. 1976

TABLE 8. Summary of Oropouche (ORO) viremia titers among 15 patients from whom the virus was isolated during an epidemic in Belém, Pará, Brazil, April and May, 1961*

Specimen number	Titer of ORO virus in sera
H 29604	4.8 $\log_{10}$SMLD$_{50}$/0.02 ml
H 29086	4.7
H 29716	4.5
H 29762	4.0; 3.0
H 30125	4.0
H 29327	3.9
H 29020	3.8
H 29516	3.5
H 29717	3.4
H 29872	3.4
H 30079	3.2
H 29329	2.9
H 29761	2.4
H 29559	1.8
H 29718	0.8

* From Pinheiro et al., 1962

TABLE 9. Summary of Oropouche viremia titers among 6 patients infected during an epidemic in Mojui dos Campos, Pará, Brazil in 1975. Also shown is a comparison of the sensitivity of different methods for titrating Oropouche virus. *

Specimen Numbers	Vero cells			Baby mice ic
	PFU/0.1 ml	TCD_{50}/0.01 ml		LD_{50}/0.02 ml
		Tubes	Microplates	
H 275932	4.9**	5.5	-	4.3
H 273999	4.5	4.5	-	4.0
H 274000	-	4.7	-	3.5
H 274053	-	3.5	3.5	4.0
H 274055	5.7	6.0	5.0	5.2
H 274082	4.6	-	4.5	4.8

* From Pinheiro et al., 1976

** Log_{10}

I. ECOLOGY OF OROPOUCHE VIRUS

A. Studies on the Epidemic Cycle

3. The Tomé Açú epidemic

OBJECTIVE: The objective of this section is to conduct concurrent epidemiological and entomological surveys during an Oropouche (ORO) virus epidemic in Tomé Açú and contiguous rural settlements. Additional objectives of the survey were to:

a. determine the prevalence of ORO virus in the inhabitants of Tomé Açú and surrounding rural areas

b. define the geographical distribution of clinical cases of ORO virus

c. quantify and identify the hematophagous insects biting man in a peridomiciliary environment

d. identify the epidemic vector of ORO virus.

BACKGROUND: During the second quarter of 1978, reports of clinical cases of Yellow Fever (YF) virus from Tomé Açú area were referred to the Institute Evandro Chagas Virology Section. In August, members of the Virology Section and the PAHO team traveled to the areas where YF virus was reported. During a general epidemiological survey of the inhabitants in the area, the Tomé Açú hospital was visited to determine if febrile patients were present. An adolescent female patient had been admitted on the day of the visit with clinical signs of fever and general discomfort. Blood was drawn for virus isolation attempt. The virus isolated was identified as ORO virus. Thus, it became apparent that two epidemics of arboviral agents were occurring concurrently, both YF and ORO. Subsequently, field investigations for ORO virus activity in the Tomé Açú area were initiated by a joint effort of Instituto Evandro Chagas and the PAHO team.

DESCRIPTION: Tomé Açú is a small village located due south of Belém, in the state of Pará. The area of Tomé Açú is divided into various jurisdictional areas, in which the village of Quatro Bocas lies. Early clinical cases of ORO virus were reported primarily from Quatro Bocas. From early case reports it was felt that the disease focus was located in the vicinity of Quatro Bocas. This village serves as a commercial center for the large agriculture area surrounding Quatro Bocas.

Epidemiological efforts were concentrated within the village and nearby agriculture farms: however, reports of persons with fever in nearby

settlements were also investigated when feasible. A house-to-house survey of Quatro Bocas to identify those persons exhibiting clinical signs of ORO virus infection was conducted by a 2 man epidemiological survey team. Case histories were recorded and blood samples were drawn for virus isolation attempts. Blood samples were stored in liquid nitrogen and transferred to the Institute Evandro Chagas for virus isolation attempts. White mice and hamsters were utilized to isolate ORO virus from febrile cases.

Entomological surveys for hematophagous insects were conducted primarily in two areas of the village and two peripheral areas. Capture sites are shown in Figure 7. Sites were chosen based on epidemiological evidence of ORO virus activity in those areas. Systematic man-biting captures for insects were performed by two man teams making collections near the houses. Night-time man-biting collections and CDC light traps baited with carbon dioxide were used. Captured hematophagous insects were gathered periodically and transferred to the field laboratory where they were separated into general taxonomic groups and subdivided into blood fed and non-engorged. Only non-engorged insects were tested for virus isolation. Field material was conserved in liquid nitrogen and returned to the main laboratory for virus isolation attempts.

In Belém, collected insects were identified and pooled for virus isolation attempts. Initial pool size for Culicoides paraensis was approximately 100 individuals. When it later became apparent that several thousand Culicoides would need to be tested, the pool size was raised to 200.

Culicoides were assayed for virus in Vero cells grown in tubes. Pools of Culicoides were triturated with tissue grinders in 1.0 ml of 25% Bovine plasma albumin in phosphate buffered saline with antibodies. Triturated pools were centrifugated at low speeds for 15 minutes, then 0.1 ml of supernatant fluids inoculated into duplicate drained tubes of Vero cells. Tubes were then incubated for 1 hr at 37°C, rinsed, and 1.0 ml of fresh maintenance media added. Tubes were observed daily for evidence of viral cytopathic effect (CPE) for 15 days post inoculation. Oropouche virus normally causes CPE on initial passage in Vero cells between days 10 and 14.

PROGRESS: Table 10 presents a summary of the age, sex, occupation and area of residence for confirmed cases of ORO virus in the area of Tomé Açú.

During the initial ORO virus survey in the Tomé Açú area, 20 strains of ORO virus were isolated from human blood samples. Twelve of the isolates were from persons residing within the village of Quatro Bocas, with one case occurring on cacao plantations bordering the village. One case was identified in Tomé Açú, which is located approximately 13 kilometers west of Quatro Bocas. Two cases were reported from Arraia, a small village located to the north of Quatro Bocas. Four additional cases were recorded from field technicians investigating the epidemic in Quatro Bocas and one case recorded from a female visiting the area during the epidemic.

Entomological survey results show that 15 species of insects were collected by man-biting captures in the village of Quatro Bocas (Table 11). Capture data show that the dominate diurnal peridomiciliary species were C. paraensis, with Culex fatigans and Culex coronator being the dominate nocturnal species. Numbers of hematophagous insects collected during the survey were fairly variable when comparing spatial distribution of insects within the village and surrounding agriculture areas. The greatest difference noted in spatial distribution of insects was for C. paraensis, the suspect vector of ORO virus. The largest number of C. paraensis recorded was associated with those areas involved in cacao production, with the fewest numbers being collected from bordering areas.

Figure 8 shows the diurnal biting pattern for C. paraensis near a house located on a cacao plantation. This area was found to have a large population of biting midges. This biting activity was found to be a trimodal pattern with the highest activity occurring between 16:00 and 18:00 hours.

Preliminary results are available for virus isolation attempts from insects collected from Quatro Bocas. To date a total of 155 pools representing 24,465 individuals of C. paraensis have been tested for virus in cell culture. Of these, 4 pools have produced CPE characteristic of ORO virus. All 4 have been successfully passed in cell culture, all are neutralized by antibody specific to ORO virus. All 4 pools were collected from the same locality, a Japanese cacao plantation which had several persons ill with ORO virus infection.

Figure 9 graphically depicts the location of residencies for 12 persons from whom ORO virus was isolated. In addition, the collection site of the 4 pools of C. paraensis from which ORO virus was isolated is also presented. Clearly ORO virus activity was dispersed throughout the village of Quatro Bocas, and not localized in any particular area.

COMMENTS: Epidemiological findings indicate that an epidemic of ORO virus was being experienced by the inhabitants of the Tomé Açú area, with an apparent focus of disease activity occurring in Quatro Bocas and adjoining agricultural farms. However, data are not available to determine the initial onset of the disease, since it is a common practice in small communities of the Amazon Basin, for ill persons to remain in the home and not seek medical attention.

It was apparent from the preliminary epidemiological findings that ORO virus was more prevalent among the inhabitants than was indicated by the number of confirmed cases. When the house-to-house survey was being conducted numerous households indicated that febrile symptoms similar to ORO virus disease had been experienced by family members; however, the etiological disease was unknown. It would be reasonable to assume that a proportion of

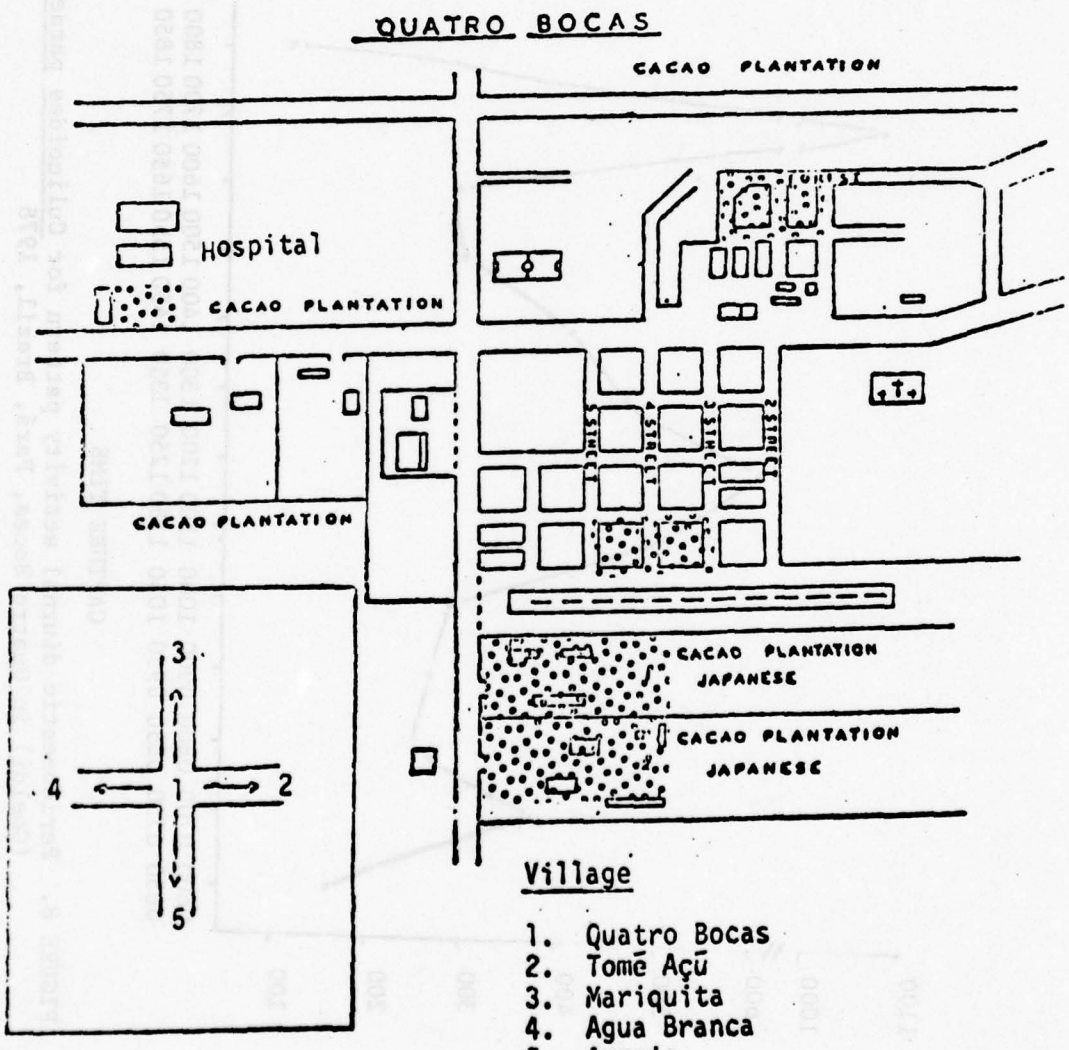
these febrile reports could be attributed to ORO virus. Due to the general dispersion of ORO virus isolates within the village, it was not felt that the disease prevalence could be associated with a particular residential area (Figure 9). This was expected since the village area is small and the inhabitants are quite transient.

From the available data derived from the case histories of confirmed ORO virus isolates, it was found that 85% (17/20) of the cases were males with an age range of 7 to 58. Five of the male cases were persons associated with agriculture related work, and since cacao is one of the main agriculture crops, it can be assumed that these individuals were working in areas of high populations of Culicoides. Only 15% (3/20) of the cases were female, thus the data tend to suggest that activities or occupations are important factors in determining the risk of exposure to ORO virus infection.

It should be noted that the residential location was an important factor when considering the spatial distribution of hematophagous insects (Table 11). An approximately two fold variation was noted for Culex fatigans and Culex coronator in two areas of the village. However, the most marked differences were noted for C. paraensis.

Since the epidemiological and laboratory transmission data accumulated to date indicate that Culicoides paraensis is the most probable insect vector, it is important to note that this specie was the dominant specie collected in all capture areas. However, the number of C. paraensis was found to be considerable higher in the agriculture areas producing cacao than in the village. This findings were not unexpected, since prior studies at a cacao research station in Belém demonstrated that rotting cacao husk provides a natural breeding substrate for several species of midges including that of C. paraensis. Samples of the cacao husks collected at the cacao plantations yielded several individuals of C. paraensis. Since Quatro Bocas is primarily an agricultural center, several large cacao plantations border the small village; therefore, it is proposed that main population of C. paraensis observed within the village can be attributed to the dispersion of this biting midge from nearby cacao plantations. The other recognized breeding site for C. paraensis, decomposing banana tree substrate, does not appear to be important in Quatro Bocas since few banana trees were observed within the village.

FIGURE 7. Spatial distribution of capture sites in Quatro Bocas Pará, Brazil, 1978



Areas where man biting insects were collected.

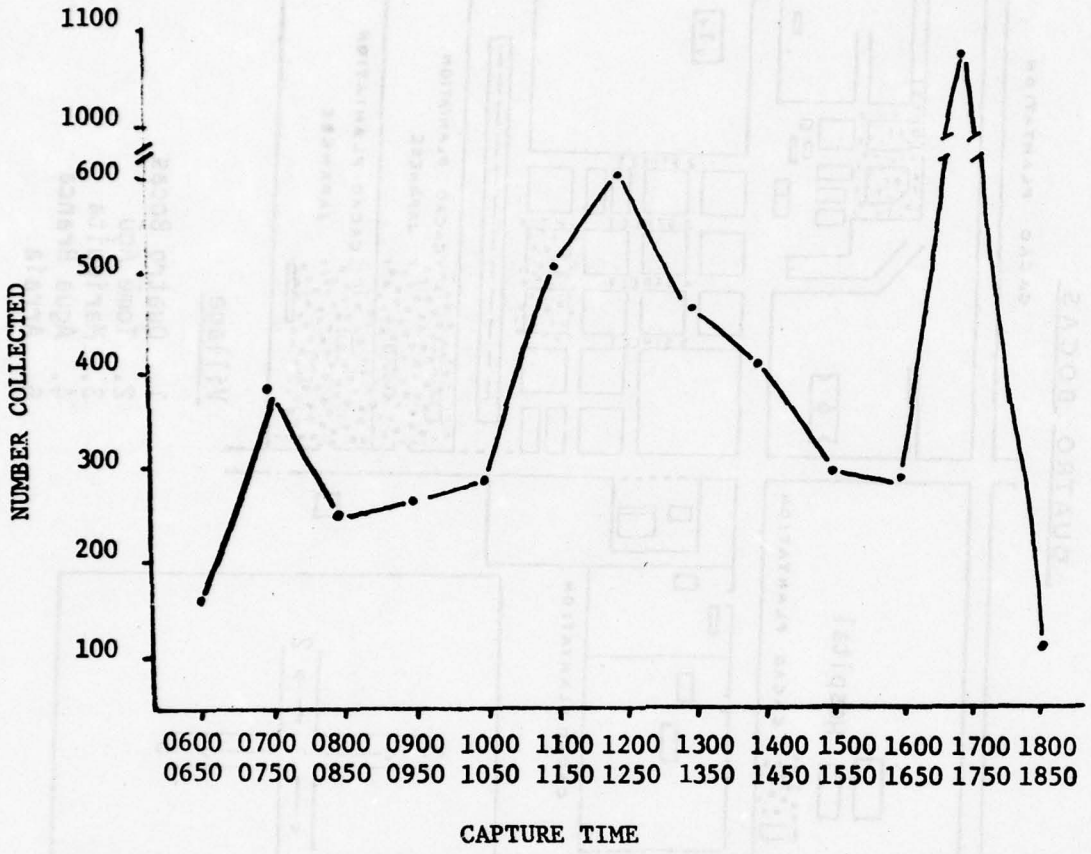
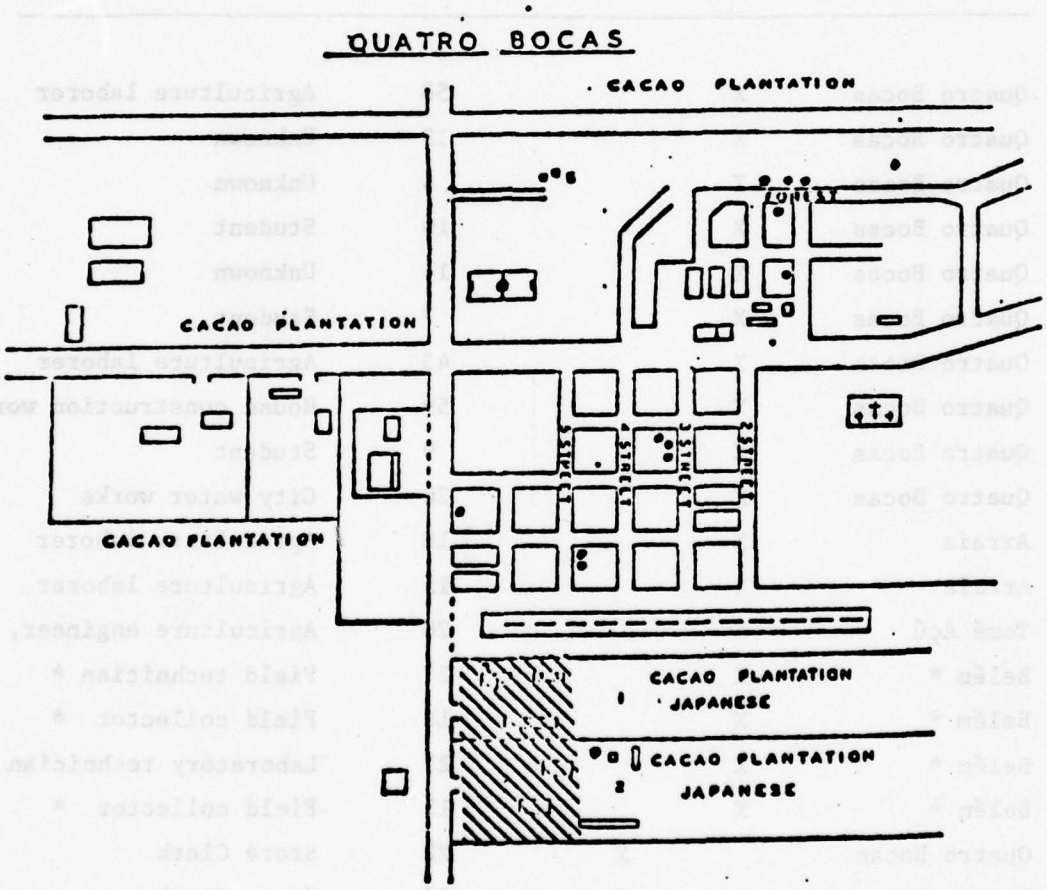


FIGURE 8. Peridomestic diurnal activity pattern for Culicoides paraensis (Goeldi) in Quatro Bocas, Pará, Brazil, 1978

FIGURE 9. Spatial distribution of Oropouche virus isolates from man and C. paraensis in Quatro Bocas, Pará, Brazil, 1978



Area in which four strains of Oropouche virus were isolated from pools of C. paraensis

Spatial distribution of Oropouche virus isolates from man

TABLE 10. Age, sex and occupation data for Oropouche cases determined by virus isolation

City of residence	Sex M	Sex F	Age	Occupation
Quatro Bocas	X		58	Agriculture laborer
Quatro Bocas	X		19	Unknown
Quatro Bocas	X		6	Unknown
Quatro Bocas	X		10	Student
Quatro Bocas	X		14	Unknown
Quatro Bocas	X		7	Student
Quatro Bocas	X		43	Agriculture laborer
Quatro Bocas	X		59	House construction worker
Quatro Bocas	X		9	Student
Quatro Bocas	X		20	City water works
Arraia	X		18	Agriculture laborer
Arraia	X		15	Agriculture laborer
Tomé Açú	X		28	Agriculture engineer, cacao
Belém *	X		27	Field technician *
Belém *	X		16	Field collector *
Belém *	X		27	Laboratory technician *
Belém *	X		15	Field collector *
Quatro Bocas		X	22	Store Clerk
Quatro Bocas		X	23	Store Clerk
Belém		X	27	Visitor

* Members of field teams investigating the epidemic

TABLE 11. List of hematophagous insects collected in an initial survey of Quatro Bocas during an Oropouche virus epidemic, Pará, Brazil, 1978

Location and species collected	Diurnal man-biting captures * (peridomiciliary)	Nocturnal captures		Total
		Man-biting	CDC**	
Forest area				
Culicoides paraensis	171	0	0	171
Aedes Scapularis	1	0	0	1
Culex fatigans	14	67	7	88
Culex coronator	18	15	16	49
Culex declarator	9	0	17	26
Culex corniger	10	0	2	12
Limatus durhamii	2	0	0	2
Psorophora cingulata	0	2	0	2
Trichoprosopon digitatum	1	0	0	1
Wyeomyia spp	4	0	0	4
Village Street #5				
Culicoides paraensis	652	0	0	652
Aedes scapularis	0	1	1	2
Anopheles (Nys) numeztovari	0	0	0	0
Culex fatigans	22	111	39	172
Culex coronator	42	4	29	75
Culex sp #21	0	0	1	1
Culex declarator	0	4	0	4
Culex (Mel.) sp	1	0	0	1
Culex corniger	5	0	5	10
Culex spp	0	3	4	7
Psorophora ferox	2	0	0	2
Psorophora cingulata	0	2	5	7
Japanese Cacao Plantation				
Culicoides paraensis	5056	NT***	NT	5056
Hospital				
Culicoides paraensis	1001	NT	NT	1001

* Capture times were 0700 to 1850 hrs.
** Light traps were operated from 1900 to 0700 hrs.
*** Nocturnal captures were not performed

I. ECOLOGY OF OROPOUCHE VIRUS

B. Studies of the Endemic Cycle

1. Curuá-Una Study

a. Vectors present

OBJECTIVE: The objectives of this study were to conduct field surveillance of hematophagous insects in forested areas in an effort to incriminate natural vector(s) of Oropouche virus, and when possible, to determine seasonal and ecological associations of potential insect vectors.

BACKGROUND: In May 1977 an entomological and ecological field surveillance program was initiated in Curúa-Una to investigate Oropouche (ORO) virus activity in a sylvatic environment. Curúa-Una is located approximately 44 km south and 40 km west of Santarém, Pará, Brazil. This area was selected for study because in 1975 an epidemic of ORO virus was investigated in a small village, Mojui dos Campos, which lies near the Curúa-Una forest. It was felt that perhaps ORO virus was endemic in this forested area, and that ORO virus may have been introduced into Mojui dos Campos from it.

Available epidemiological and entomological information tends to support the hypothesis that the disease is maintained in a sylvatic vertebrate-insect cycle of transmission. This theory is supported by serological evidence of ORO virus activity among forest inhabiting birds and mammals.

DESCRIPTION: The field surveillance program was designed to monitor hematophagous insects for virus activity and to obtain basic entomological information concerning insect ecology. Entomological information was gathered by multiple collecting techniques (light traps, Shannon trap, and man bitting collections). A routine ground and canopy collecting program was established at three separate field collecting stations (Figure 10). The systematic collecting program was designed to study both the diurnally and nocturnally feeding insect activity. Al medically important insects collected are in the process of being classified and assayed for virus.

A sentinel hamster animal program was also conducted for a short period of time during the surveillance. Sentinel animals were marked by digital clipping and exposed at two forest levels, ground and canopy. Hamsters exposed during the diurnal period (06:00-18:00 hrs.) were replaced by a second group of hamsters which were exposed during the nocturnal period (18:00-06:00 hrs.). After being exposed for 2 weeks in the sylvatic environment, they were maintained in the laboratory for two additional weeks and observed for overt clinical signs of virus activity. Subsequently, they were sacrified for serological testing for virus antibodies.

The ecological descriptions for the study area are included in a subsequent section of this report.

PROGRESS: The sylvatic surveillance program was concluded after one season of observations. However, material from this investigation is still being identified and processed for virus isolation attempts. Therefore, only general results will be presented here.

Table 12 presents a preliminary list of hematophagous insects collected by various collecting methods at the three Curúa-Una forested collecting stations. To date, 43 species of Culicidae have been identified and 7 species of biting midges (Ceratopogonidae). The largest number of hematophagous insects collected belong to the Culicinae and Phlebotomus taxonomical groups.

Table 13 summarizes the results of the sentinel hamster program utilized to survey sylvatic virus activity. Seventy nine (79) hamsters were exposed for 6 days each. Only one isolation of virus was obtained, Kwatta virus, and although all sera were tested against a block of 19 antigens, no sero-conversions were noted.

Tables 14, 15 and 16 present the monthly geometric mean of the numbers of taxonomical groups (Culicidae, Psychodidae, Ceratopogonidae) of insects by two collecting methods (man biting, shannon trap). Mosquitoes (Culicidae) and sandflies (Psychodidae) were the dominant group collected.

COMMENTS: As is shown in the species list, the sylvatic environs of Curuá-Una produce a significant number of hematophagous insect species, and thus, demonstrate a wide potential disease vector spectrum. It would therefore seem reasonable to assume that if there exists a sylvatic vector-vertebrate cycle of ORO virus within the study environment, then the basic entomological information obtained during this study would be useful when a vector specie is eventually identified. However, to date none of the sylvatic species have been shown to be involved in ORO virus transmission.

The sentinel hamster program resulted in the isolation of Kwatta virus from a hamster exposed at night at ground level. Kwatta virus was first isolated in Surinam from a Culex mosquito pool. Little is known regarding the natural history of the virus. The isolation of the virus here from the sentinel hamster tends to support a theory of vector-host transmission.

Collection data in Tables 14, 15 and 16 demonstrate that the mosquito fauna represents the largest number of sylvatic hematophagous insects in these areas. Within this group, the number of Culicinae collected was superior to those of the Anophelinae fauna. This was representative of the environment. Ecologically, in areas 1 and 2, the primary breeding sites for Culicinae species were temporary rain water habitats: rain pools, three holes, fruit pod containers, etc. Breeding habitats for Anopheles, were primarily confined to near the river, which was relatively fast flowing and had few aquatic plants, and consequently rather unfavorable for Anopheles.

The collections of Culicinea at area 3 were notably higher than the number recorded for areas 1 and 2. This can be attributed to the large number of Culicinae mosquitoes breeding in the near by river habitat. The river near this site expands into a cove which has numerous aquatic plants covering most of the water surface. This type of habitat is quite favorable for the mosquito species of Mansonia and Coquillettidia, which were the dominant genera collected in this area.

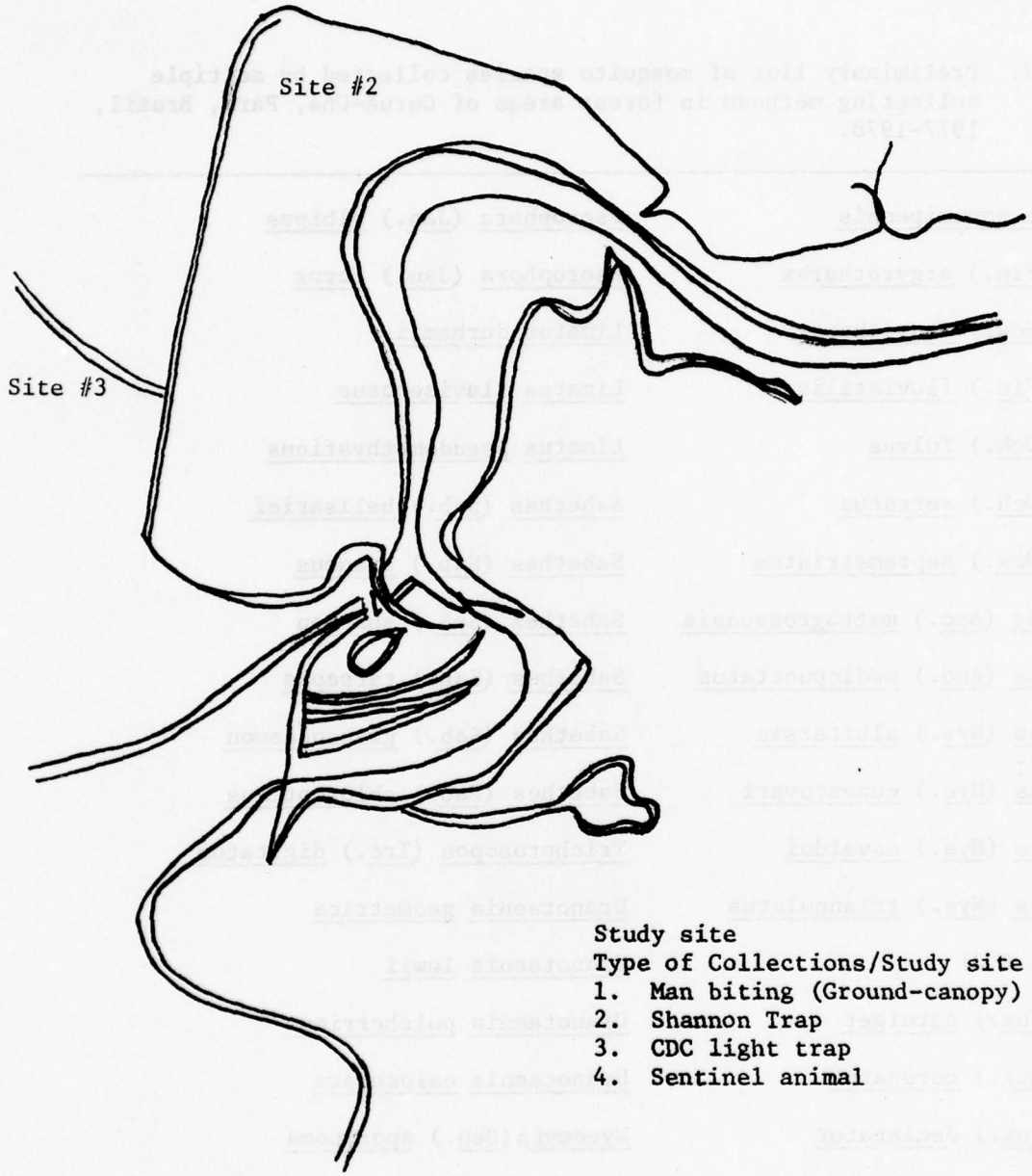


Fig. 10. Geographical distribution of Entomological study sites in the sylvatic area of Curuá-Una, Pará, Brazil, 1977-1978.

Table 12. Preliminary list of mosquito species collected by multiple collecting methods in forest areas of Curuá-Una, Pará, Brazil, 1977-1978.

Aedeomya squamipennis	Psorophora (Jan.) albipes
Aedes (Fin.) argyrothorax	Psorophora (Jan.) ferox
Aedes (How.) fluvithorax	Limatus durhamii
Aedes (Fin.) fluviatilis	Limatus flavisetosus
Aedes (Och.) fulvus	Limatus pseudomethysticus
Aedes (Och.) serratus	Sabethes (Sab.) belisarioi
Aedes (How.) septemstriatus	Sabethes (Sab.) cyaneus
Anopheles (Ano.) mattogrossensis	Sabethes (Sab.) shannon
Anopheles (Ano.) mediopunctatus	Sabethes (Sab.) tarsapus
Anopheles (Nys.) albitarsis	Sabethes (Sab.) glaucodaemon
Anopheles (Nys.) nuneztovari	Sabethes (Sab.) chloropterus
Anopheles (Nys.) oswaldoi	Trichprosopon (Trc.) digitatum
Anopheles (Nys.) triannulatus	Uranotaenia geometrica
Culex sp B 21	Uranotaenia lowii
Culex (Cux.) corniger	Uranotaenia pulcherrimus
Culex (Cux.) coronator	Uranotaenia calosomata
Culex (Cux.) declarator	Wyeomyia (Den.) aporonoma
Culex (Mel.) vomerifer	Anopheles (Nys.) sp.
Culex (Mel.) vomerifer complex	Culex spp.
Coquillettidia nigricans	Culex (Carrollia) sp.
Coquillettidia venezuelensis	Culex (Mel.) sp.
Mansonia (Man.) titillans	Haemagogus spp.
Mansonia (Man.) pseudotitillans	Psorophora spp.
Mansonia (Man.) amazonensis	Uranotaenia spp
Orthopodomyia fascipes	Limatus spp.
	Wyeomyia spp.

Table 13. Summary of the sentinel hamster surveillance program monitoring virus activity in the Curuá-Una forest study areas, Pará, Brazil, 1978.

Month	Location of sentinel animal - forest level			
	Ground		Canopy	
	Day	Night	Day	Night
November	14*	13**	2	2
December	6	6	2	2
January	8	8	2	2
February	4	4	2	2

* Kwatta virus was isolated from one sentinel hamster.
** Number of sentinel animals monitored for 6 day exposure.

Table 14. Seasonal variation of hematophagous insects collected at a field station (Area I) within the sylvatic area of Curuá-Una. Collections were preformed by man-biting and Shannon-trap. Curuá-Una, Pará, Brazil, 1977-1978.

Taxonomic Group	JUL 77	AUG 77	SET 77	OCT 77	NOV 77	DEC 77	JAN 78	FEB 78	MAR 78	APR 78	MAY 78	JUN 78	JUL 78
Man biting collections - Capture Time (17:30 - 18:30)													
Culicidae (Anophelinae)	2.0	2.8	2.2	1.7	1.3	1.1	1.0	1.1	1.2	1.8	2.5	1.9	2.5
Culicidae (Culicinae)	10.7	11.7	18.0	19.9	2.3	27.4	29.4	11.6	13.9	10.3	29.2	38.8	36.1
Psychodidae (Phlebotomus)	6.3	5.3	2.8	3.9	1.1	1.8	1.4	1.2	1.6	2.3	2.9	4.3	1.5
Cerotopogonidae (Culicoides)	1.1	1.0	1.0	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.1	1.1	1.0
Shannon Trap - Capture Time (19:30 - 20:30)													
Culicidae (Anophelinae)	1.5	3.1	1.9	2.4	1.0	2.4	1.2	1.7	1.2	1.6	1.4	2.1	7.3
Culicidae (Culicinae)	9.3	56.1	43.5	29.2	10.0	32.8	19.4	13.3	5.4	10.1	22.7	14.5	47.9
Psychodidae (Phlebotomus)	4.3	5.8	1.1	1.0	2.6	1.0	1.0	1.6	1.3	2.3	2.7	3.1	3.0
Ceratopogonidae (Culicoides)	1.3	1.0	1.0	1.0	1.0	1.0	1.0	1.3	1.0	1.0	1.0	1.5	1.0

Table 15. Seasonal variation of hematophagous insects collected at a field station (Area 2) within the sylvatic area of Curuá-Una. Collections were preformed by man-biting and Shannon Traps. Curuá-Una, Pará, Brazil, 1977-1978.

Taxonomic Group	JUL 77	AUG 77	SET 77	OCT 77	NOV 77	DEC 77	JAN 78	FEB 78	MAR 78	APR 78	MAY 78	JUN 78	JUL 78
Man biting collections - Capture Time (17:30 - 18 30)													
Culicidae (Anophelinae)	1.2	1.2	1.4	1.1	1.3	1.0	1.4	1.0	1.1	-	1.8	1.0	1.0
Culicidae (Culicinae)	5.3	3.8	4.5	4.3	2.3	1.8	13.5	9.3	6.8	-	10.4	7.4	13.2
Psychodidae (Phlebotomus)	11.9	3.6	1.1	1.1	1.1	1.0	5.1	1.8	13.6	-	4.1	1.6	1.3
Ceratopogonidae (Culicoides)	1.6	1.0	1.0	1.0	1.0	1.0	1.0	1.9	1.4	-	1.3	1.0	1.1
Shannon Traps - Capture Time (19:30 - 20:30 hrs.)													
Culicidae (Anophelinae)	1.2	1.3	1.0	1.4	1.0	1.1	1.7	1.6	1.3	-	1.3	1.2	1.0
Culicidae (Culinae)	6.7	20.5	58.7	12.1	10.0	9.1	57.2	13.3	8.4	-	8.0	9.4	18.9
Psychodidae (Phlebotomus)	31.3	8.7	1.3	1.1	2.6	1.0	6.6	3.4	4.7	-	14.5	14.5	1.8
Ceratopogonidae (Culicoides)	2.7	1.0	1.0	1.0	1.0	1.0	2,9	1.0	1.9	-	1.6	12.2	1.6

Table 16. Seasonal variation of hematophagous insects collected at a field station (Area 3) within the sylvatic area of Curuá-Una. Collections were performed by man-biting and Shannon trap. Curuá-Una, Pará, Brazil, 1977 - 1978.

Taxonomic Group	JUL 77	AUG 77	SEP 77	OCT 77	NOV 77	DEC 77	JAN 78	FEB 78	MAR 78	APR 78	MAY 78	JUN 78	JUL 78
Man biting collections - Capture Time (17:30 - 18:30 hrs.)													
Culicidae (Anophelinae)	-	-	8.5	6.8	1.8	1.4	1.1	1.0	1.0	-	1.3	1.0	1.0
Culicidae (Culicinae)	-	-	30.5	132.9	29.3	46.1	26.9	30.5	71.2	-	139.6	135.0	79.2
Psychodidae (Phlebotomus)	-	-	1.0	1.0	1.0	1.2	1.0	1.0	1.5	-	1.0	1.1	1.0
Ceratopogonidae (Culicoides)	-	-	1.0	1.0	1.0	1.0	1.0	1.0	1.0	-	1.0	1.0	1.0
Shannon Trap - Capture Time (19:00- 20:30 hrs.)													
Culicidae (Anophelinae)	-	-	19.9	6.6	4.6	1.7	1.3	1.4	1.0	-	1.2	2.0	4.9
Culicidae (Culicinae)	-	-	91.5	47.5	32.4	34.5	15.8	15.3	15.0	-	99.5	229.0	214.0
Psychodidae (Phlebotomus)	-	-	1.0	1.4	1.1	1.3	1.9	2.0	1.0	-	1.1	1.0	1.0
Ceratopogonidae (Culicoides)	-	-	1.0	1.0	1.0	1.0	1.0	1.0	1.0	-	1.0	1.0	1.0

I. ECOLOGY OF OROPOUCHE VIRUS

B. Studies on the Endemic Cycle

1. Curúa-Una study

b. Vertebrate host serology

OBJECTIVE: Objectives of the program are to determine which species of mammals and birds may serve as a reservoir to Oropouche (ORO) virus in a sylvatic environment, and to elucidate their role in the disease cycle. Habitat preferences, breeding cycles, and annual population fluctuations of incriminated species will also be documented.

BACKGROUND: Antibody to ORO virus has been found in sera of various mammals and birds. The only recorded isolation from a non-human vertebrate was from a three-toed sloth (Bradypus variegatus) collected 150 km southeast of Belém. As part of the general study of the ecology of ORO virus, mammals and birds were collected by trapping, hunting and mist-netting from 15 June 1977 through 10 February 1978 in the Curúa-Una study area.

DESCRIPTION: The Curúa-Una study site is located 44 km south and 40 km west of Santarém, Pará at the Curúa-Una Hydroelectric Plant (latitude 2° 50'S, longitude 54°22'W). Mammals and birds were collected in the forest on both sides of the Curúa-Una River which flows from west to east and is approximately 100 m wide. The general topography of the area ranged from flatland to steep slopes. The river below the hydroelectric plant is 47 m above sea level and the hills rise to approximately 100m. The forest is classified as a tropical semi-evergreen seasonal forest (Beard, 1944). The soil ranges from a reddish-brown sandy clay to clay-sand. The A_1 horizon (humus layer) varies from 1 to 2 cm in thickness and contains many fine hair-like roots. The A_0 horizon (litter layer) is from 4 to 7 cm thick and composed of fallen leaves and twigs. Moss cover on fallen trees and exposed rocks is common. A shrub layer of thin woody plants, 0.5 to 3 m tall is fairly thick, but it is not necessary to cut a trail to walk through the forest. Small trees and palms, to 10 m in height make up an open canopy. Larger trees, from 15 to 20 m tall with slim trunks, are common, and the emergents to 30 m in height are scattered throughout. Small scattered thickets of thin stemmed bamboo are also present. Epiphytes are not common, but small vines and lianas are plentiful. Some of the smaller trees have stilled roots, and buttressing is evident on a few of the emergents.

Small mammals were collected in National live traps (150x150x485 mm) and Rinker live traps (80x80x255 mm) which were set at 10 m intervals along established trails in the forest. The traps were left in place until trapping success began to diminish, usually within 2 to 4 weeks. The Rinker traps were also placed in trees to collected arboreal rodents and marsupials.

The traps were placed in the forest described above. Trapping sites 1, 2, 3 and 4 (Figure 11) were in a flat upland forest. Sites 5 and 6 were in an upland forest with gradual slopes. Sites 7 and 8 were in a forest with steep slopes, and sites 9 and 10 were in an upland hillside forest.

The traps were baited with corn and bananas, and checked early in the morning to reduce the number of animals dying. Larger animals, monkeys, sloths, anteaters, etc were hunted. Hunting was conducted both during the night and during the day.

Bats were collected in mist nets, 12 m long and 2.6 m in height, which were usually set across natural flyways through the forest, such as trails and narrow roads (Figure 11). Two mist nets, numbers 17 and 18 were set in a park-like area along the river. The nets were normally tended from dusk until 22:00 hrs. Nets were not opened while the moon was shining brightly.

The mist nets were also set in continous lines cut through the forest to capture birds (Figure 11). The trail, although kept as narrow as possible, was wide enough to allow passage on both sides of the nets. From 30 to 40 mist nets were opened before dawn and usually tended until 11:00 hrs.

The captured mammals and birds were transferred from the traps and nets to bags and taken to the field laboratory for processing. Each animal was given a collection number at the field laboratory and all specimens taken from it, whole blood, sera ectoparasites, endoparasites, viscera, etc, were given the same number. After processing in the field lab, all laboratory specimens were preserved and shipped to the base laboratory in Belém. Each animal specimen was preserved either as skin and skull, skull only, or in formalin and shipped to Belém for tentative identification, and later to taxonomists specializing in South American animals to confirm the identification. All information was recorded on field forms which were described in the 1975 Annual Report. A more detailed description of the methods of processing the specimens is in the 1976-1977 Annual Report.

Bird and mammal sera were tested in the Belém laboratory for antibody to viruses by the hemagglutination inhibition (HI) tests. Samples of whole blood and viscera were tested for virus by intracerebral inoculation into suckling mice.

PROGRESS: From 15 June 1977 through 9 February 1978 1,032 mammals representing 62 species, and 777 birds of 26 families were collected. Three viruses, Urucuri, Flexal and Kwatta, were isolated from these animals. Antibody to ORO, Mayaro and other viruses were found among these area as well.

Flexal virus was isolated from two species of arboreal rodents. Seventeen percent (2/12) of the Oryzomys bicolor and 25% (2/8) of the O. concolor bloods or viscera tested had Flexal virus. Urucuri virus was isolated from 0.62% (1/162) of the spiny rats, Proexhimys longicaudatus (Table 17). Of the 321 Formacariidae tested, Kwatta virus was isolated from one scale-backed antibird, Hylophylax poecilonota (Table 18).

The sera from 824 mammals were tested by HI tests and three serum two monkey sera and one rodent serum contained antibody to ORO virus while 11 sera, one marsupial serum, six bat sera and 5 monkey sera, were positive for Mayaro virus (Table 19). Of the 624 bird sera treated with protamine to nullify the influence of heparin and tested by HI tests, 42 sera, three Dendrocolaptidae sera, 35 Formacariidae sera, and four Troglodytidae sera, had antibody to ORO virus, while 15 sera, one Dendrocolaptidae serum, 12 Formicariidae sera and two Troglodytidae sera, contained antibodies to Mayaro virus (Table 18).

During the 109 nights of trapping, 348 marsupials and rodents were collected. The spiny rat (Proechimys longicaudatus) was the most commonly trapped mammal (Table 20). The trapping success of the sites north of the river was considerably higher than those south of the river. This may have been due to the northern sites being farther from the hydroelectric plant and the forest less disturbed. Mist nets were tended for 63 nights, and 639 bats were collected (Table 21). Carolla brevicaudata, a fruit eating bat, was the most common bat collected. Forty-three mammals, monkeys, sloths, anteaters, etc., were collected by hunting or by hand.

The bird nets were opened for 59 mornings, and collected 777 specimens. The members of the antibird family, Formicariidae, were the most commonly captured (Table 22). The woodpeckers, Dendrocolaptidae, although half as common as the antibirds, were caught three times more often than any of the remaining families. Bird collecting site B, located north of the river, produced almost twice the amount of birds per morning trapped as either of the two sites south of the river.

COMMENT: Primates, although not common in the Curúa-Una area, had the highest rates of antibody to both ORO virus and Mayaro virus. The two larger species of monkeys, Callicebus torquatus and Alouatta belzebuth, had a higher percent of individuals with antibody than did the marmoset, Callithrix argentata. Very little is known about the longevity of this marmoset in the wild. Although one individual has been reported to have lived for 16 years in captivity, its normal life span may be much less. The larger monkey species may live considerably longer in the wild than the marmosets, thus the probability of being exposed to a virus during its lifetime would be greater.

The members of the antibird family (Formicariidae) were the most common birds collected, and had the highest antibody rate to both ORO virus and Mayaro virus of all birds collected. Although this is a very difficult group of birds to keep in captivity, experimental inoculation with ORO virus and Mayaro virus to determine if they can circulate the viruses at a sufficient titer to infect insects feeding on them are warranted, and initial attempts to do this are reported in a following section of this report.

Although the forest vegetation north of the river appears very similar to that south of the river, the mammal and bird collecting results indicate the density of the animals is higher in the northern sites. The hydroelectric plant was completed in 1977 and many construction workers spent their free hours hunting in the surrounding forest. The forest south of the river was the most accessible and consequently the most heavily disturbed.

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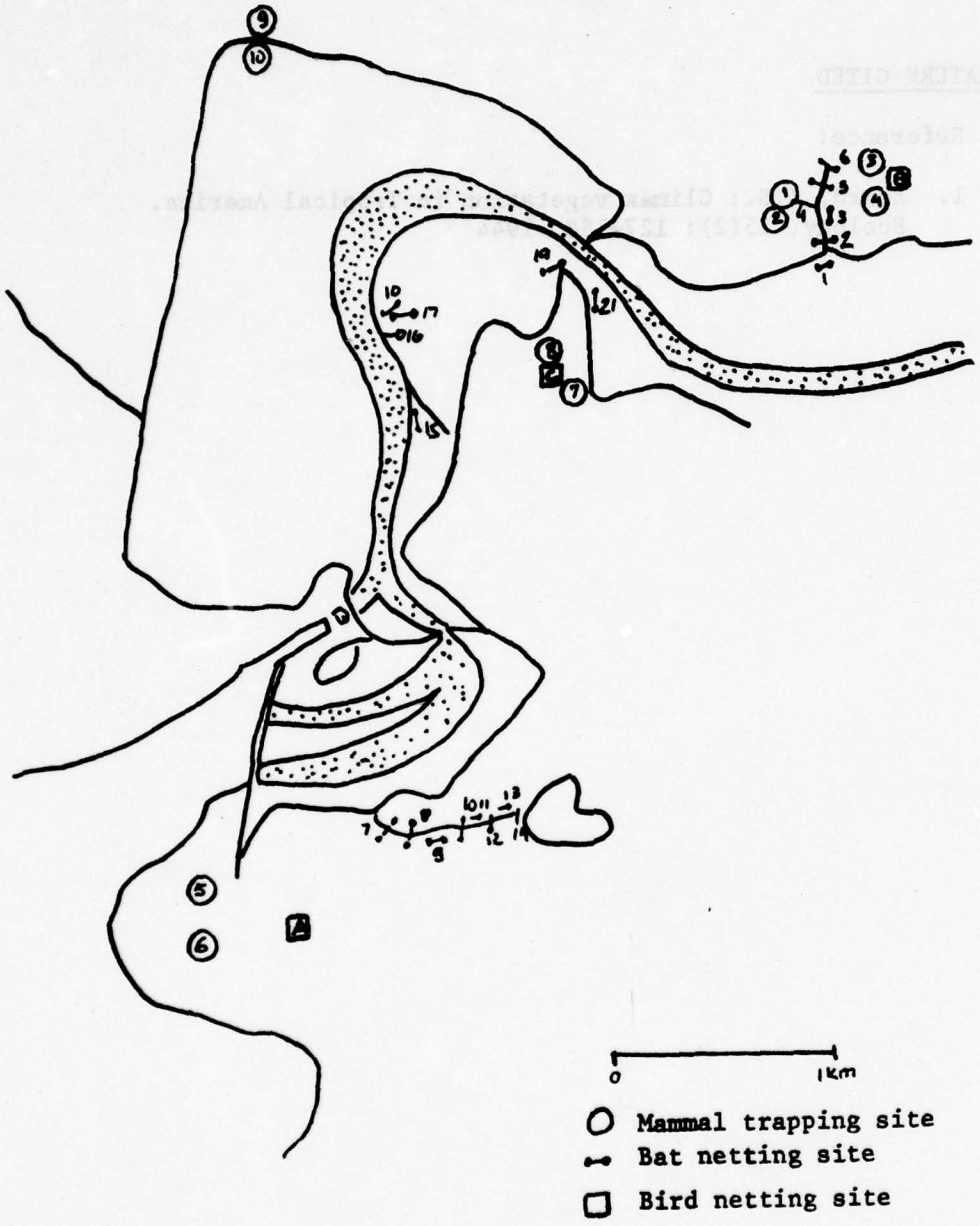

FIGURE 11. Mammal and bird collecting sites in the Curúa-Una study area, Pará, Brazil.

TABLE 17. Viruses isolated from mammals collected in the Curuá-Una study area, Pará, Brazil 15 June 1977 through 9 February 1978

Species	Total collected	Blood pos/tested	Viscera pos/tested
Marsupialia			
Monodelphis brevicaudata	10	0/10	0/10
Marmosa cinerea	6	0/5	0/6
M. murina	3	0/2	0/3
M. parvidens	3	-	0/3
Metachirus nudicaudatus	22	0/21	0/22
Didelphis marsupialis	21	0/20	0/21
Chiroptera			
Saccopteryx leptura	1	-	0/1
Peropteryx macrotis	2	0/1	0/2
Pteronotus parnellii	18	0/15	0/17
P. personatus	2	0/1	0/2
Tonatia bidens	2	0/2	0/2
T. brasiliensis	3	0/2	0/3
T. silvicola	2	0/2	0/2
Phyllostomus discolor	13	0/11	0/13
P. elongatus	5	0/4	0/5
P. hastatus	2	0/2	0/2
P. latifolius	1	0/1	0/1
Trachops cirrhosus	3	0/2	0/3
Glossophaga soricina	12	0/8	0/12
Lionycteris obscura	1	-	0/1
L. spurrellii	3	0/2	0/3
Lonchophylla thomasi	5	0/3	0/5
Carollia brevicauda	360	0/338	0/359
C. castanea	1	0/1	0/1
Rhinophylla fischerae	19	0/13	0/18
R. pumilio	3	0/3	0/3
Sturnira lilium	76	0/73	0/76
S. tildae	17	0/15	0/17
Uroderma bilobatum	4	0/3	0/4
Vampyrops helleri	8	0/8	0/8
Vampyressa bidens	9	0/8	0/9
Artibeus cinereus	15	0/11	0/15
A. concolor	1	0/1	0/1
A. fuliginosus	7	0/6	0/7
A. jamaicensis	2	0/2	0/2
A. literatus	24	0/24	0/24
A. sp.	10	0/9	0/10
Ametrida centurio	3	0/1	0/3

(Cont.)

TABLE 17. Viruses isolated from mammals collected in the Curuá-Una study area, Pará, Brazil, 15 June 1977 through 9 February 1978. (Cont.)

Species	Total collected	Blood pos/tested	Viscera pos/tested
Desmodus rotundus	8	0/8	0/8
Eptiscus Brasiliensis	2	-	0/2
Molossus sp	2	0/1	0/2
Primates			
Callicebus torquatus	1	0/1	0/1
Alouatta belzebul	5	0/4	0/5
Callithrix argentata	8	0/5	0/7
Edentata			
Tamandua tetradactyla	3	0/2	0/2
Bradypus variegatus	3	0/3	0/3
Choloepus didactylus	1	0/1	0/1
Dasypus novemcinctus	3	0/2	0/3
Rodentia			
Sciurus gilvigularis	3	0/3	0/3
Oryzomys bicolor	12	0/12	2/12*
O. capito	38	0/37	0/38
O. concolor	9	1/17*	1/8 *
Rhypidomys	1	-	0/1
Rattus rattus	1	0/1	0/1
Agouti paca	1	0/1	0/1
Dasyprocta prymnolopha	2	0/2	0/2
Proechymis guyannenis	48	0/48	0/48
P. Tongicaudatus	172	0/162	1/161+
Mesomys hispidus	4	0/4	0/3
Dactylomys dactylinus	1	0/1	0/1
Carnivora			
Nasua Nasua	5	0/1	0/5
Felis wiedii	1	0/1	0/1
TOTAL	1,032	937	1,014

* Flexal virus
\+ Urucuri virus

TABLE 18. Distribution of hemagglutination inhibiting antibody to Oropouche and Mayaro viruses in birds collected in the Curuá-Una study area, Pará, Brazil, 15 June 1977 through 9 February 1978

Family	Oropouche pos/tested	Mayaro pos/tested	Isolation/ tested
Tinamidae	0/1	0/1	0/1
Accipitridae	0/1	0/1	0/1
Falconidae	0/1	0/1	0/3
Columbidae	0/5	0/5	0/8
Cuculidae	0/2	0/2	0/2
Caprimulgidae	-	-	0/3
Trochilidae	0/3	0/3	0/5
Trogonidae	0/7	0/7	0/8
Alcedinidae	0/1	0/1	0/1
Momotidae	0/5	0/5	0/5
Galbalidae	0/5	0/5	0/5
Bucconidae	0/18	0/18	0/23
Picidae	0/8	0/8	0/8
Dendrocolaptidae	3/130	1/130	0/159
Furnariidae	0/22	0/22	0/23
Formacariidae	35/259	12/259	1*/321
Conopophadidae	0/1	0/1	0/1
Cotingidae	0/12	0/12	0/14
Pipridae	0/41	0/41	0/48
Tyrannidae	0/38	0/38	0/54
Troglodytidae	4/45	2/45	0/52
Turdidae	0/2	0/2	0/3
Sylviidae	0/1	0/1	0/1
Coerebidae	-	-	0/1
Thraupidae	0/6	0/6	0/7
Fringillidae	0/10	0/10	0/18
TOTAL	42/624	15/624	1*/775

* Kwatta virus

TABLE 19. Results of hemagglutination inhibition (HI) tests for antibody to Oropouche and Mayaro viruses among mammals collected from the Curuá Una study area, Pará, Brazil, 15 June 1977 through 9 February 1978

Species	Oropouche pos/no. tested	Mayaro pos/no. tested
Marsupialia		
Monodelphis brevicaudata	0/9	1/9
Marmosa cinerea	0/6	0/6
M. murina	0/1	0/1
Metachirus nudicaudatus	0/22	0/22
Didelphis marsupialis	0/20	0/20
Chiroptera		
Peropteryx macrotis	0/1	0/1
Pteronotus parnellii	0/3	0/3
Tonatia bidens	0/1	0/1
T. silvicola	0/3	0/3
Phyllostomus discolor	0/9	0/9
P. elongatus	0/3	0/3
P. hastatus	0/2	0/2
Glossophaga soricina	0/5	0/5
Lionycteres spurrelli	0/1	0/1
Lonchophylla thomasi	0/2	0/2
Carollia brevicauda	0/295	5/295
C. castanea	0/1	0/1
Rhinophylla fischerae	0/4	0/4
R. pumilio	0/1	0/1
Sturnira lilium	0/69	0/69
S. tildae	0/14	0/14
Uroderma bilobatum	0/3	0/3
Vampyrops helleri	0/5	0/5
Vampyressa bidens	0/5	0/5
Artibeus cinereus	0/6	0/6
A. concolor	0/1	0/1
A. fuliginosus	0/6	0/6
A. jamaicensis	0/2	0/2
A. literatus	0/24	0/24
A. sp.	0/5	0/5
Desmodus rotundus	0/7	0/7
Molossus sp.	0/1	0/1
Primates		
Callicebus torquatus	1/1	1/1

(Cont.)

TABLE 19. Results of hemagglutination inhibition (HI) tests for antibody to Oropouche and Mayaro viruses among mammals collected from the Curuá-Una study area, Pará, Brazil, 15 June 1977 through 9 February 1978 - Cont.

Species	Oropouche pos/no. tested	Mayaro pos/no. tested
Alouatta belzebul	1/4	3/4
Callithrix argentata	0/5	1/5
Edentata		
Tamandua tetradyactila	0/3	0/3
Bradypus variegatus	0/3	0/3
Choloepus didactylus	0/1	0/1
Dasypus novencinctus	0/2	0/2
Rodentia		
Sciurus gilvigularis	0/3	0/3
Oryzomys bicolor	0/6	0/6
O. capito	0/37	0/37
O. concolor	0/4	0/4
Rattus rattus	0/1	0/1
Agouti paca	0/1	0/1
Dasyprocta prymnolopha	1/2	0/2
Proechimys guyannensis	0/43	0/43
P. longicaudatus	0/165	0/165
Mesomys hispidus	0/3	0/3
Carnivora		
Nasau nasua	0/2	0/2
Felis wiedii	0/1	0/1
TOTAL	3/824	3/824

TABLE 20. Mammals collected by trapping in the Curuá-Una study area, Pará, Brazil, 15 June 1977 through 9 February 1978.

Species	Number collected per trapping site				
	1 - 4	5 - 6	7 - 8	9 - 10	Total
Marsupialia	21	6	1	34	62
Monodelphis brevicaudata	2	-	-	8	10
Marmosa cinerea	3	-	-	3	6
M. murina	1	-	-	1	2
M. parvidens	-	1	-	1	2
Metachirus nudicaudatus	7	-	1	14	22
Dedelphis marsupialis	8	5	-	7	20
Rodentia	111	6	1	168	286
Sciurus gilvigularis	-	-	-	2	2
Orizomys bicolor	11	-	-	1	12
O. capito	7	-	1	29	37
O. concolor	8	-	-	1	9
Rhypidomys	1	-	-	-	1
Rattus rattus	-	-	-	1	1
Proechimys guyanensis	28	1	-	19	48
P. longicaudatus	53	5	-	114	172
Mesomys hispidus	3	-	-	1	4
TOTAL	132	12	2	202	348
Nights trapped	37	11	7	54	109
Trap nights	6,135	2,024	1,200	9,870	19,229
Mammals/1000 trapnites	21.5	5.9	1.7	20.5	18.1

TABLE 21. Bats collected in mist nets in the Curuá-Una study area, Pará, Brazil, 15 June 1977 through 9 February 1978

Species	Number collected per trapping site				
	1 - 6	7 - 14	15 - 18	19 - 21	Total
Saccopteryx leptura	-	1	-	-	1
Peropteryx macrotis	-	1	-	-	1
Pteronotus parnellii	15	2	1	-	18
P. personata	-	1	-	1	2
Tonatia bidens	-	2	-	-	2
T. brasiliensis	1	-	1	1	3
T. silvicola	-	1	1	-	2
Phyllostomus discolor	1	3	4	5	13
P. elongatus	1	2	1	-	4
P. hastatus	-	2	-	-	2
P. latifolius	1	-	-	-	1
Trachops cirrhosus	-	3	-	-	3
Glossophaga soricina	-	4	3	4	11
Lyonycteris spurrelli	-	3	-	-	3
Lonchophylla thomasi	-	4	-	-	4
Carollia brevicauda	46	178	79	57	360
C. castanea	-	1	-	-	1
Rhinophylla fischerae	6	11	1	1	19
R. pumilio	2	-	-	1	3
Sturnira lilium	3	28	31	14	76
S. tildae	1	11	-	5	17
Uroderma bilobatum	-	-	1	3	4
Vampyrops helleri	-	6	1	1	8
Vampyressa bidens	-	5	4	-	9
Artibeus cinereus	1	11	2	1	15
A. concolor	-	1	-	-	1
A. fuliginosus	-	4	3	-	7
A. jamaicensis	1	1	-	-	2
A. literatus	1	16	2	5	24
A. sp.	-	9	1	-	10
Ametrida centurio	-	1	2	-	3
Desmodus rotundus	1	4	1	2	8
Eptesicus brasiliensis	-	1	-	1	2
Total	81	317	139	102	639
Nights netted	16	22	12	13	63
Net hours	234	378	194	130.5	936.5
BAts/net hour	0,34	0.84	0.72	0.78	0.68

TABLE 22. Birds collected in the Curuá-Una study area, Pará, Brazil, 15 June 1977 through 9 February 1978.

Family	Site A	Site B	Site C	Total
Tinamidae	-	1	-	1
Accipitridae	-	1	-	1
Falconidae	-	2	1	3
Columbidae	5	-	3	8
Cuculidae	-	2	-	2
Caprimulgidae	2	1	-	3
Trochilidae	2	2	1	5
Trogonidae	1	4	3	8
Alcedinidae	1	-	-	1
Momotidae	-	2	3	5
Galbalidae	2	1	2	5
Bucconidae	14	6	3	23
Picidae	2	5	1	8
Dendrocolaptidae	30	92	38	160
Furnariidae	10	9	4	23
Formacariidae	119	129	74	322
Conopophadidae	-	1	-	1
Cotingidae	6	7	1	14
Pipridae	28	10	10	48
Tyrannidae	17	21	16	54
Troglodytidae	9	32	11	52
Turdidae	-	1	2	3
Silviidae	-	1	-	1
Coerebidae	1	-	-	1
Thraupidae	1	-	6	7
Fringillidae	1	8	9	18
TOTAL	251	338	188	777
Mornings netted	23	18	18	59
Birds/morning	10.9	18.8	10.4	13.2

I. ECOLOGY OF OROPOUCHE VIRUS

B. Studies on the Endemic Cycle

2. The Cachoeira Porteira Study

a. Potential insect vectors.

OBJECTIVE: The objective of the program of study in Cachoeira Porteira is to investigate the ecology of arboviruses in an undisturbed tropical forest. This section deals with entomological investigations made near Cachoeira Porteira, Pará, Brazil, with the specific objective of presenting a checklist of potential insect vectors found there.

BACKGROUND: When construction of the Transamazon Highway south of the Amazon River was initiated, a sister plan was also finder consideration. This plan called for the development of a second highway parallel to the Amazon River, but to the north. As part of this plan, a base camp was established at Cachoeira Porteira on the eastern bank of the Trombetas River. This camp served to house work crews, professional staff, and equipment used in the construction of the highway.

From this base camp an access road was constructed. This road passes almost due north from Cachoeira Porteira for more than 100 km, and cuts through some of the least disturbed tropical forest of the world. No one lived in this area when this road was constructed.

Shortly after completion of the access road, the entire northern highway program was abandoned. As a result, this area has remained almost unspoiled, yet relatively easy access to this undisturbed tropical forests is available. Even now, almost no human activity is evident in this area.

The base camp has been leased by Andrade Gutiérrez, a firm which is developing a large bauxite deposit south of Cachoeira Porteira, also on the Trombetas River. They now use the camp as permanent housing for the professional staff of the mine. The road is maintained by the Brazilian government and occasionally the military conducts training exercises along it. No plans have been made public to renew construction of the highway.

DESCRIPTION: Collections have been made at various sites along the access road. Generally field teams construct a camp along the highway, then collect mammals, birds and insects from several localities within a 2 or 3 km radius from the camp. Collections are usually conducted for approximately 15 consecutive days during each trip, and trips are usually made about 3 times per year.

Habitats vary considerably along the highway and it would not be practical to provide a detailed description of collection sites here. General characteristics of the area are predominated by the near total

absence of human activity. Aside from the effects of road construction itself, little else has been disturbed. One result of road building has been to slow or stop the flow of some small streams, which has caused the formation of several small impoundments of water. These impoundments flood low-lying areas and kill the vegetation there, resulting in ideal breeding sites for certain species of mosquitoes, especially some Anopheles.

Little or no logging has taken place along the highway, consequently the forest is taller than that seen in most other areas, and the canopies are more distinct. Aside from immediately adjacent to the road, few indicators of secondary growth such as Cecropia are evident in the area.

Insect collections are conducted nightly using man-biting collections, suction traps and Shannon traps.

PROGRESS: Tabe 23 presents a summary of the number and species of potential insect vectors collected during trips made in September and November-December, 1976 to Cachoeira Porteira. Clearly the vast majority of insects collected were mosquitoes of the genus Culex. Relatively few canopy species are represented in these collections, most likely because no attempt was made to actively sample this habitat. Consequently, those canopy species presented here represent the few individuals which were caught on the forest floor while collections were being made. Likewise, diurnal species are also under represented.

The absence of Culicoides and significant numbers of Phlebotomus from this check list probably also represents a failure on the part of the collectors to actively seek these groups, rather than their true absence from this area.

TABLE 23. Summary of numbers and species of potential insect vectors collected from Cachoeira Porteira, municipality of Oriximiná, Pará, Brazil, 1976.

Species	Data and number collected	
	September	Nov-Dec
Aedes hortato	1	-
Aedes serratus	5	1
Coquillettidia sp.	1	-
Coquillettidia albicosta	3	-
Culex spp.	124	895
Culex sp. B 1	8	455
Culex sp. B 8	6	-
Culex sp. B 17	1	-
Culex sp. B 26	23	-
Culex (Aedinus) sp.	4	-
Culex (Culex) sp.	9	25
Culex coronator	2	15
Culex declarator	12	79
Culex (Lut.) bigoti	1	-
Culex (Melanoconion) sp.	9931	2627
Culex (Mel.) portesi	1	-
Culex (Mel.) spissipes	5	3
Culex (Mel.) taeniopus	5	1
Culex (Mel.) vomerifer	2	-
Culex (Microculex) sp.	2	-
Haemagogus spp.	50	1
Uranotaenia calosomata	11	-
Uranotaenia lowii	10	-
Uranotaenia leucoptera	2	-
Uranotaenia geometrica	18	57
Limatus flavisetosus	30	4
Limatus paraensis	10	-
Limatus pseudomethysticus	13	-
Phoniomyia spp.	1	-
Sabethes (Sab.) amazonicus	6	-
Sabethes (Sab.) belizarioi	14	-
Sabethes (Sab.) quasicyaneus	2	-
Sabethes (Sab.) cyaneus	21	1
Sabethes (Sab.) tarsopus	2	-
Sabethes (Sab.) chloropterus	14	7
Sabethes (Sab.) glaucodaemon	37	-
Trichprosopon digitatum	1	-
Wyeomyia spp.	70	4
Wyeomyia aporonoma	2	-

(Cont.)

TABLE 23. Summary of numbers and species of potential insect vectors collected from Cachoeira Porteira, municipality of Oriximiná, Pará, Brazil, 1976. - (Cont.)

Species	Data and number collected	
	September	Nov-Dec
Anopheles albimanus	-	5
Anopheles benarrochi	-	9
Anopheles intermedius	2	-
Anopheles nunez-tovari	47	51
Anopheles oswaldoi	7	22
Anopheles triannulatus	14	3
Phlebotomus app.	1	1
TOTAL	10,531	4,266

I. ECOLOGY OF OROPOUCHE VIRUS

B. Studies on the Endemic Cycle

2. Cachoeira Porteira study

b. Vertebrate host serology

OBJECTIVES: The objective of this section is to define through a serological survey those feral vertebrate hosts which have been exposed to Oropouche virus.

BACKGROUND: The environs and program of study at Cachoeira Porteira have been presented elsewhere. Briefly, investigations in this area attempt to study the ecology of arboviruses in a habitat essentially devoid of human inhabitants.

DESCRIPTIONS: Three separate trips to Cachoeira Porteira were made during 1977. On each trip, teams were away from Belém a maximum of 30 days, and usually 15 to 20 actual collecting days were managed within this period. During each trip birds, mammals and insects were collected.

Birds were collected using Japanese mist-nets placed along cleared trails in the forest. All nets were placed at ground level, and no attempt was made to sample birds in the canopy. Nets were opened before sunrise, and were closed between 11 a.m. and 12 noon. Nets were checked at about 30 minute intervals, and captured birds were removed and taken to a field station where they were bled from the jugular vein using a heparinized needle and syringe. A portion of the blood taken was diluted immediately in nutrient media and stored in liquid nitrogen until processed in Belém. The remaining blood was allowed to clot and sera were saved to test for the presence of antibody. Surviving birds were leg banded and released.

Mammals were trapped or shot. Like birds, each mammal captured was bled, and an aliquot stored in liquid nitrogen to be tested for the presence of virus, and the remaining blood allowed to clot and the serum saved to test for antibody. Only very small mammals were bled with heparinized syringes.

Sera were tested for the presence of antibody by the standard hemagglutination inhibition (HI) test, as described by Shope[1]. Sera were treated with acetone, then tested at 1:20 dilution against 4 hemagglutinating units of different viral antigens. Whenever a serum was inhibited at the 1:20 dilution, it was retested at additional dilutions of 1:40 through 1:320. The strain of Oropouche (ORO) virus used in all tests was Be An 19991 from infected hamster sera.

Most, but not all, sera which were positive by HI tests were confirmed by neutralization tests conducted in microtiter plates using Vero cells.

At one point during the study, several bird sera could not be confirmed by neutralization tests. Close examination of the blooding techniques used by the field personnel revealed that they were using a highly concentrate solution of heparin to moisten their syringes. To avoid the possibility of non-specific inhibition due to contaminant heparin, all sera were treated with protamine prior to testing.[2]

PROGRESS: A total of 508 bird sera were collected during three separate trips to Cachoeira Porteira in 1977. These sera represent 25 separate families of birds. Formicariidae birds contributed the largest number of individuals, with 194. All bird sera collected were tested by HI for antibody to ORO virus, and all sera were found to be negative. Table 24 presents a list of bird sera collected by family during each of the three trips made to Cachoeira Porteira during 1977.

A total of 273 mammal sera were collected during the three trips made in 1977. Of these, 74 represented sera from five different species of marsupials. All these sera were negative for HI antibody to ORO virus. Rodents contributed 133 sera from 10 different species, and again, all sera were negative for HI antibody to ORO virus.

Thirty-seven sera were collected from primates hunted in Cahoeira Porteira, and of these 10 (27%) contained HI antibody to ORO virus. All positive sera were confirmed by netralization tests. Positive species of primates included Alouatta seniculus (2 pos/5 tested), Ateles paniscus (2/3), Cebus apella (3/15), and Chiropotes satanas (3/10). Of the remaining mammals tested, 6 carnivores, 15 ungulates and 8 other, all lacked HI antibody to ORO virus. A summary of the numbers and species of mammals tested for HI antibody to ORO virus is presented in Table 25.

COMMENT: The only vertebrate group tested which had HI antibody to ORO virus was the primates. Among the primates tested, four of the six species collected had individuals which possessed HI antibody. It appears that the endemic vector of ORO virus must share a habitat utilized by primates, most likely the forest canopy. Further investigations are needed to define where monkeys are becoming infected, and by what vector.

The very promising high HI antibody prevalence rates to ORO virus among Formicariidae and other families of birds previously reported appears now to be artifactual. With the protamine treatment of bird sera, all sera originally positive for HI antibody to ORO virus were uniformly negative on retesting.

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TABLE 24. Families and numbers of birds tested for hemagglutination inhibiting (HI) antibody to Oropouche virus from Cachoeira Porteira, km 71, municipality of Oriximiná, Pará, Brazil, 1977. All birds lacked HI antibody to Oropouche virus.

FAMILY	Date and number sampled			TOTAL
	March-April	July-August	November-December	
Alcediridae	-	2	-	2
Bucconidae	-	1	3	4
Coerobidae	-	-	1	1
Columbidae	-	1	4	5
Conopophagidae	2	3	-	5
Cotingidae	-	1	2	3
Cracidae	1	-	-	1
Dendrocolaptidae	16	21	16	53
Falconidae	-	1	1	2
Formicariidae	78	71	45	194
Fringillidae	1	6	2	9
Furnariidae	8	17	12	38
Galbulidae	1	3	-	4
Momotidae	7	3	2	12
Parulidae	3	-	2	5
Picidae	2	1	-	3
Pipridae	35	20	5	60
Ramphastidae	1	-	-	1
Sylvilidae	3	-	-	3
Thraupidae	6	-	-	6
Tyrannidae	12	9	13	34
Trochilidae	1	-	-	1
Troglotididae	3	5	6	14
Turdidae	13	20	7	40
Vireonidae	-	1	-	1
TOTAL	201	186	121	501

TABLE 25. Distribution of hemagglutination inhibiting antibody to Oropouche virus among mammals captured at Cachoeira Porteira, km 71 municipality of Oriximiná, Pará, Brazil, 1977

Species	March-April	July-August	Nov-Dec	Total
Marsupials				
Didelphis marsupialis	0/2*	0/6	0/6	0/14
Marmosa cinerea	-	-	0/1	0/1
M. murina	0/4	0/3	0/2	0/9
Monodelphis brevicaudata	0/4	0/3	0/10	0/17
Philander opossum	0/9	0/11	0/13	0/33
TOTAL	0/19	0/23	0/32	0/74
Rodents				
Agouti paca	-	0/1	-	0/1
Dasyprocta aguti	-	0/5	0/2	0/7
Hydrochaeris hydrochaeris	-	-	0/1	0/1
Myoprocta acouchi	0/2	-	0/1	0/3
Neacomys spinosus	-	0/3	0/1	1/4
Nectomys squamipes	0/1	0/7	0/1	0/9
Oryzomys bicolor	-	0/1	-	0/1
O. capito	-	0/15	0/7	0/22
Proechimys guyannensis	0/34	0/28	0/21	0/83
Sciurus gilvigulares	0/1	0/1	-	0/2
TOTAL	0/38	0/61	0/34	0/133
Primates				
Alouatta seniculus	2/2	-	0/3	2/5
Ateles belzebul	-	0/3	-	0/3
A. paniscus	1/1	-	1/2	2/3
Cebus apella	-	0/11	3/4	3/15
Chiropotes satanas	-	2/6	1/4	3/10
Pithecia pithecia	-	-	0/1	0/1
TOTAL	3/3	2/20	5/14	10/37
Carnivores				
Eira barbara	0/1	-	-	0/1
Felis concolor	-	0/1	-	0/1
F. pardalis	0/1	-	-	0/1
Nasua nasua	-	0/1	0/2	0/3
TOTAL	0/2	0/2	0/2	0/6

(Cont.)

TABLE 25. Distribution of hemagglutination inhibiting antibody to Oropouche virus among mammals captured at Cachoeira Porteira, km 71 municipality of Oriximiná, Pará, Brazil, 1977.-Cont.

Species	March-April	July-August	Nov-Dec	Total
Ungulates				
Mazama americana	0/1	0/1	0/3	0/5
Tapirus terrestris	-	-	0/1	0/1
Tayassa pecari	-	0/5	0/5	0/8
Dicotyles tajacu	-	0/1	-	0/1
TOTAL	0/1	0/7	0/7	0/15
Other mammals				
Dasypus novemcinctus	0/1	-	-	0/1
Bats	-	0/5	0/1	0/6
Tamandua tetradactyla	-	-	0/1	0/1
TOTAL	0/1	0/5	0/2	0/8

* Number pos/number tested.

I. ECOLOGY OF OROPOUCHE VIRUS

B. Studies on the Endemic Cycle

3. Mojui dos Campos study

OBJECTIVE: The objective in this study was to determine the antibody level to Oropouche virus in the various wild mammals and birds from Mojui dos Campos area.

BACKGROUND: During January and February of 1975 an Oropouche (ORO) virus epidemic occurred in Mojui dos Campos, and the Instituto Evandro Chagas personnel collected wild mammals and birds in the area during February and March 1975. Their results showed an ORO virus HI antibody prevalence rate of 32.5% among Formicariidae at that time. The overall HI antibody prevalence to ORO virus among all birds and mammals tested was 4.9% (34 /681) and less than 1% (1/361) respectively.

The PAHO ecology team collected wild mammals and birds in the Mojui dos Campos area from 16 February 1978 through 10 March 1978. The mammals and birds were trapped, hunted and mist-netted in the same sites as during the 1975 collections. The objective of this collecting program was to determine if a significant change in the antibody prevalence rates to ORO virus had occurred. An equal or higher prevalence rate would suggest continued transmission of ORO virus in the area, while a lower rate would suggest that vertebrates were infected only during the outbreak, and a drop in prevalence rates could be attributed to normal attrition.

DESCRIPTION: Mojui dos Campos is located 22 km south of Santarém, Pará (latitude 2°37'S, longitude 54°42'W). This area is inhabited by many colonists, without title to their lands, who practice slash and burn agriculture. The entire area is consequently secondary scrub with fruit trees and banana plants near the houses. Mango (Mangifera indica), piquiá (caryocar villosum) trees and babaçú (Orbygnia martiana) palms are very common. Various types of citrus trees and coffee trees are scattered throughout. Manioc and beans are the most common cultivated crops.

The methods of processing the mammal and bird specimens in the field laboratory located in Mojui dos Campos has been described in previous annual reports.

The bird collecting site was located 5.7 km northwest of Mojui dos Campos and south of the road which runs between Mojui dos Campos and the Santarém-Cuiabá road. Birds were collected from before dawn until 11:00 hrs with mist nets 12 m long and 2.6 m in height. Thirty five nets were placed along a trail which ran through thick secondary scrub. The secondary scrub contained mainly woody plants with some bamboo and other grasses along the edge of the trail. The woody plants ranged from ground

level to about four meters tall. Low palms and trees to about 10 meters in height were scattered throughout. It was impossible to walk through the thicket without cutting a trail. The area was about 50 meters from an overgrown fruit orchard described in bat netting areas 1, 2, and 3.

Bats were collected in mist nets set under fruit trees, and across natural flyways such as trials, edges of clearing, etc. The nets were usually tended from dusk until 22:00 hrs.

Bat nets 1, 2 and 3 were set in an overgrown orchard 5.7 km northwest of Mojui dos Campos and south of the road. The orchard consisted of mango, piquiá and coffee trees, and banana plants. The underbrush was quite thick, although it was possible to walk through without cutting a trail.

Bat nets 4, 5 and 6 were set in the back yard of a house 6.4 km northwest of Mojiu dos Campos and north of the road. The nets were set under mango, orange and piquiá trees, and banana plants. The yard was cleared of underbrush, and only some grass and many leaves covered the ground.

Small mammals were collected in National live traps (150x150x485mm) and in Rinker live traps (80x80x255mm) which were set out at 10 m intervals. The traps were baited with corn and banana, and were checked early in the morning. Larger mammals, such as monkeys and sloths, were hunted.

Trapping area one was located 2.9 km northwest of Mojui dos Campos and south of the road. The traps were set on the edge of a manioc field bordered by a secondary scrub thicket. The secondary scrub consisted of woody plants from ground level to 3 m in height Babaçú palms to 7 m tall were common. It was not possible to walk without cutting a trail.

Trapping area two was located south of the road 1.6 km norhtwest of Mojui dos Campos. The traps were placed along trails cut in the very thick secondary scrub vegetation which was composed of woody plants and vines from ground level to about 6 m in height, some scattered cecropia (Cecropia sp.) trees were taller.

Trapping area three was located south of the road, 1 km northwest of Mojui dos Campos. Traps were set along the edge of an overgrown manioc field, which contained many shrubs to 4 m in height, and in a thick secondary scrub which bordered the field. The secondary scrub was made up of shrubs from 3 to 5 m tall and babacú palms to about 6 m in height. Trails were cut through the scrub vegetation for the trap lines.

The fourth trapping area was located behind the house which was used as a field laboratory in Mojui dos Campos. The traps were set in a citrus grove in which the fruit trees were 1 to 2 m in height. The ground was covered with grass, and the grove was surrounded by large mango trees.

PROGRESS: During the 3 1/2 weeks of collecting in the Mojui dos Campos area, 170 mammals representing 20 species, and 305 birds of 14 families were trapped, hunted or mist netted (Table 26 and 27). No viruses were isolated from the 158 whole blood and 166 viscera specimens tested from mammals, nor the 105 whole blood specimens from birds tested. None of the 136 mammal sera tested contained HI antibody to ORO or Mayaro viruses. Nine of the 304 bird sera tested contained HI antibodies to ORO virus (Table 27). The sera from 18.8% (3/16) of the antbirds (Formicariidae, Formicivora griseu), 4.8% (1/21) of the manakins (Pipridae, Manacus manacus) and 14.7% (5/34) of the wrens (Troglodytidae, Thryothorus leucotis , T. coraya-4) were positive to ORO virus.

Traps were set out for 14 nights in each of the four trapping sites which produced seven marsupials and 64 rodents (Table 28). Zygodontomys lasiurus (35 specimens) and Oxymycteris sp. (11 specimens) were the most commonly trapped mammal. Mist nets were set out three nights in each of the two netting sites and collected 92 bats. Site 4-6, which had more fruit trees and less undergrowth, produced a higher netting success (3.06 bats/net hour) than net site 1-3 (1.76 bat/net hour).

The nets for collecting birds were operated for 11 days and produced 305 birds, of which the tyrant-flycatcher family, Tyrannidae (75 collected) were the most common (Table 27).

COMMENTS: The results of this serological survey indicate that the ORO virus HI antibody prevalence rates are lower than those detected following the epidemic investigated during 1975. These results suggest that active transmission of ORO virus is no longer occurring in the Mojui dos Campos area.

The secondary scrub vegetation of the Mojui dos Campos area contrasted greatly with the disturbed primary forest vegetation of Curuá-Una. The species make up of the mammals and birds between the two areas differed accordingly. The two species of Proechimys, normally a forest dwelling rodent, were the most common mammals collected in Curuá-Una, while Zygodontomys, the most common rodent in Mojui dos Campos, was not collected in Curuá-Una. The antbirds (Formicariidae) were the most commonly collected in Curuá-Una but one of the least common in Mojui dos Campos, where the tyrant-flycatchers (Tyrannidae) were the most common.

TABLE 26. Total mammals collected and tested for virus from the Mojui dos Campos study area, Pará, Brazil, 16 February through 10 March, 1978.

Species	Total collected	Sera tested	Blood tested	Viscera tested
Marsupialia				
Caluromys philander	3	2	3	3
Monodelphis brevicaudata	2	2	2	2
Metachilus nudicaudatus	1	1	1	1
Marmosa murina	1	1	1	1
Chiroptera				
Tonatia bidens	1	1	1	1
T. brasiliensis	1	0	1	1
Phyllostomus latifolius	2	2	2	2
Glossophaga soricina	2	0	1	2
Carollia brevicauda	37	23	33	36
Rhynophylla fischerae	1	0	0	1
Sturnira lilium	28	23	26	28
Uroderma bilobatum	6	2	6	6
U. magnirostrum	1	1	1	1
Artibeus cinereus	13	9	11	11
Edentata				
Bradypus variegatus	7	7	7	6
Rodentia				
Zygodontomys lasiurus	35	33	35	35
Oxymycteris sp.	11	11	11	11
Rattus rattus	2	2	2	2
Proechimys guyannensis	13	13	11	13
P. longicaudatus	3	3	3	3
	170	136	158	166

TABLE 27. Birds collected and tested for HI antibody to Oropouche (ORO) virus and processed for virus isolation from the Mojui dos Campos study area, Pará, Brazil, 16 February through 10 March, 1978

Family	Total collected	Sera tested	Blood tested	HI antibody to ORO virus
Tinamidae	1	1	1	
Rallidae	1	1	1	
Columbidae	44	44	44	
Cuculidae	1	1	1	
Caprimulgidae	1	1	1	
Dendrocolaptidae	3	3	3	
Furnariidae	1	1	1	
Formacariidae	16	16	16	3/16 (18.7%)
Pipridae	21	21	21	1/21 (4.7%)
Tyrannidae	75	74	75	
Troglodytidae	34	34	34	5/34 (14.7%)
Virionidae	13	13	13	
Thraupidae	44	44	44	
TOTAL	305	304	305	9/305(2.9%)

TABLE 28. Mammals collected by trapping and by mist netting in the Mojui dos Campos study area, Pará, Brazil, 16 February through 10 March, 1978.

Species	Number collected per site			
	Trap sites 1-4	Net sites 1-3	Net sites 4-6	Total
Marsupialia	7			7
Caluromys philander	3			3
Monodelphis brevicaudata	2			2
Marmosa murina	1			1
Metachirus nudicaudatus	1			1
Chiroptera		37	55	92
Tonatia bidens		1		1
T. brasiliensis			1	1
Phyllostomus latifolius			2	2
Glossophaga soricina			2	2
Carollia brevicauda		9	28	37
Rhinophylla fischerae		1		1
Sturnira lilium		14	14	28
Uroderma bilobatum			6	6
U. magnirostrum		1		1
Artibeus cinereus		11	2	13
Rodentia	64			64
Zygodontomys lasiurus	35			35
Oxymycteris sp.	11			11
Rattus rattus	2			2
Proechimys guyannensis	13			13
P. longicaudatus	3			3
TOTAL	71	37	55	163
Trap nights	1,680			
Net hours		21	18	
Mammals/1000 trap nights	42.3			
Bats/net hour		1.76	3.06	

I. ECOLOGY OF OROPOUCHE VIRUS

B. Studies on the Endemic Cycle

4. Experimental infections of vertebrates

a. Mammals

OBJECTIVE: These studies are an attempt to define those vertebrates hosts which are capable of producing a substantial viremia following infection, and thus could potentially act as amplifying hosts of Oropouche virus. The section deals with experimental infections of mammals.

BACKGROUND: Mammals collected from localities throughout much of the state of Pará have been tested for the presence of antibodies to Oropouche (ORO) virus. While several thousand individuals have been tested, antibody has only been detected consistently among primates. Primates may serve as the principal vertebrate host of ORO virus in nature, but the sparse abundance of primates and their low reproductive potential indicate that this is a questionable hypothesis, and additional information is required before a conclusion can be drawn on this theory.

Since virtually no rodents have been found to contain antibody to ORO virus, one might conclude that rodents are not involved. One would expect, however, that even if rodents are not actively involved in the maintenance of this virus, an occasional individual would have become infected. Consequently, before they can be discontinued completely, it is essential to document that if infected, rodents would produce a normal immune response. This is to insure that the laboratory techniques currently in use, hemagglutination inhibition and neutralization tests, would detect previously infected individuals and that these negative results are in fact a true measurement of exposure.

DESCRIPTION: The rodents most frequently collected in our studies have been members of the genus Proechimys. Consequently this group was selected as the initial hosts for experimental infections. Nine Proechimys rodents were collected from study sites in Curúa-Una, Pará, Brazil and transported live to Belém for experimental infection in the laboratory. Each individual was bled prior to exposure and tested by HI for pre-existing antibody to ORO virus. They were then inoculated subcutaneously with 5.5 $\log_{10}TCID_{50}/0.02$ ml of ORO virus. Rodents were bled daily for seven days post infection, and their whole blood diluted 1:10 in bovine plasma albumin diluent, then frozen at -70°C pending assay for virus. On days 7, 14 and 29 post inoculation each animal was bled for serology as well. Sera were tested by HI and NT using Vero cells grown in microtiter plates. Attempts to isolate virus from potentially viremic hosts were made by inoculating 0.1 ml of diluted whole blood into duplicate tubes of Vero cells. Cells were observed for 8 days for evidence of CPE.

PROGRESS: No viremia was detected in any of the Proechimys experimentally infected with ORO virus. In addition, even though a relatively high titered inoculum was used, no animal produced HI or N antibody to ORO virus.

COMMENT: These results suggest that Proechimys rodents are refractory to infection with ORO virus. Since data are only available from this single attempt to infect rodents, it is essential that these experiments be repeated before any generalizations can be made. These preliminary results do, however, suggest a plausible explanation for our failure to detect antibody to ORO virus among rodents.

I. ECOLOGY OF OROPOUCHE VIRUS

B. Studies on the Endemic Cycle

4. Experimental infections of vertebrates

b. Birds

OBJECTIVE: These studies are an attempt to define those vertebrate hosts which are capable of producing a substantial viremia following infection, and thus could potentially act as amplifying hosts of Oropouche virus. This section deals with experimental infections of birds.

BACKGROUND: Serological surveys have found high HI antibody prevalence rates to Oropouche (ORO) virus among certain groups of birds, especially members of the family Formicariidae. Such results suggest that these birds are frequently exposed to feeding by the endemic vector, and that they may provide one means of virus amplification, should they produce a significant viremia following infection. An attempt is made in this study to experimentally infect Formicariidae birds and thereby define their potential to serve as vertebrate amplifying hosts of ORO virus.

Birds of the family Formicariidae are quite common in the forested areas of the New World. Several species have been described, and 23 different species were collected from Cachoeira Porteira during recent trips. Most, if not all, species are insectivores. Their family name is derived from their behavior of following army ants as the ants forage, and feeding on the insects that are flushed up. They apparently do not feed on the ants themselves. They are common in both undisturbed forests and dense secondary growth. Their activity periods generally coincide with that of the ants, and they are especially active in the early morning. They frequent the lower scrubs closest to the forest floor, where they often sit motionless awaiting insects. Few species are found in the forest canopy. Their nesting and roosting sites are unknown, but hey are most likely in thickets near the forest floor. They are generally small in size and quite delicate, although some of the larger species approach the size of blackbirds. Formicariidae birds do not migrate.

Two authorities were contacted regarding the biology of Formicariidae birds, Dr. Philip S. Humphrey, Director, Museum of Natural History, University of Kansas, Lawrence, Kansas, USA 66045 and Dr. Edwin Willis, Department of Zoology, UNICAMP, Caixa Postal 1170, Campinas, Sao Paulo, Brazil, 13100. Both were quite cooperative and provided much of the summary of Formicariidae biology discussed above. They were also questioned regarding the possibility of keeping these birds in captivity, and both agreed that this is quite difficult, and that the odds of success are very slim.

DESCRIPTION: Formicariidae birds were collected by mist nets from the Utinga forest near Belem. All birds were bled to detect pre-existing HI antibody to ORO virus, then inoculated with approximately 400 plaque forming units of ORO virus. Birds were bled to detect viremia at 24 hour intervals post inoculation and brains, livers and hearts were assayed for virus following death. Whole blood was tested as a 1:10 dilution in bovine plasma albumin; organs as a 10% triturated solution. Blood and organ suspensions were assayed undiluted and at 10^{-2}, 10^{-3} and 10^{-5}. These dilutions were tested at the suggestion of Dr. Pinheiro, who indicated that occasionally ORO virus can only be detected in diluted samples. Blood and organ suspensions were tested in triplicate tubes of Vero cell culture and were observed for cytopathic effect (CPE) for 7 days post-inoculation.

PROGRESS: A total of 14 Formicariidae birds were collected and inoculated with ORO virus. Unfortunately, 10 of these birds died shortly after being infected. Only 4 birds survived at least 24 hours, and all birds died within 48 hours of infection. Cause of death in all cases was due to either the trauma associated with bleeding or failure to feed while in captivity.

Of the 4 birds surviving 24 hours or longer, none demonstrated a detectable viremia following infection, although all lacked pre-existing ORO HI antibody. Assay of livers, brains and hearts also failed to detect ORO virus. These results are summarized in Table 29.

COMMENT: Birds tested in this experiment survived for too short a period to produce meaningful results. Additional work is needed to clarify the role of Formicariidae birds in the maintenance of ORO virus.

This experiment was discontinued due to our inability to maintain the birds in the laboratory. Attemps may be made in the future to construct a large cage to house infected birds in the forest. This may result in longer survival times, but will also present the problem of how to infect potential amplifying hosts without introducing virus into the free-living vector population.

TABLE 29. Summary of virus isolation attempts from Formicariidae birds experimentally infected with approximately 400 plaque forming units of Oropouche virus. Birds were bled at 24 hour intervals post-inoculation, and brain, liver and heart were assayed for virus isolations attempts following death. Material was assayed for virus in Vero cells. All birds tested lacked pre-existing antibody to Oropouche virus.

Species	Virus isolation: Hrs. post-inoculation 24 hrs.	48 hrs.	Liver	Heart	Brain
Formicarius analis	-	-	-	-	-
Pyriglena leuconota	-	dead	-	-	-
Pyriglena leuconota	-	dead	-	-	-
Thamnophilus aethiops	-	-	-	-	-

II. ECOLOGY OF MAYARO VIRUS

A. Review of the Literature

OBJECTIVE: The objective of this section is to provide a succinct summary of the literature which deals with Mayaro virus. This summary will serve as a preface to the studies reported in the following sections.

BACKGROUND: Mayaro (MAY) virus was first characterized by Casals and Whitman (1957) and found to be closely related to, but distinguishable from Semliki Forest virus. Today it is recognized as an arbovirus of the family Togaviridae, genus Alphavirus (Berge 1975).

Mayaro virus was originally isolated from five humans resident in Southeastern Trinidad and takes its name from Mayaro County, Trinidad, the county in which these people resided. Anderson et al. (1957) described the clinical illness associated with these original five cases, which consisted of fever of several days duration, generalized systemic complaints of headache, chills and general body pain. One patient had a loose bowel movement, and another complained of joint pains and swelling. No rash was reported, and all patients recovered without complications or relapses.

An outbreak of MAY virus which occurred at a rock quarry on the Guamá River in Pará, Brazil was described by Casey and Maroja (1957). Six strains of MAY virus were recovered during investigations of the outbreak, and these strains were also included by Casals and Whitman (1957) in their initial characterization of MAY virus. Clinical illness associated with Guamá River outbreak was very similar to that seen in Trinidad.

A third outbreak was described by Schaeffer et al. (1959). This study reported on epidemic jungle fevers found in a newly formed colony of Okinawan settlers in eastern Bolivia. While several different etiologic agents were probably responsible for this outbreak, only MAY virus was actually isolated. The clinical summary of the single patient from whom MAY virus was isolated is not significantly different from that originally described by Anderson et al. (1957), with the exception that this patient had a mild, generalized maculopapular erythema which appeared on the 6th day of illness and persisted for 2 days. A serological survey of those settlers indicated that 10-15% of the the epidemic jungle fevers seen in this settlement could be attributed of MAY virus infection.

Ecological investigations have failed to define the natural cycle of MAY virus. Several species of mosquitoes have been the source of MAY virus isolations, including Mansonia venezuelensis, Haemagogus spp., Culex sp., Sabethes sp. and Psorophora sp. (Berge 1975). However, no clear association between a vector species and MAY virus has yet been demonstrated. Likewise, no vertebrate host has been suggested as a principal amplifying host for MAY virus, although it was once isolated from an orchard oriole (Icterus spurius) migrating into Louisiana, USA (Calisher et al. 1974).

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II. ECOLOGY OF MAYARO VIRUS

B. Studies on the Epidemic Cycle - the Belterra Outbreak

1. Description of Belterra

OBJECTIVE: The objective of this section is to provide a detailed description of Belterra, Pará, Brazil. This description will then serve as background information for the discussions concerning the epidemiology and epizootology of Mayaro virus in Belterra which follows.

BACKGROUND: In February and March of 1978, several cases of an acute febrile disease were observed in Belterra, and three fatalities were recorded. The virus section of the Institute Evandro Chagas was requested to investigate the apparent outbreak. Members of the PAHO team were then asked by the Institute Evandro Chagas to assist in the investigations.

Investigations were begun in March and two arboviruses were identified as responsible for this outbreak: Yellow Fever (YF) and Mayaro (MAY). All deaths were attributed to YF virus. A discussion of preliminary results of clinical, ecological and epidemiological investigations of these outbreaks is included herein.

DESCRIPTION: Belterra is the name given to a rubber plantation originally founded by the Ford Motor Company of Brazil in 1934. This plantation covers a total of 281,000 ha, of which 7,200 have been used for a rubber plantation. The plantation lies on a plateau about 5 km from the eastern bank of the Tapajós River in the state of Pará, Brazil. The plateau is 175 m above sea level, and the lowland between the plantation and the river is 75 m above sea level. Belterra is approximately 40 km south of Santarém, the nearest large city (Figure 1).

The plantation was founded prior to World War II, but rubber production has been hampered due to a persistent fungal disease which has attacked the rubber trees. The plantation has, however, remained open and productive although it changed hands in 1945 and is currently owned and managed by the Brazilian Ministry of Agriculture. During the last 20 years much of the plantation has been allowed to be overgrown by secondary vegetation, consequently the ecology of the area is now a mixture of rubber trees and secondary forest, surrounded by more mature, less disturbed forest.

Belterra lies as a rectangle with its length running east to west. The plantation is divided lengthwise by a road (Road 5) which runs through the center of the plot, and is bordered on the north by Road 1 and the south by Road 7. The plantation is further divided by Roads 2, 4, 6, 8 and 10 which run north-south. The greatest concentration of housing and administration buildings is found in the north-west corner of the plantation. A small hospital is also located in this area. Additional housing is interspersed throughout the plantation, as shown in Figure 2. Housing is provided by the

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administration of the plantation for all its employees at minimal cost. Medical care is free to employees. Approximately 50% of the housing districts have electricity and all districts have running water, though not inside the houses. Houses are usually constructed of wood, and aside from the established vilas, nearly all are in close proximity to the forest. Few houses have screening, and mosquito netting is generally not used at night.

Most residents of Belterra are associated with some aspect of the plantation. Many people, both men and women, are employed to collect the latex from the rubber trees and are required to enter the forest almost daily. Latex is generally collected in the morning. Some workers maintain their trails in the afteroon, while others tend to their private gardens. Many families have private gardens away from their houses, and frequently the entire family goes to the garden to work. Hunters frequent both the plantation and the adjacent forests. As a result, many residents of Belterra, representing both sexes and all ages, enter the forests frequently and are thus pottentially exposed to infected sylvatic vectors.

The administrators of the plantation conducted a census of Belterra in December of 1977 and found a total of 4,083 people resident in the area. A population pyramid showing the age and sex distribution of Belterra as measured by this census is presented in Figure 3, and Table 1 presents the number of individuals seen in each age group. It will be noted that a significant portion of the population is over fifty years of age. This can be explained in part by the fact that employees injured during service for the plantation, or those reaching retirement age, are allowed to remain in housing provided by the plantation and continue to receive free medical care. Consequently, there is no pressure for this segment of the population to leave. In addition, much of the population below the age of 41 years old was born in Belterra, consequently, established families are present with whom the older segments of the population may remain. Table 2 presents a summary of the total residents found in each district of Belterra, and Tables 3 through 8 present a summary of the age structure of each district.

Table 9 presents a summary of the place of birth of 397 persons as questioned in a stratified random sample of 10% of the occupied houses of Belterra made during July, 1978. Clearly the majority of persons currently residing in Belterra were born there. Since the plantation at Belterra has only been in operation for slightly over 40 years, it seems apparent that the conditions at Belterra have resulted in a very stable population.

Table 10 shows the number of households questionned and the number of people residing in those households, as well as the average number of residents per household. This information was also collected from the 10% stratified random sample of occupied households made during July, 1978.

The climate in Belterra is classified as humid tropical according to the Holdridge life zone classification system. A meteorological station which has been operated by the Instituto Nacional de Meteorología since 1972 is located in Belterra. The average annual rainfall during the 7 year period was 2,109.5 mm. The normal amount of rainfall changes drastically between the 4 month dry season, August, through November, in which less than 55.5 mm of precipitation falls per month, and the 8 month wet season, December through July, in which the monthly average is more than 100 mm. The average monthly temperature normally differs only slightly throughout the year. The dry season month of October is usually warmest with the 26.2°C average temperature, and July is usually the coolest with an average of 23.8°C. During the dry season the average monthly extreme temperatures range from a low of 20.0°C to a high of 32.0°C. The average monthly extreme temperatures range from a low of 19.4°C to a high of 31.3°C during the wet season. According to Gaussen's formula of plotting rainfall against temperature (20 mm of precipitation equals 10°C), a drought period occurs when the rainfall curve falls below that of the temperature. Using this formula (Figure 4), only the month of October normally has a water deficiency.

The actual rubber plantation is located on a flat plateau which was cleared of small trees and shrubs during the late 1930's. The rubber trees which naturally occurred in the area were left and others were planted. The trees of commercial value were cut for lumber. The remaining vegetation was nearly a monoculture of rubber trees, thereby creating a favorable habitat for parasites and diseases. During the last 20 years invading trees and shrubs have been allowed to grow until the plantation now has a continuous secondary scrub undergrowth, which reaches to approximately 15 m in height and contains some lianas and small vines. The soil is mainly a reddish brown clay-sand with a very thin humus layer. The litter layer, composed of fallen leaves and twigs, is from 1 to 5 cm thick. Trails have been maintained throughout the plantation to permit easy access to the rubber trees. Grapefruit, orange, mango, cupuacu (Sterculiacea), avocado, cacao and banana trees have been planted, both behind and in front of the houses. The secondary growth vegetation has encroached to the edge of the yards, placing many houses in very close contact with the forest.

The lowland forest which occupies the area north and west of Belterra, although subjected to slash and burn agriculture in years past, is less disturbed than the upland forest. The soil there is a brown sandy clay with a very thin humus layer which contains many hair-like roots. The litter layer, from 1 to 5 cm thick, is composed of leaves and twigs. Fallen trees covered by thick moss are common. Woody plants from 1 to 5 m tall are numerous. Shrubs with thin trunks and scattered palms from 3 to 4 m in height make up an open intermediate canopy, and larger trees form a higher canopy approximately 15 m from the ground. The emergents, some exhibiting burn scars, rise about 30 m in height. No buttressing is evident on the emergents, and only scattered trees have stilt roots. Lianas are common, but not numerous. The vegetation is fairly open, and one can walk in most places without cutting a trail. The inhabitants of Belterra utilize this area for hunting, and thus the trails remain open.

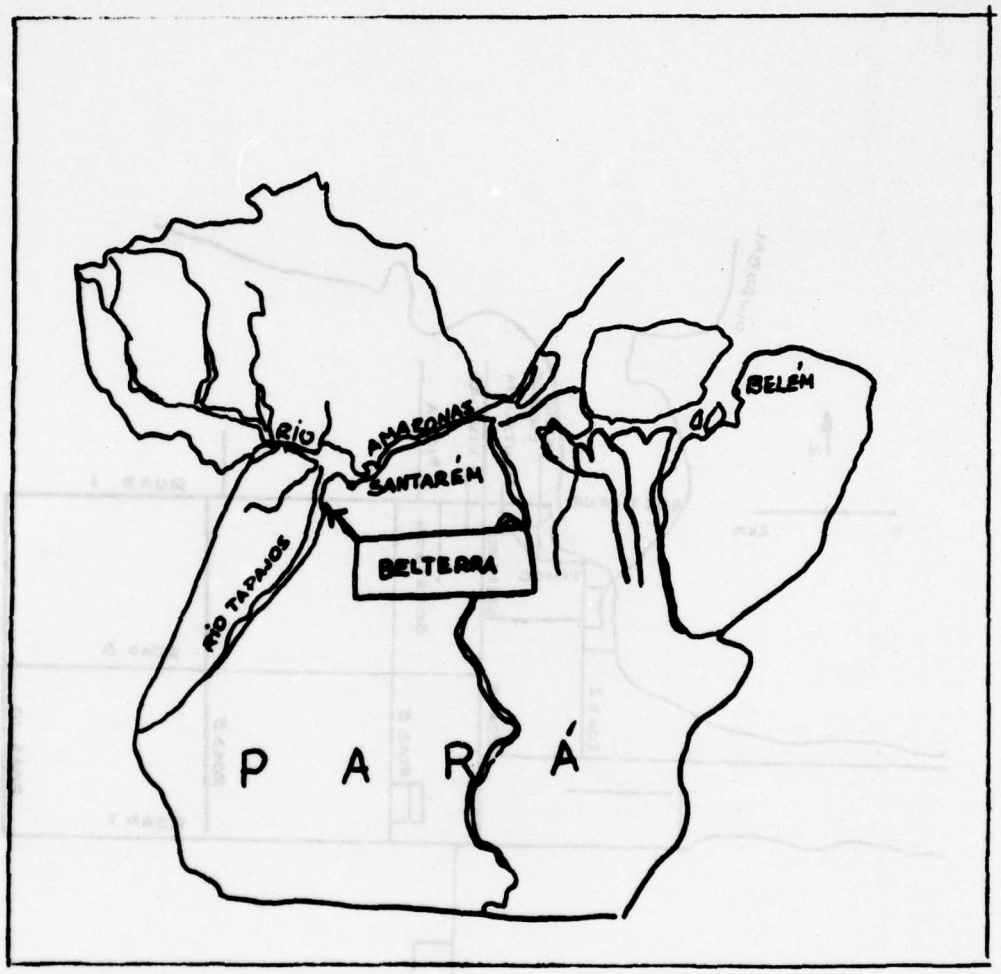


FIGURE 1. Map of the state of Pará, Brazil, showing the location of Belterra.

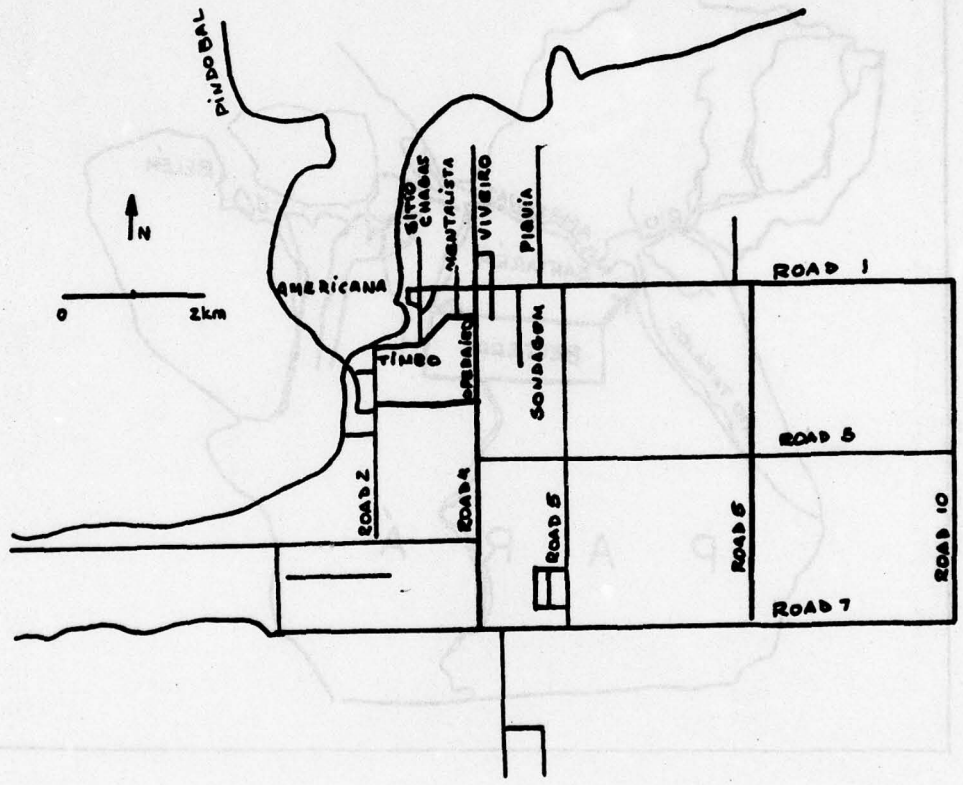


FIGURE 2. Map of Belterra, Pará, Brazil, showing the major roads and names of various residential districts.

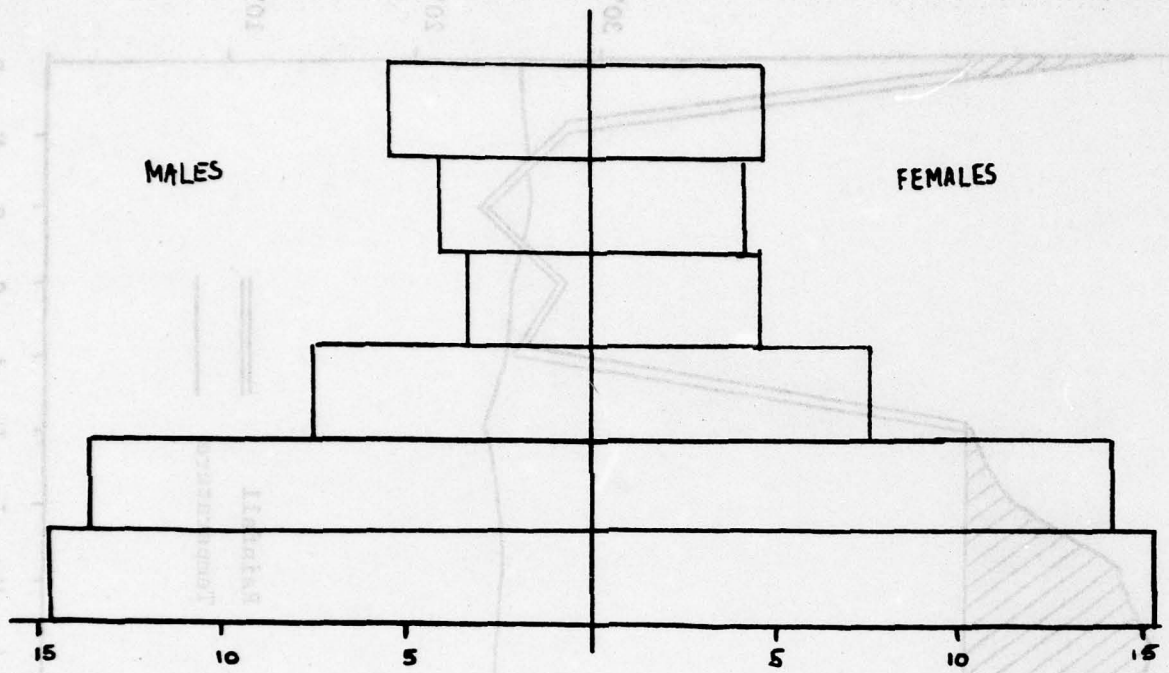


FIGURE 3. Percent of population in each age group, Belterra, Pará, Brazil, December, 1977

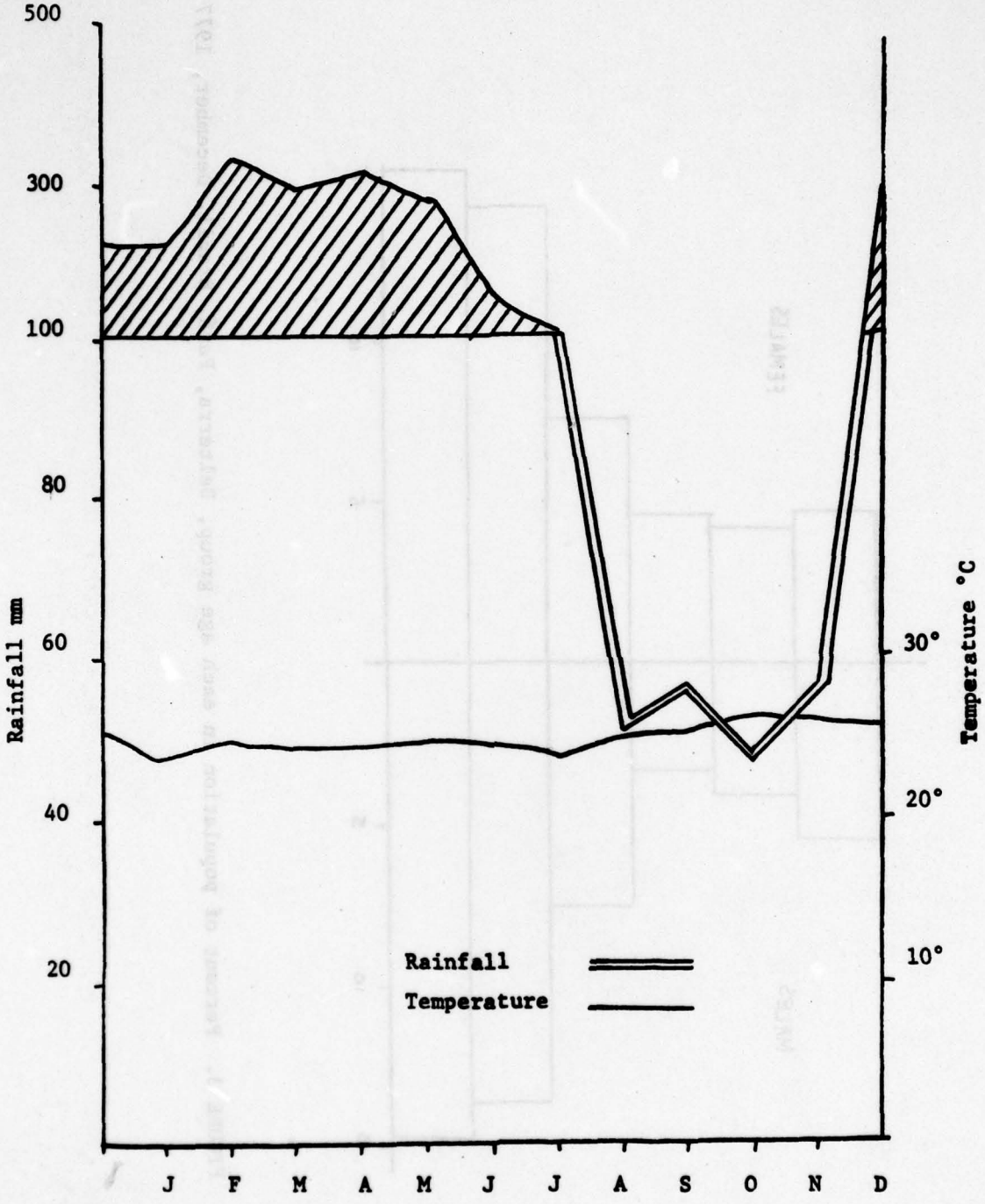


FIGURE 4. Climatic diagram of Belterra, Pará. The shaded areas of the curve indicate rainfall over 100 mm per month. This part of the scale has been reduced by 1/10. The monthly temperatures and rainfall were averaged from 6 to 7 years data. (1972-1978).

II. ECOLOGY OF MAYARO VIRUS

B. Studies on the Epidemic Cycle - the Belterra Outbreak

2. Clinical disease in man

OBJECTIVE: The objective of this section is to present a summary of the clinical disease caused by Mayaro virus infection in man as seen in the Belterra outbreak.

BACKGROUND: In February and March of 1978, several cases of an acute febrile disease were observed in Belterra, and three fatalities were recorded. Investigations were begun in March and two arbovirus were identified as responsible for this outbreak: Yellow Fever (YF) and Mayaro (MAY). All deaths were attributed to YF virus. A discussion of preliminary results of clinical investigations of MAY virus is reported here.

DESCRIPTION: Suspect cases of MAY virus infection were actively sought throughout Belterra during investigations of the epidemics. Febril patients were bled and a clinical history was taken. Blood samples were frozen in liquid nitrogen and returned to Belém where attempts to isolate virus were made by inoculation into suckling mice. In four cases where the clinical history clearly indicated MAY virus infection, but virus was not recovered in suckling mice, attempts were made to isolate virus by directly plaquing whole blood on confluent monolagers of Vero cells grown in 25 cm^2 plastic flasks.

Selected patients from whom MAY virus was isolated were followed throughout the course of their illness and recovery. These individuals were bled periodically to determine the duration of viremia and onset of detectable antibody. These sera will be used to document the antibody pattern of IgM and IgG production, and samples have been forwarded to the Department of Virus Diseases, WRAIR, for IgM/IgG level determinations. Detailed case histories were also taken from these patients to determine the duration of clinical signs and symptoms of MAY virus infection

PROGRESS: Infection with MAY virus was confirmed in 55 (76.4%) of 72 cases seen during the peak of the epidemic. Confirmation was accomplished by isolation of virus from 43 cases, and serologically in an additional 12. Of the 43 cases from whom virus was isolated, 39 isolations were made in suckling mice and 4 were made in Vero cell culture. Virus was identified by hemagglutination inhibition tests using certified reference reagents. The range of ages among patients from whom MAY virus was recovered varied from 2 to 62 years, and both sexes were represented.

Clinical manifestation of disease due to MAY virus included fever, arthralgia and exanthema. Table 11 presents clinical signs and symptoms associated with MAY virus infection as seen in the 43 patients from whom virus was isolated. Of the clinical symptoms, arthralgia was most predominant and usually manifested in the fingers, hands, feet and ankles,

and occasionaly affecting the knee or elbow joints. Exanthema was either small maculopapular or micropapular, and was most commonly seen in the thorax, back, upper and lower extremities.

Clinical manifestations persisted for 3-5 days, except exanthema, which could be seen until the 8th or 9th day of illness. Arthralgia persisted in some patients, especially older patients, for 2 months or more. No deaths were attributed to infection with MAY virus.

Figure 5 presents a schematic summary of the duration of clinical manifestations of infection with MAY virus, as well as the magnitude and duration of viremia and the onset of detectable hemagglutination inhibiting antibody, as seen in 21 patients bled periodically and from whom MAY virus was isolated. Values for temperature, viremia and antibody are presented as mean values (circles) with ranges superimposed (brackets). Viremia data are presented on a $\log_{10}$ scale, while antibody is presented on a $\log_2$ scale. Temperatures were measured externally in the axilla, consequently temperatures recorded are somewhat lower than those expected from oral measurements.

COMMENT: In this outbreak, the combined conditions of fever, arthralgia and exanthema were pathognomonic for MAY virus infection. Both fever and arthralgia were seen in all patients from whom virus was isolated, and exanthema was seen in two-thirds of those cases. No other disease was seen which presented itself as a combination of these three conditions.

This is in contrast to the other reported epidemics of MAY virus, where no distinctive clinical syndrome was detected. Arthralgia was recorded in one of five cases in Trinidad described by Anderson et al. (1957), but not among the six patients from whom MAY virus was isolated in the Guamá River, Brazil, outbreak reported by Causey and Maroja (1957), or in the single patient from whom MAY virus was isolated in the Bolivian study of Schaeffer et al. (1959). Likewise, exanthema was also reported for a single case, in this instance the Bolivian patient. Clearly MAY virus may present itself in a spectrum of clinical syndromes. The fact that all confirmed patients seen in the Belterra outbreak complained of arthralgia, and two-thirds presented with an exanthema, suggests that the strain of MAY virus which caused this outbreak was especially virulent.

II. ECOLOGY OF MAYARO VIRUS

B. Studies on the Epidemic Cycle - the Belterra Outbreak

3. Viremia in man

OBJECTIVE: The objective of this section is to quantify the viremic stage of Mayaro (MAY) virus infection in man. The underlying consideration is the question, can man serve as an amplifying host to infect feeding vectors?

BACKGROUND: All patients considered in this section were naturally infected in Belterra during the epidemic which occurred between December 1977 and June, 1978. Previous sections of this report have characterized Belterra and described the clinical syndrome of MAY virus infection in man.

DESCRIPTION: Febrile patients suspected of being infected with MAY virus were bled as described earlier and their blood tested for the presence of virus by inoculation in suckling mice or cell culture. Each sample from which virus was isolated was titrated by directly plaquing 0.1 ml of whole or diluted blood on Vero cells grown in 25 cm^2 plastic flasks. Cells were incubated for 1 hr. at 37°C, then overlaid with nutrient agar. Flasks were stained after 4 or 5 days and plaques counted 24 hrs. later. In certain instances when patients presented with symptoms characteristic of MAY virus infection and were bled during the first or second day of illness, but virus was not recovered in suckling mice the samples were then assayed by directly plaquing on Vero cells as described above.

PROGRESS: Mayaro virus was recovered from a total of 43 patients seen during the Belterra outbreak. Virus was isolated from 99.9% (32 pos/33 tested) patients bled during the first 24 hrs. after onse symptoms. Recovery rates then decreased to 82.3% (14/17) on day 2; 22.2% (4/18) on day 3; 6.6% (1/15) on day 4, and 0% (0/13) on day 5. These results are presented in Table 12. The single patient whose blood was negative on day 1 was actually bled at about 12 hrs. after the onset of symptoms. This patient was not bled again during the period of typical viremia, and was diagnosed only on the basis of seroconversion. Likewise, the three negative samples drawn on day 2 were also diagnosed solely by seroconversion.

Viremia was detected on days 1, 2, 3 and 4 after the onset of symptoms. Of those bloods tested so far, the maximum titer detected was 9.0×10^3 plaque forming units/0.1 ml whole blood, which was seen on day 1. Titers of viremia were lower on days 2 and 3. Results for day 4 are still pending. A summary of maximum titers detected on each day following the onset of symptoms is presented in Table 13.

COMMENT: Results presented here indicate that the duration of viremia in patients is at most 4 days, and significant titers can be reached in at least 3 of these 4 days. While the quantity of virus needed to infect feeding vectors has not been determined, it appears that man may circulate virus in sufficient quantities to infect some feeding vectors. However, most patients observed during this stage of illness were not continuing their daily activities, and many were bedridden. Consequently, patients would only be expected to be exposed to those vectors found in or near their residencies.

II. ECOLOGY OF MAYARO VIRUS

B. Studies on the Epidemic Cycle - the Belterra outbreak

4. Distribution of cases

OBJECTIVES: The objectives of this section are to present the temporal and geographical distribution of cases of Mayaro (MAY) virus as seen in Belterra, and to define the rates of clinically apparent and inapparent cases.

BACKGROUND: A description of the environs and population of Belterra has been presented previously. The census of Belterra made in December, 1977, described earlier, was used as a standard population for all age adjustments.

DESCRIPTION: Information was gathered to construct an epidemic curve for the outbreak in Belterra based on the onset or clinical symptoms characteristic of MAY virus infection. In order to acquire this information, a house-to-house survey of every occupied household was conducted during the last week of May, 1978, and people were questioned for a history of illness compatible with MAY virus infection. Cases with onset in June were estimated from results acquired during a serological survey which was made in July, 1978.

A total of three serological surveys have been made in Belterra. The first was conducted in 1972, and sampled 161 people over the age of 10 years old (y.o.). No information is available as to the sampling frame used or criteria for selection. Consequently, it cannot be assumed that these sera were collected in any type of systematic fashion.

In April, 1978, during the peak of the MAY epidemic, another serological survey was made. This sample contains 327 sera representing all age groups and all residential areas of Belterra. While no formal sampling frame was established, the sample was taken in an attempted random fashion.

The final survey was made during July, 1978, after the end of the MAY outbreak. This was a stratified random sample of 10% of all occupied households in Belterra. The sample was drawn by first numbering all occupied houses by residential area in Belterra, then randomly selecting households. The number of households sampled in each residential area was determined by the percent of households which that area contributed to the total of occupied households. Houses within each residential area were then selected using a table of random numbers. People who occupied the selected houses were bled and questioned for a history of illness compatible with MAY virus. People not at home during the initial visit were actively sought, and several return visits were made when necessary to complete the sample.

Sera collected in each survey were tested for the presence of hemagglutination inhibiting (HI) antibody to MAY virus. Samples of both positive and negative results were confirmed by netralization tests using Vero cell cultures.

PROGRESS: Figure 6 presents a diagram of the epidemic curve for the outbreak of MAY virus in Belterra. Results presented here are based on clinical histories from 3,941 people questioned during the last week of May, and for the June cases from the 10% stratified random sample made in July. These results indicate that the epidemic began in December, 1977, reached its peak in April, and the last cases were detected during June, 1978. Active transmission spanned a period of approximately 6 months. The first cases recorded lived at Road 8, and a total of 807 (20.4%) clinical cases were recorded.

Figure 7 presents a summary of the number of people found with HI antibody to MAY virus by age groups. Clearly all ages were exposed to MAY virus infection, and no single age group contributed prominently to the epidemic.

Cases of MAY virus infection were seen in all residential areas of Belterra; however, as shown in Figure 8, the greatest concentration of cases was located in the eastern portions of Belterra. Mayaro virus antibody prevalence rates decreased from east to west, and were lowest in the northwest corner of Belterra, where the population density was greatest, and where housing was farthest from the forest.

In general, the closer that housing was to the forest, the higher the antibody prevalence rates. Characteristic of this association is the higher prevalence rates along Roads 7 and 10, where the population density is low and houses are immediately adjacent to the forests, as compared with Vila 129, also in the southeast part of Belterra, but where houses are clustered together and the forest is not directly adjacent to most houses. This association was not upheld everywhere, though. In Sitio Chagas, a small residential area in the northwest corner of Belterra, houses are sparse and in close contact to the forest;however, this area has a very low prevalence rate of antibody to MAY virus.

The serological survey made in 1972 found a 10.3% age adjusted HI antibody prevalence rate to MAY virus in residents of Belterra above the age of 10. Since no one questioned during the house-to-house survey made during May recalled any illness clinically similar to the present MAY virus syndrome, results of the 1972 survey may serve as an estimate of the pre-existing MAY virus antibody prevalence rate.

The survey made in April, 1978, at the peak of the epidemic, found an age adjusted HI antibody prevalence rate of about 22%, in which males outnumbered females about 2:1. The July survey, made after the epidemic had subsided, showed 29.7% of the population possessed antibody to MAY virus; and the male to female ratio was nearly equal. Table 14 presents a summary of all 3 serological surveys made in Belterra.

Based on the results of the serological surveys, the house-to-house survey and the census data previously presented, an estimation of clinically apparent and inapparent attack rates can be made. Of the 3,941 people questined in May, 807 (including 20 estimated for June) had a history of

clinical illness compatible with MAY virus infection. The serological survey made in July estimated that 29.7% of the population had antibody to MAY virus; however, the survey made in 1972 showed that 10.3% of the population over the age of 10 y.o. had MAY virus antibody at that time. Consequently, a portion of the positive reactions in the July survey represent pre-existing antibody.

Since the 1972 survey only included people over the age of 10 y.o., this must be taken into account when comparing the different surveys. If it is assumed that the 3,941 people questioned have the same age distribution as the total census for Belterra, then 70.3% or 2,770 people questioned were over 10 y.o. The July serological survey found a 29.7% age adjusted antibody prevalence rate to MAY virus, or 823 of the 2,770 people over 10 y.o. would have had MAY antibody. Of these 2,770 people, 10.3% or 285, would have had pre-existing antibody to MAY virus. Thus 538 (823-285=538) people would be new cases in the 1978 population over the age of 10 y.o.

This assumes that those under 10 y.o. in the 1972 sample had the same pre-existing antibody prevalence rate (10.3%) as did those above 10 y.o. If, however, no one under 10 y.o. had antibody to MAY virus in the 1972 population, then this entire segment would all be susceptible prior to the 1978 outbreak. The 1972 survey was made approximately 5-1/2 years prior to the 1978 survey, consequently 55% of the current 11-20 y.o. age group would be susceptible. A total of 1,121 people would be in the age group, and 55% of this equels 617 people. These subtracted from 2,770 people over age 10 y.o. leaves 2,153 persons at risk of previous antibody, of whom 10.3%, or 222 persons, would have pre-existing antibody.

The previous estimate of 823 serologically positive cases, minus those with pre-existing antibody, should equal the total number of cases of MAY virus which occurred during the outbreak. If those under 10 y.o. in 1972 had the same antibody prevalence rate as the rest of the population, then 285 would have had pre-existing antibody in 1978 and 538 would represent new cases above age 10 y.o. If those under 10 y.o. in 1972 had no antibody, then 222 would have had pre-existing antibody in 1978, and 601 would represent new cases in the above 10 y.o. age group. Thus the range of new cases above 10 y.o. in 1978 is 538 to 601.

If the 807 clinical histories consistent with MAY virus infection are distributed by age as is the population of Belterra as a whole, then 70.3% of these 807 should be above age 10 y.o., or 567 persons. The estimate for the clinically apparent attack rate is then this value divided by the estimates of new cases just calculated based on serology, or 567/538=100%, and 567/601=94.3%. Thus, within the above 10 y.o. age groups, at least 94% of the cases of MAY virus which occurred during the Belterra outbreak were clinically apparent. Table 15 presents a summary of these attack rates.

Among the under 10 y.o. age groups, 26% were serologically positive in 1978. A total of 1171 (3941-2770=1171) persons are in this age group, consequently 304 (26% of 1171) cases must have occurred. This value, added to the above estimates for the over 10 y.o. ages, represents the new cases of the population as a whole. Thus, 304+538=842, and 304+601=905, are estimates of the range of serologically positive new cases of May virus. Since 807 represents the total of clinically ill persons, then 807/582=95.8% and 807/905=89.2% are estimates for the clinically apparent attack rate of MAY virus for all ages seen in the Belterra outbreak. Table 16 presents a summary of these attack rates.

In summary, the clinically apparent attack rate for MAY virus in the whole population as studied in Belterra was between 89% and 96%, and in the segment above 10 y.o., the clinically apparent attack rates was between 94 and 100%. Most inapparent cases were then in the under 10 y.o. age group.

A second estimate of the clinical attack rate can be made based on the April serological survey. In this survey, 327 persons were questioned and bled, and 71 were found to have antibody to MAY virus. If 70.3% of the 327 are above the age of 10 y.o., then 230 may have had pre-existing antibody. As measured in the 1972 survey, 10.3% of those above 10 y.o. had antibody to MAY virus, consequently 24 of the 230 would be expected to have pre-existing antibody. The results of questions directed at past clinical illness are summarized in Table 17, and of the 71 antibody positive persons questioned 23 were asymptomatic. This is very close to the 24 expected to have per-existing antibody, and these results then also suggest a very high apparent attack rate.

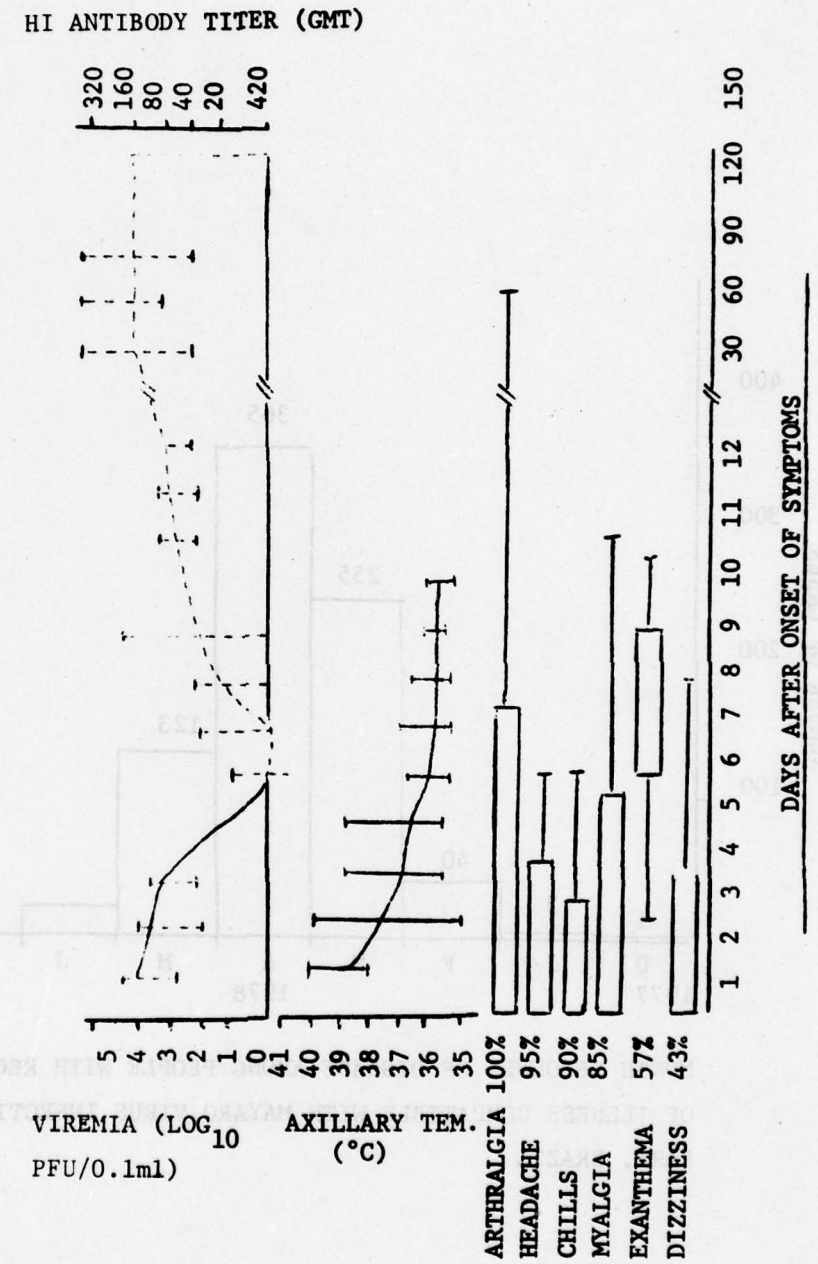
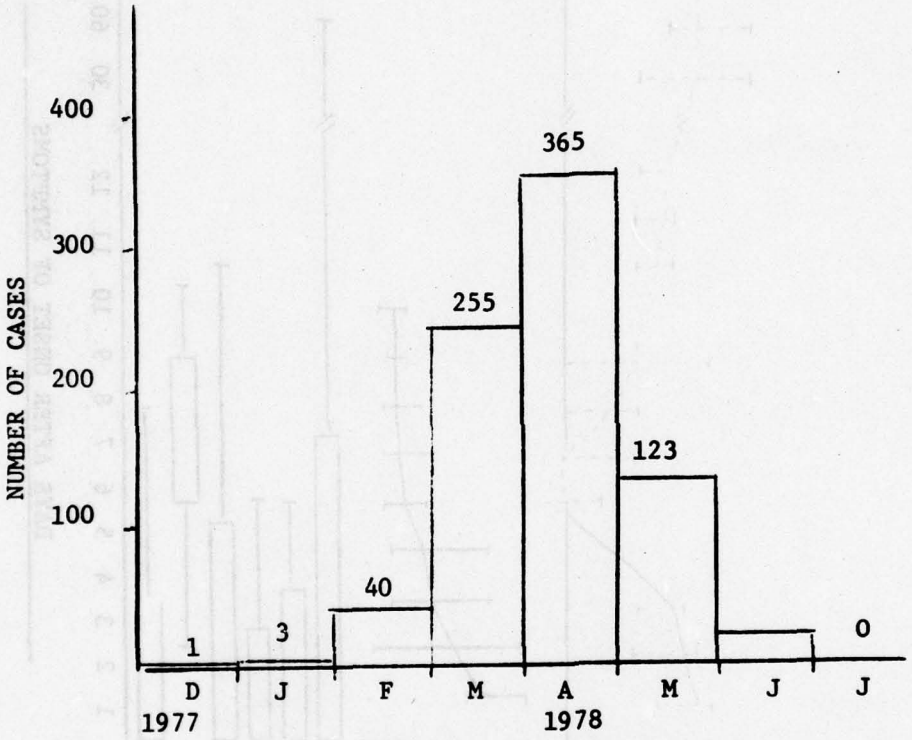


FIGURE 5: Schematic summary of the viremia temperature and clinical manifestations of Mayaro virus infection as recorded from patients infected during an epidemic in Belterra, Pará, Brazil, 1978



MONTH OF ONSET OF DISEASE AMONG PEOPLE WITH RECENT HISTORY OF ILLNESS COMPATIBLE WITH MAYARO VIRUS INFECTION, BELTERRA PARA, BRAZIL

Figure 6 Diagram of the epidemic curve for an outbreak of Mayaro virus which occurred in Belterra, Para, Brazil, 1978

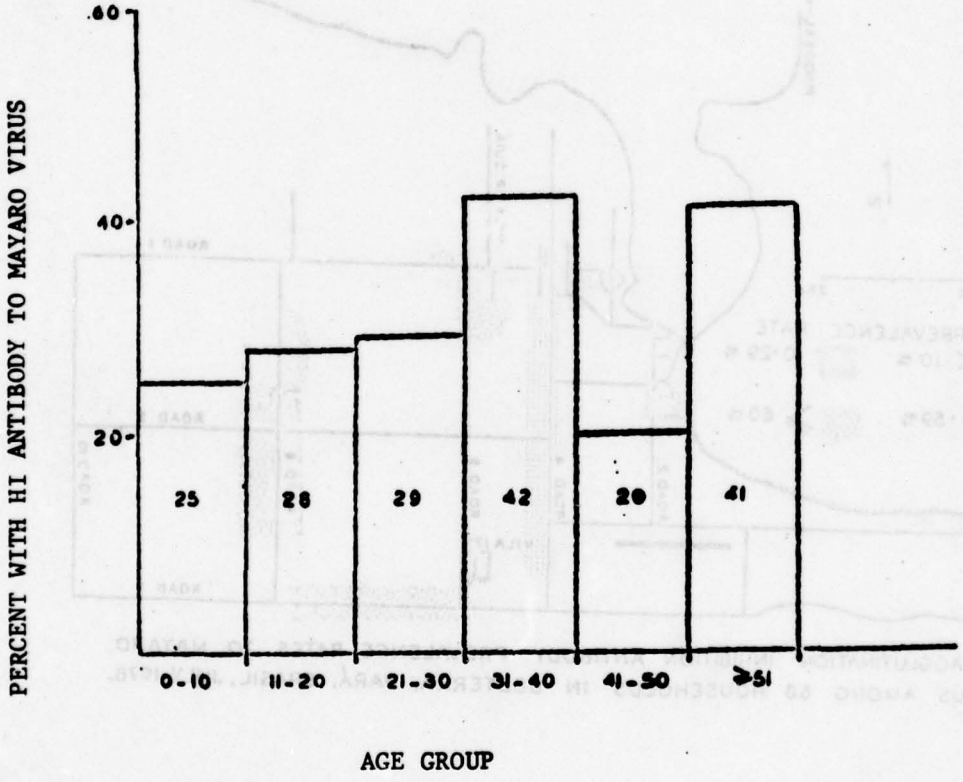
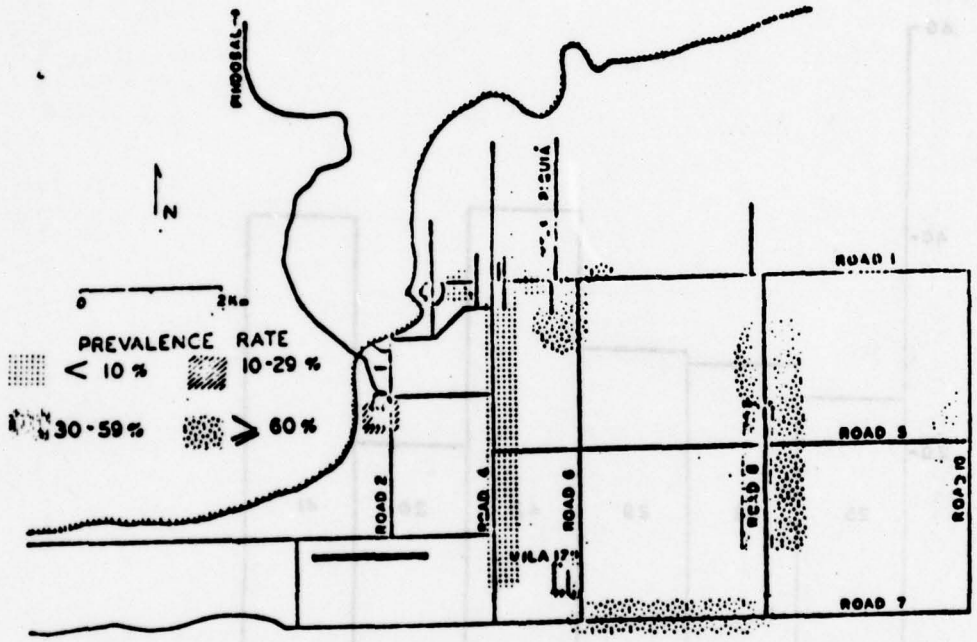


Figure 7: Diagramatic summary of the percent of people found with hemagglutination inhibiting (HI) antibody to Mayaro virus by age as seen among 327 people resident in Belterra, Pará , Brazil 1978



HEMAGGLUTINATION INHIBITION ANTIBODY PREVALENCE RATES TO MAYARO VIRUS AMONG 68 HOUSEHOLDS IN BELTERRA, PARÁ, BRASIL, JULY, 1978.

Figure 8. Distribution of hemagglutination inhibiting antibody to Mayaro virus among 68 households randomly selected from all residential areas of Belterra, Pará, Brazil, July, 1978

TABLE 1. Population of Belterra, Pará, Brazil as measured by a census taken in December, 1977.

Age Group	Males	Females	Total
0 - 5	267	286	553
6 - 10	320	339	659
11 - 20	549	613	1,162
21 - 30	296	299	593
31 - 40	141	191	332
41 - 50	165	176	341
> 50	240	201	441
TOTAL	1,978	2,105	4,083

TABLE 2. Census of Belterra, Pará, Brazil by district and sex, December, 1977.

District	Male	Female	Total
Road 1	180	172	352
Road 2	46	44	90
Road 4	247	273	520
Road 6	42	39	81
Road 7	42	34	76
Road 8	446	453	899
Road 10	75	96	171
Sitio Chagas	44	52	96
Vila Americana	20	26	46
Vila Mensalista	34	38	72
Vila Operário	100	132	232
Vila Piquiá	105	82	187
Vila Sondagem	63	75	138
Vila Timbó	61	70	131
Vila Viveiro	272	294	566
Vila 129	201	225	426
TOTAL	1,978	2,105	4,083

TABLE 3. Age and sex distribution of residents of Roads 1, 2 and 4 based on the December, 1977 census of Belterra, Pará, Brazil.

Age	Locality					
	Road 1		Road 2		Road 4	
	M	F	M	F	M	F
0 - 5 yrs	23	29	4	4	40	34
6 - 10	33	32	5	5	40	49
11 - 20	55	40	11	14	71	80
21 - 30	20	22	12	7	32	36
31 - 40	14	16	2	1	18	23
41 - 50	14	15	5	8	16	23
> 50	21	18	7	5	30	28
TOTAL	180	172	46	44	247	273
TOTAL	352		90		520	

TABLE 4. Age and sex distribution of residents of Roads 6, 7 and 8 based on the December, 1977 census of Belterra, Pará, Brazil.

Age	Locality					
	Road 6		Road 7		Road 8	
	M	F	M	F	M	F
0 - 5 yrs	6	6	3	1	50	57
6 - 10	7	10	2	2	80	72
11 - 20	13	7	14	11	110	121
21 - 30	2	5	14	10	78	68
31 - 40	6	4	2	6	34	42
41 - 50	-	3	3	2	34	43
> 50	8	4	4	2	60	50
TOTAL	42	39	42	34	446	453
TOTAL	81		76		899	

TABLE 5. Age and sex distribution of residents of Road 10, Sitio Chagas and Vila Americana based on the December, 1977 census of Belterra, Pará, Brazil

Age	Locality					
	Road 10		Sitio Chagas		Vila Americana	
	M	F	M	F	M	F
0 - 5 yrs	16	14	8	9	2	1
6 - 10	13	15	10	14	3	3
11 - 20	21	29	8	9	7	11
21 - 30	9	12	9	6	3	6
31 - 40	3	9	-	5	2	2
41 - 50	6	8	4	3	3	2
> 50	7	9	5	6	-	1
TOTAL	75	96	44	52	20	26
TOTAL	171		96		46	

TABLE 6. Age and sex distribution of residents of Vila Mensalista, Vila Operária and Vila Piquiá based on the December, 1977 census of Belém, Pará, Brazil

Age	Localities					
	Vila Mensalista		Vila Operário		Vila Piquiá	
	M	F	M	F	M	F
0 - 5 years	3	3	15	16	13	17
6 - 10	2	5	22	31	14	13
11 - 20	7	12	28	35	28	23
21 - 30	1	3	11	17	24	12
31 - 40	8	6	4	17	9	2
41 - 50	5	6	11	7	10	6
> 50	8	3	9	9	7	9
TOTAL	34	38	100	132	105	82
TOTAL	72		232		187	

TABLE 7. Age and sex distribution of residents of Vila Sondagem, Vila Timbó and Vila Viveiro based on the December 1977 census of Belterra, Pará, Brazil.

Age	Localities					
	Vila Sondagem		Vila Timbó		Vila Viveiro	
	M	F	M	F	M	F
0 - 5 yrs.	8	9	5	7	38	40
6 - 10	9	11	7	8	53	40
11 - 20	20	21	24	30	74	107
21 - 30	5	9	8	7	40	44
31 - 40	7	8	2	7	19	23
41 - 50	6	7	7	4	24	18
> 50	8	10	8	7	24	22
TOTAL	63	75	61	70	272	294
TOTAL	138		131		566	

TABLE 8. Age and sex distribution of residents of Vila 129 based on the December, 1977 census of Belterra, Pará, Brazil.

Age	Vila 129	
	M	F
0 - 5 yrs.	33	39
6 10	20	29
11 - 20	38	62
21 - 30	28	35
31 - 40	11	20
41 - 50	17	21
> 50	34	18
TOTAL	201	225
TOTAL	426	

TABLE 9. Place of birth of full and part time residents of Belterra by age group as sampled by a stratified random sample of 10% of the occupied houses in Belterra, Pará, Brazil, July, 1978.

Age group	Place of birth		
	Belterra	Elsewhere	Total
0-10	103 (77%)	30 (23%)	133
11 - 20	82 (71%)	34 (29%)	116
21 - 30	30 (67%)	15 (33%)	45
31 - 40	15 (50%)	15 (50%)	30
41 - 50	3 (11%)	24 (89%)	27
> 50	1 (2%)	45 (98%)	46
	234 (59%)	163 (41%)	397

TABLE 10. Number of households sampled and the number of full time residents per district and average number of residents per household by district in Belterra, Pará, Brazil based on a 10% stratified random sample of occupied household.

District	# Households	Residents	X
Road 1	6	34	5.7
Road 2	1	7	7.0
Road 4	9	36	4.0
Road 6	2	12	6.0
Road 7	1	7	7.0
Road 8	16	80	5.0
Road 10	3	13	4.3
Sitio Chagas	2	4	2.0
Vila Americana	1	3	3.0
Vila Mensalista	3	11	3.7
Vila Operário	3	19	6.3
Vila Piquiá	4	19	4.8
Vila Sondagem	2	7	3.5
Vila Timbó	2	12	6.0
Vila Viveiro	8	53	6.6
Vila 129	7	47	6.7
TOTAL	70	364	5.2

TABLE 11. Clinical signs and symptoms among 43 patients from whom Mayaro virus was isolated during the epidemic in Belterra, Pará, Brazil, 1978

Signs or symptoms	%
Fever	100
Arthralgia	100
Headache	86.0
Chills	81.3
Myalgia	74.4
Exanthema	66.6
Enlarged lymph nodes	52.6
Dizziness	41.8
Eye pain	37.8
Nausea	34.8
Articular edema	23.0
Vomiting	20.9
Photophobia	6.9
Diarrhea	4.6
Conjunctival congestion	2.3

TABLE 12. Number of bloods tested and number of Mayaro virus isolations made by days after onset of clinical symptoms as sampled during an outbreak in Belterra, Pará, Brazil, 1978.

Days of illness	Number of isolations	Number tested	% positive
1	32	33	96.9
2	14	17	82.3
3	4	18	22.2
4	1	15	6.6
5	0	13	0

TABLE 13. Number of bloods titrated for Mayaro virus and the maximum titers found by days after onset of symptoms as sampled during an outbreak in Belterra, Parâ, Brazil, 1978.

Days of illness	Maximum titer*	Number tested
1	9.0×10^3	14
2	6.0×10^3	4
3	2.2×10^3	2
4	none tested	0

*Plaque forming units/0.1 ml. on Vero cells.

TABLE 14. Summary of HI antibody prevalence rates to Mayaro virus among Humans residing in Belterra, Pará, Brazil prior to, during and after an epidemic of Mayaro virus disease. All rates are age adjusted to the 1977 census of Belterra.

Date	Sample size	Mayaro by prevalence rate		
		Males	Females	Total
Nov-Dec. 1972	164*	10.8%	3.2%	10.3%
April 1978	327+	32.0%	15.3%	22.3%
July 1978	365+	32.2%	26.1%	29.7%

* Included only above age 10 yrs.; rates based on these denominators.

+ All ages included.

TABLE 15. Maximum and minimum clinical attack rates of Mayaro virus infection among persons over 10 y.o. in Belterra, Pará, Brazil, 1978.

Assumed prevalence of antibody in < 10 y.o. in 1972	Estimated seroconversions in 1978	Actual clinical incidence in 1978	Resulting clinical attack rate
10.3%	538	567	100%
0 %	601	567	94.3%

TABLE 16. Maximum and minimum clinical attack rates of Mayaro virus infection among all persons in Belterra, Pará, Brazil, 1978.

Assumed prevalence of antibody in <10 y.o. in 1972	Estimated seroconversions in 1978	Actual clinical incidence in 1978	Resulting clinical attack rate
10.3%	582	807	95.8%
0 %	905	807	89.2%

TABLE 17. Clinical manifestations among 71 persons with hemagglutination inhibiting antibody to Mayaro virus as found in a sample of 327 persons interviewed and bled during April, 1978 in Belterra, Pará, Brazil.

Clinical manifestations	No. of persons
Fever and arthralgia with or without rash	40
Fever and rash	4
Fever	4
Asymptomatic	23

II. ECOLOGY OF MAYARO VIRUS

B. Studies on the Epidemic Cycle - the Belterra Outbreak

5. Vectors in the epidemic cycle

OBJECTIVE: The entomological program was designed to systematically sample hematophagous insects feeding on man by multiple sampling techniques in an effort to identify the principal epidemic vectors of Mayaro (MAY) virus. Additional objectives of the entomological program were:

a. to identify and quantify the hematophagous insects feeding on man in both the peridomiciliary and working sylvatic environment

b. to establish systematic collecting programs and thereby define the temporal and spatial distribution of MAY virus vectors

c. to define the geographical distribution and ecological association of MAY virus vector(s)

d. to identify the epidemic vector(s) of Yellow Fever (YF) virus and, if possible, characterize it as above as well.

BACKGROUND: Simultaneous epidemics of MAY and YF viruses occurred in Belterra, Pará, Brazil. Both epidemics began in December, 1977. The YF virus epidemic was halted by a vaccination campaign which began in mid-April, and the last human case was seen at the end of April. The MAY virus epidemic ended in June, with the last human case seen at that time. A description of the environs of Belterra has been presented previously, as has a summary of the clinical manifestations of MAY virus infection in man. This section deals with investigations of potential insect vectors of both YF and MAY viruses in the Belterra epidemics.

DESCRIPTION: A field entomological surveillance program was initiated in April, 1978 to assist with an epidemiological study of an ongoing epidemic of MAY and YF viruses. Several habitats were sampled to determine the ecological associations of potential insect vectors in Belterra. Those habitats can be considered under two separate headings, the peridomiciliary habitat and the sylvatic habitat.

Peridomiciliary Insect Survey: From the initial epidemiological survey information on current cases of MAY and YF viruses within the Belterra rubber plantation, it was apparent that all members of the family unit were at risk of infection, and that both diseases were widespread and not limited to a single definable ecological or geographical area. Therefore, it was felt that the peridomestic environment should be considered sampled throughout Belterra. Peridomestic surveys were conducted primarily during the day, with limited sampling at night. In each area, paired habitats were chosed to be sampled. One site was chosen within 10 to 20 meters of the house, and

the other approximately 50 to 100 meters away in the forests. A team of two collectors was located at each site.

"Peridomiciliary" captures were defined as those collections conducted within 10 to 20 meters of the house, normally to the rear of the house within the confies of backyard. "Peridomiciliary-forest" collections were defined as those collections conducted between 50 to 100 meters from the rear of the house, thus, generally penetrating into the leading edge of the denser growth of the rubber three plantation. Ecologically these two habitats shared some similar features, for example, rubber trees were present in both habitats. However, fewer rubber trees were observed within 20-30 meters of the houses and most active households which bordered on the plantation had cleared the lower secondary forest growth near their houses. Banana and mango trees were generally associated with most of the households, and were not present in the forests. "Domiciliary" captures were defined as those collections conducted within a house.

Night Time Surveys: Surveillance for nocturnally active hematophagous insects in the domiciliary and peridomiciliary environments was accomplished by utilizing two survey techniques: man-biting captures and CDC light traps.

Domiciliary surveys were conducted periodically from 18:00-24:00 hrs. of rom 21:00 to 03:00 hrs. by 2 men capture teams. Landing-counts were recorded for 50 minutes per hour capture time. In each collection area, 3 to 4 houses were surveyed simultanneously by different teams. Normally, night time collections were made in households where cases of MAY virus had been reported.

Noctural peridomiciliary surveys were accomplished by placing CDC light traps near the houses and in adjacent forested areas. When feasible, the light traps were baited with dry ice.

Forest Insect Survey: Sylvatic man-biting insects were surveyed in two distinct ecological habitats: the rubber tree plantation on the plateau the lowland disturbed primary forest nearer to the Tapajós River. These areas have been described earlier.

In the rubber tree plantation three tree towers were constructed in order to survey the canopy insect fauna. Selection of tree tower locations was based on the following criteria: (1) type of trees available for tower construction; (2) density of tree canopy; (3) being representative of the surrounding forest habitat, and (4) located within the forest areas where rubber latex was being actively removed.

When possible, concurrent collections in the canopy and at ground level were made, each by two men collection teams. Teams were rotated hourly to prevent fatigue and differences in capture team efficiency. Captures were normally performed from 07:00 to 14:00 and 16:00 to 18:00 hrs. each capture day; however, a rigid program was impossible due to uncontrollable environmental and personnel problems.

In the lowland forested area, two tree towers were constructed. One was located on the plain of the lowland area while the second was constructed on the mid-slope down from the rubber plantation plateau. Paired canopy-ground captures were performed at each tree tower from 07:00 to 18:00 hrs with 50 minute capture periods. Ground and canopy teams were rotated hourly as stated previously.

Processing of Insects Specimens: Preliminary processing of insect specimens was conducted at the field station laboratory, with definitive taxonomical determination and virus isolations being performed at the Institute Evandro Chagas.

In order to prevent excessive mortality of collected insects and possible loss of viral infectivity, insect collections were gathered periodically from all the field sites. Periodic gathering of the material also facilitated the routine processing of the insects. At the field lab, all insects were lightly anesthetized with chloroform, organized in general taxonomic groups and placed in labeled vials. Information pertaining to each collection period was recorded on a field control form. Labeled vials containing the specimens were immediately placed into liquid nitrogen and were transferred to Belém laboratories biweekly. Timely handling and rapid preliminary processing of field specimens collected during the epidemic was a major task and could not have been accomplished without an efficient and dedicated Brazilian staff.

At the Belém laboratories, insects were identified to species and pooled for virus isolations. Sandflies (Psychodidae) and Culicoides (Ceratopogonidae) were grouped into pool sizes of 50, while the pool size for mosquitoes (Culicidae) was 50 individuals or less. Mixed pooling of insects from different collecting areas was performed when the areas were in close proximity, or when only a few specimens of a species were represented. Blood engorged insects were identified; however, these were not pooled for virus isolation attempts.

PROGRESS: The entomological survey at Belterra was initiated on 5 April 1978 and was concluded on 5 May 1978. During this time approximately 12,000 man biting insects were captured and identified; however, only 9,000 ca. of these were processed for virus isolations due to the presence of undigested blood in many of the insects. Table 18 presents a summary of the species collected and the number of groups tested for virus isolations.

Virus isolations were obtained from 3 species of mosquitoes: Haemagogus janthinomys, Limatus, flavisetosus, and Wyeomyia aporonoma; however, only Haemagogus janthinomys yielded isolates of YF and MAY virus. Wyeomya complex viruses were isolated from Limatus flavesetosus and Wyeomytia aporonoma.

Table 19 and Figure 9 present a summary of the geographical distribution and ecological association of arbovirus isolates from Haemagogus janthinomys. A review of the data show that only 2 MAY virus isolates were obtained from H. janthinomys collected from the peridomiciliary environs,

while 7 strains of MAY virus were isolated from H. janthinomys in the forest further from the residential areas. Seventy seven percent /7/9) of the MAY virus isolates were obtained from Haemagogus captured in the forest canopy. It is also noted that approximately 50% (5/9) of the MAY virus isolates were recovered from the disturbed primary forest lowland bordering the rubber plantation plateau.

Only two strains of YF virus were isolated from 64 pools of Haemagogus tested. One isolate was obtained from collections made in the rubber plantation forest, while the second isolate was from collections made in the lowland forest area. Table 19 indicates that the isolates of MAY and YF viruses appear to be dispersed in a variable pattern among the areas sampled.

Tables 20 and 21 summarize the nocturnal endophilic and exophilic insect species captured in the domiciliary environs. Culex fatigans and Culex coronator were the most abundant man-biting mosquito species collected within the houses, with Culex fatigans being the dominant of the two. Five endophilic species of mosquitoes (Culex, 2 spp; Mansonia, 3 spp.) were recorded during the epidemic.

Light trap collections yielded 7 species of mosquitoes in the peridomiciliary environs. Culex coronator was the dominant mosquito species collected. Man-biting and light trap collections indicated that the mosquito populations were low in the peridomiciliary environs.

Figures 10, 11, 12 and Table 22 show the diurnal activity for Haemagogus, janthinomys in different geographical and ecological areas, which represent paired canopy and ground captures for Haemagogus. Figure 10 indicates that the forested areas near the house exhibited significantly higher numbers of Haemagogus than the environs immediately adjacent to the house. Haemagogus activity near the residential areas was quite low and relatively stable throughout the times sampled, while Haemagogus activity in the nearby forested areas showed a definite increase from midday to approximately 16:00 hours.

Table 22 summarized the diurnal activity pattern for the three (3) tree towers located on the rubber plantation plateau. The number of Haemagogus captured from these areas was low; however, the data show a moderate increase of activity around midday, which continued until approximately 16:00 hors. Figures 11 and 12 illustrated paired canopy and ground captures in the taller lowland forest areas. Canopy collections at the two tree tower stations exhibited marked differences in Haemagogus numbers, with station #5 showing the highest numbers. When comparing canopy and ground captures, it is clearly demonstrated that the canopy collections yielded significantly larger numbers than the ground collections. Temporally, Haemagogus activity as measured by the ground captures was relatively stable, while Haemagogus activity in the canopy sharply increased at approximately 1300, followed by a drop in activity around 1600 hours.

COMMENTS: When reviewing the available information concerning the epidemic in Belterra, the data strongly indicate that Haemagogus janthinomys was the principal, if not only vector involved in the transmission of MAY and YF viruses. A total of 9,122 hematophagous insects representing 26 species were tested for virus isolation during the epidemic; however, all species with the exception of Haemagogus were negative for MAY and YF virus.

Nine strains of MAY and 2 strains of YF were isolated from 64 pools of Haemagogus. Two of the MAY isolates were recorded from Haemagogus pools collected in the peridomiciliary environment. The Haemagogus activity in this environment was considered to be low as demonstrated by man capture data (Fig. 10). Figure 10 shows that Haemagogus was only near the houses (10-20 meters) during the hours of 11:30 to 18:15. It is noteworthy that the appearance of Haemagogus near the domiciliary areas corresponds to the peak activity period for Haemagogus in the peridomiciliary forested areas. Therefore, it would be reasonable to assume that transmission of MAY and YF viruses to man in the peridomiciliary environs was most likely to occur in the latter portion of the day (12:00 to 18:00 hrs.) Due to the low numbers of Haemagogus occurring near the residential areas, it would appear that most transmission to man was actually occurring in the sylvatic environment where the highest activity of Haemagogus was localized.

Activity of Haemagogus at ground level in the sylvatic environment began at approximately 09:00 hrs. and continued until 18:00 hrs., with a moderate increase in this activity occurring during the latter part of the day. Therefore, any human activity within the sylvatic habitat would be excluded to active Haemagogus for most of the day, with a moderate increase in exposure during the latter part of the day.

The vertical distribution of MAY and YF viruses shows that 7 MAY strains and 2 YF strains were recovered from Haemagogus inhabiting the forest canopy, while two MAY isolates were made from mosquitoes collected at ground level. These results suggest that an arboreal host, perhaps primates, might be involved in the epizootic cycle of MAY virus.

TABLE 18. Summary list of insects species tested for virus isolation attempts collected furing a Yellow Fever and Mayaro epidemic in Belterra, Pará, Brazil, 1978.

List of species	No. of pools	Total No. tested	Virus isolation
Culicoides paraensis	50	2,303	
Limatus durhamii	61	1,472	
Culicoides debilipalpis	24	758	
Haemagogus janthinomys	62	732	(2) Yellow Fever (9) Mayaro
Limatus flavisetosus	32	720	(1) Wyeomyia complex
Phlehotomus spp.	18	574	
Wyeomyia aporonoma	23	472	(1) Wyeomyia complex
Culicoides spp.	11	425	
Culex (C) coronator	22	378	
Trichoprosopon digitatum	14	244	
Sabethe (Sab) belizarioi	9	174	
Wyeomyia spp.	11	158	
Sabethe (Sab) glaucodaemon	12	157	
Culex (C) fatigans	1	20	
Culex (M) sp.	4	26	
Culicoides insinuatus	4	123	
Torcypomyia	6	88	
Haemagogus trucocélaneus	1	6	
Psprophera cingulata	6	77	
Sabethe (Sab) chloropterus	2	12	
Sabethe (Sab) cyaneus	1	8	
Sabethe (Sab) quasicyaneus	14	102	
Sabethe (Sab) shannonii	3	13	
Orthopodomyia fascipes	3	69	
Aedes (How) septemstriatus	1	5	
Aedes (How) fulvithorax	1	6	

Total species - 26

Total grupos - 396

Total mosquito - 9,122

TABLE 19. Geographical distribution of arboviruses isolated from Haemogogus janthinomys (Dyar) collected during epidemics of Yellow Fever and Mayaro in Belterra, Pará, Brazil, 1978.

Collection	Habitats sampled				Pools inoculated	Specimens inoculated	Virus isolation	
	Peri-domiciliary	Forested area near house	Forested area				Mayaro	Yellow Fever
			Ground	Canopy				
Vila 129	X	X			3	27	(1)	
Vila Piquiá			X		1	3		
Vila Sondagem	X	X			5	32	(1)	
Vila Oracao	X	X			2	18		
Vila Golf	X	X			1	2		
Road 4	X	X			5	16		
Road 8	X	X			4	32		
Road 7	X	X			3	19		
Road 1 Station 1				X	5	132	(2)	(1)
Road 1 Station 1			X		5	66		
Road 6 Station 2				X	1	17		
Road 6 Station 2			X		1	7		
Station 4				X	5	71	(1)	
Station 4			X		2	34		
Station 5				X	14	193	(4)	(1)
Station 5			X		2	20		
Station 4-5			X		5	38		
TOTALS					64	727	9	2

TABLE 20. Summary of peridomiciliary CDC light trap collections conducted during Yellow Fever and Mayaro epidemics in Belterra, Pará, Brazil, 1978.

Species	Forested area near house (50-100 meters)			(Peridomiciliary collections (10-20 m.)		
	No. collected	No. Light-Trap Nights	No. Coll./ L-T Night	No. collected	No. Light-Trap Nights	No. Coll./ L-T Night
Culex						
coronator	22	8	2.7	20	23	.9
corniger	1	8	.1	5	23	.2
complexo-vomerifer	1	8	.1	2	23	
sp B # 21	1	8	.1	1	23	< .1
Psorophora						
cingulata				1	23	< .1
Uranotaenia						
lowii	1	8	.1	1	23	< .1
calosomata				1	23	< .1
Phlebotomus spp.	9	8	1.1	14	23	.6

TABLE 21. Summary of nocturnal encephilic insects collected during Yellow Fever and Mayaro epidemics in Belterra, Pará, Brazil, 1978.

Species	Time of Capture							
	19:00-19:50	20:00-20:50	21:00-21:50	22:00-22:50	23:00-23:50	24:00- 00:50	01:00-01:50	02:00-02:50
Culex								
fatigans	6/6*(1)**	1/6 (.2)	6/6 (1)	11/6(.7)	17/10 (1.7)	6/10 (.6)	3/10 (.3)	3/10 (.3)
coronator	3/6 (.5)		2/6 (.3)		3/10 (.3)			2/10 (.2)
Mansonia								
titillans					1/10 (.1)			
amazonensis				1/6 (.06)				
humeralis				1/6 (.06)				
Phlebotomus spp.					4/10 (.4)		3/10 (.3)	4/10 (.4)

*No. of insects collected/No. of 2 man team captures for 50 minutes capture periods.
**No. of insects collected per unit capture (2 man capture for 50 minutes).

TABLE 22. Diurnal activity pattern of Haemacocus janthinomys (Dyar) in the forested area of a rubber plantation in Belterra during epidemics of Yellow Fever and Mayaro viruses, 1978.

	06:40 07:10	07:20 07:50	08:00 08:30	08:40 09:10	09:20 09:50	10:00 10:30	10:40 11:10	11:20 11:50	12:00 12:30	12:40 13:10	13:20 13:50	15:30 16:00	16:10 16:40	16:50 17:20	17:20 18:00	18:10 18:40	Total
								Station # 1									
Canopy	0/6*	2/7 (.28)**	1/5 (.20)	0/3	1/3 (.33)	4/4 (1.0)	7/7 (1.0)	12/7 (1.71)	13/6 (2.16)	4/5 (.80)	13/5 (2.6)	6/4 (1.5)	24/6 (4.0)	28/7 (4.0)	16/7 (2.3)	3/5 (.60)	134/87 (1.54)
Ground	0/5	0/6	0/7	1/6 (.17)	2/7 (.28)	2/6 (.33)	2/6 (.33)	13/6 (2.2)	11/5 (2.2)	14/4 (3.5)	4/4 (1.0)	3/4 (.75)	8/7 (1.1)	11/7 (1.6)	5/6 (.83)	2/2 (1.0)	78/53 (.87)
								Station # 2									
Canopy	0/2	0/4				0/4	3/4 (.75)	4/4 (1.0)	2/4 (.50)	1/4 (.25)	1/3 (.33)	0/1	4/3 (1.33)	4/4 (1.0)	3/4 (.75)	0/2	22/43 (.51)
Ground	0/2	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	3/3 (1.0)	0/1	1/4 (.25)	2/4 (.50)	1/4 (.25)	0/2	7/56 (.13)
								Station # 3									
Canopy	0/3	0/6	0/6	0/6	0/6	0/6	0/6	0/5	0/5	2/5	1/5	0/4	0/4	0/4	0/4	0/4	3/73 (.04)
Ground	0/6	0/12	0/12	0/12	0/12	0/12	0/12	0/10	4/10	0/10	0/10	0/8	0/8	0/8	2/8	0/8	6/79 (.08)

* a/b a = Total number of Haemagogus spp. collected; b = Number of capture periods.

** ( ) = Number of Haemagogus per 2 man capture teams.

OBS.: Collections were not made between the hours of 14:00 to 15:00 hrs.

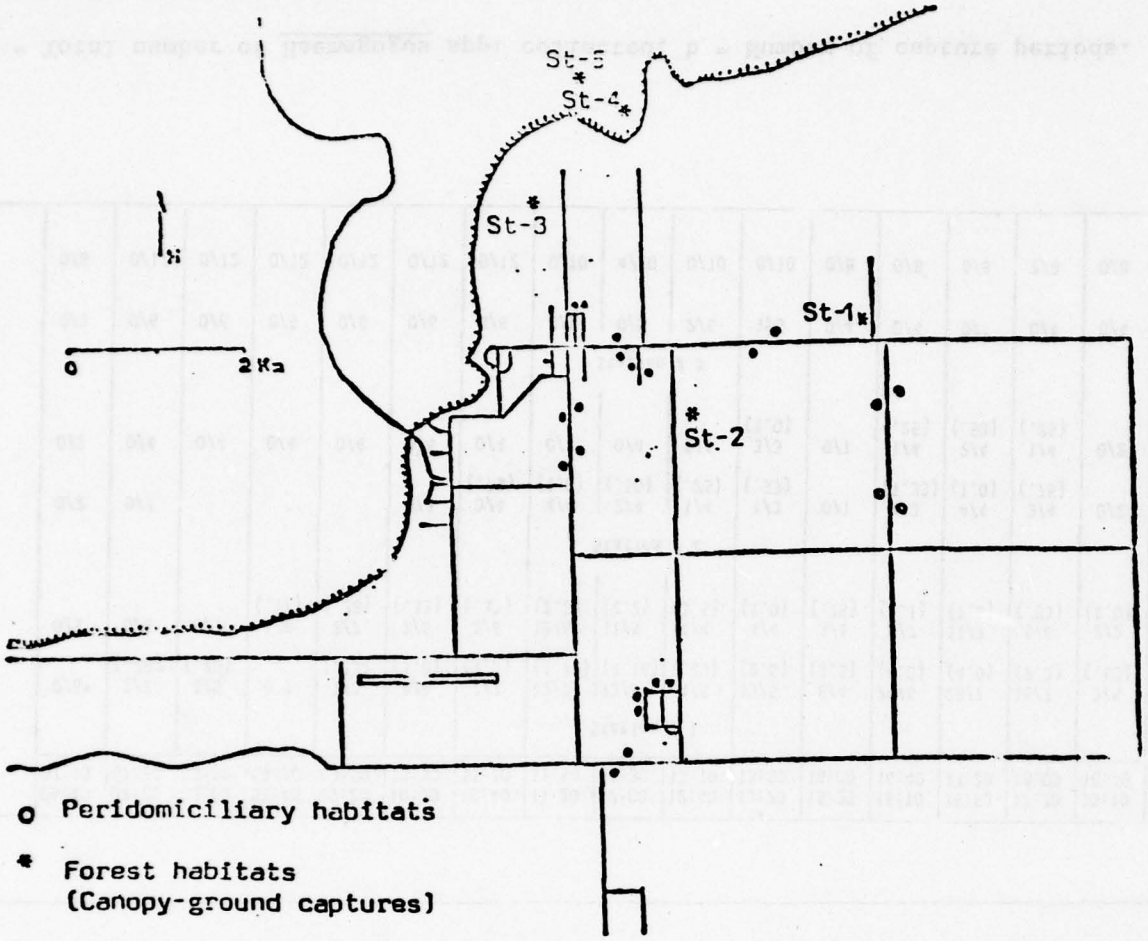


Fig. 9. Spatial distribution of sylvatic and peridomiciliary collecting sites in Belterra, Pará, Brazil, 1978.

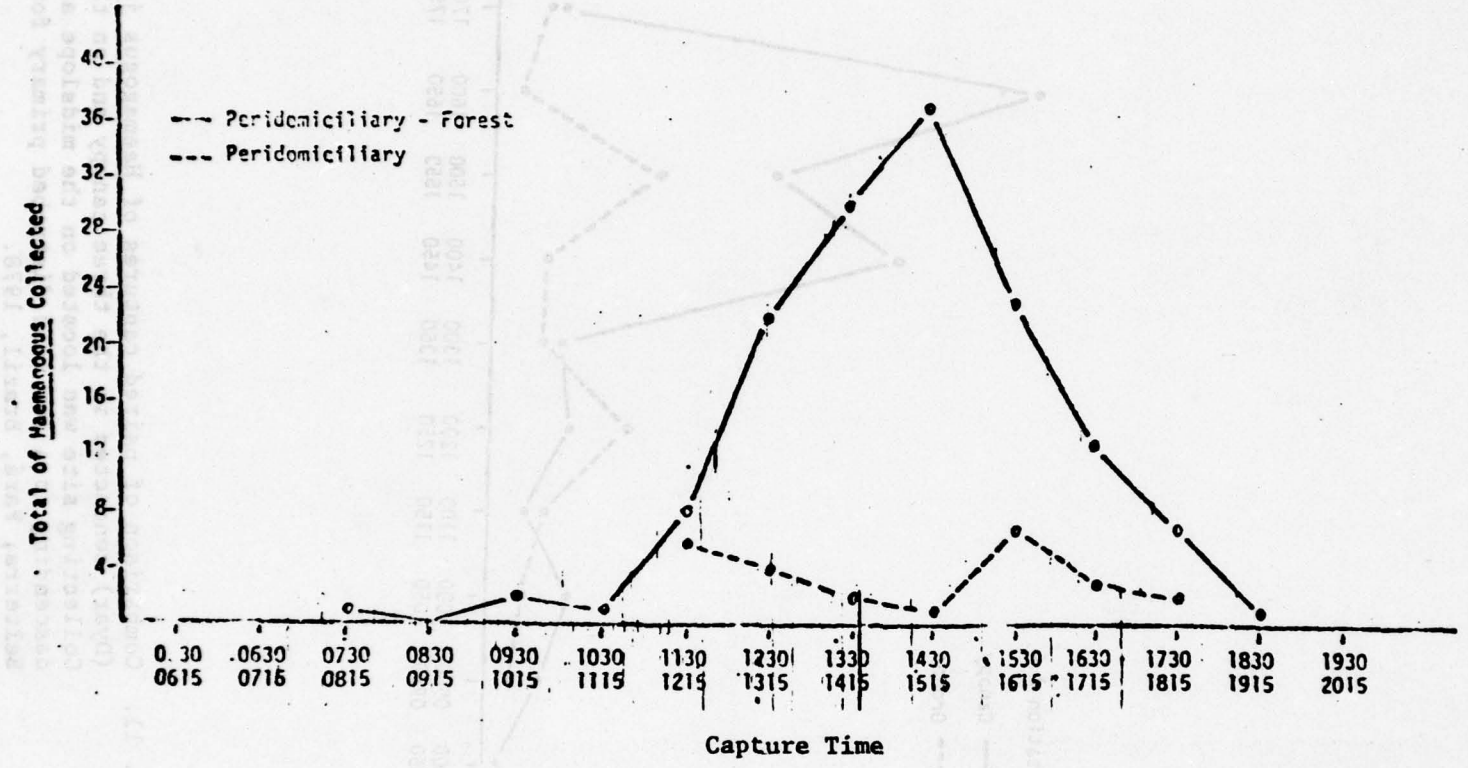


Fig. 10. Paired captures of Haemagogus janthinomys (Dyar) in two peridomestic habitats (near the house-yard, 10-20 meters and forested area near the house, 50-100 meters). Captures were conducted in a rubber plantation, Belterra, Pará, Brazil, 1978.

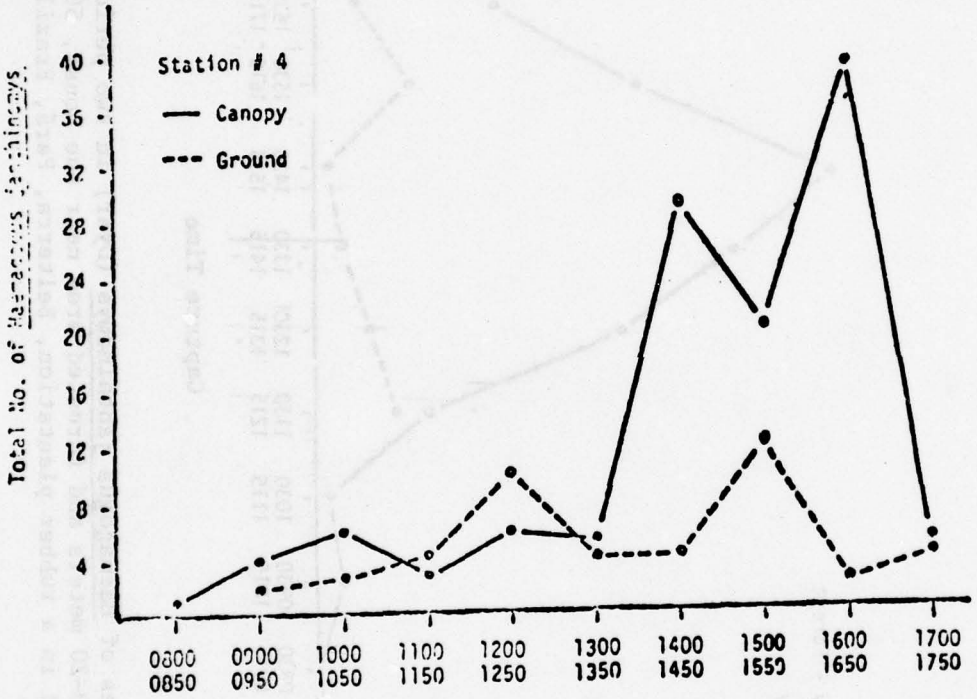


Fig. 11. Comparison of paired captures of Haemagogus janthinomys (Dyar) conducted in the three canopy and on the ground. Collecting site was located on the midslope a plateau descending to a low land disturbed primary forest, Belterra, Pará, Brazil, 1978.

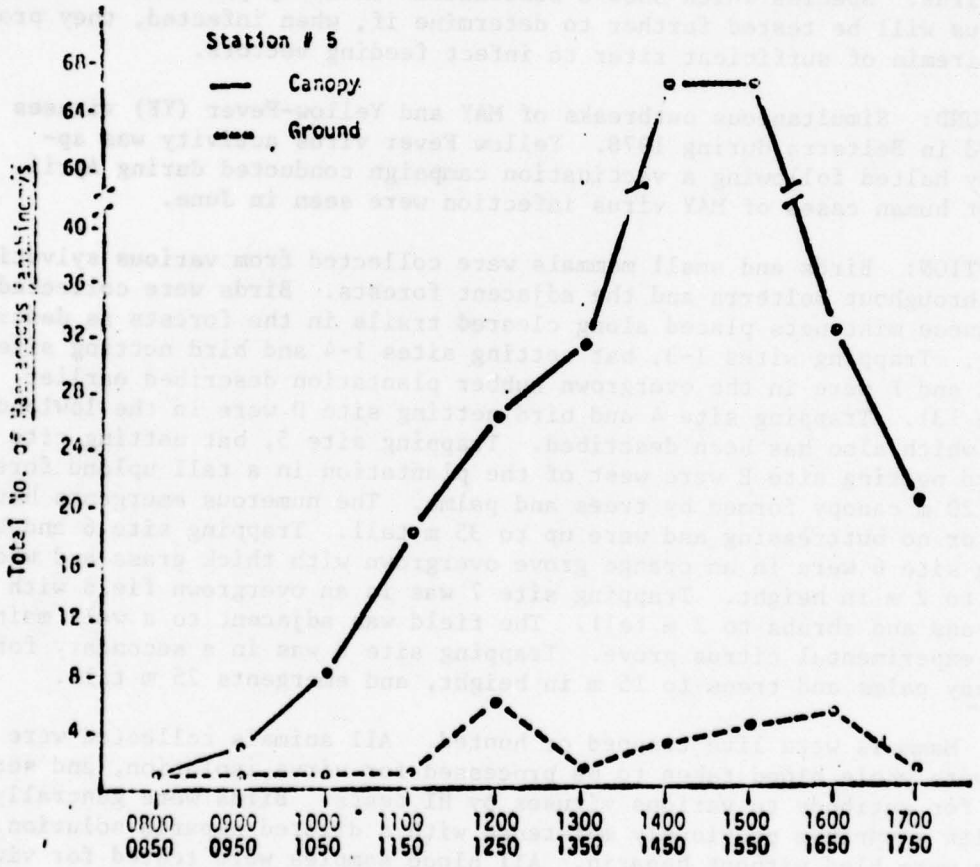


Fig. 12. Comparison of paired captures of Haemagogus janthinomys (Dyar) conducted in the tree canopy, and on the ground in a low land disturbed primary forest habitat, Belterra, Pará, Brazil, 1978.

II. ECOLOGY OF MAYARO VIRUS

B. Studies on the Epidemic Cycle. The Belterra Outbreak

6. Vertebrate hosts in the epidemic cycle

a. Results of a serological survey

OBJECTIVE: The objective of this section is to identify by a serological survey those vertebrate hosts which have been previously exposed to Mayaro (MAY) virus. Species which show a substantial antibody prevalence rate to MAY virus will be tested further to determine if, when infected, they produce a viremia of sufficient titer to infect feeding vectors.

BACKGROUND: Simultaneous outbreaks of MAY and Yellow-Fever (YF) viruses occurred in Belterra during 1978. Yellow Fever virus activity was apparently halted following a vaccination campaign conducted during April. The last human cases of MAY virus infection were seen in June.

DESCRIPTION: Birds and small mammals were collected from various sylvatic areas throughout Belterra and the adjacent forests. Birds were collected in Japanese mist nets placed along cleared trails in the forests as described earlier. Trapping sites 1-3, bat netting sites 1-4 and bird netting sites A, B, C and F were in the overgrown rubber plantation described earlier (Figure 13). Trapping site 4 and bird netting site D were in the lowland forest which also has been described. Trapping site 5, bat netting site 5 and bird netting site E were west of the plantation in a tall upland forest with a 20 m canopy formed by trees and palms. The numerous emergents have little or no buttressing and were up to 35 m tall. Trapping site 6 and bat netting site 6 were in an orange grove overgrown with thick grass and woody plants to 2 m in height. Trapping site 7 was in an overgrown field with tall grass and shrubs to 2 m tall. The field was adjacent to a well maintained experimental citrus grove. Trapping site 8 was in a secondary forest with many palms and trees to 15 m in height, and emergents 25 m tall.

Mammals were live trapped or hunted. All animals collected were bled, with whole blood taken to be processed for virus isolation, and sera tested for antibody to various viruses by HI tests. Birds were generally bled with a syringe previously moistened with a diluted heparin solution. Mammals were bled without heparin. All blood samples were tested for virus by intracerebral inoculation into suckling mice. Viruses isolated were identified by hemagglutination tests. Sera were tested for the presence of antibody by standard HI tests as described previously. Results of many, but not all, primate sera were confirmed by neutralization tests using Vero cells grown in microplates.

PROGRESS: A total of 754 birds were collected from Belterra and adjacent areas during the period 15 March through 21 July 1978. The mist nets collected 723 birds of 20 families during 53 mornings of netting (Table 23). Blood samples were also taken from three domestic ducks and 28 chickens belonging to the local inhabitants. The forest sites produced twice the number of birds per netting period as the plantation. The families of Formicariidae and Pipridae were the most commonly collected birds.

From 15 March through 25 August 1978, 661 mammals of 45 species were collected (Table 24). During 81 nights of trapping 253 marsupials, rodents and carnivores were captured (Table 25). The mist nets collected 251 bats during 20 nights of netting (Table 26). Monkeys, sloths, armadillos and other mammals were collected by hunting. The overgrown Orange grove and field (sites 6 and 17) produced more rodents per trap effort than the forest. The spiny rats (Proechimys) were the most common rodents in all sites except the orange grove and field where Zygodontomys was the most common.

Serological tests on specimens from these animals are still in progress and results currently available are incomplete. Antibody to MAY virus was found in sera from eight birds, and to Oropouche (ORO) virus in sera from 64 of 724 birds (Table 27). No viruses were isolated from 737 bird blood specimens tested. Tests on sera from 332 mammals found no antibody to Oropouche virus, and only the primates had antibody to MAY virus.

Among primates, two species were collected, a marmoset, Callithrix argentata, and a howler monkey, Alouatta belzebul. Among 69 marmosets collected, 13 (19%) had HI antibody to MAY virus. In addition, MAY virus was isolated from an adult female marmoset collected near Vila 129 on 19 April 1978. Only two howler monkeys were collected, and of these, only one had HI antibody to MAY virus. These results are summarized in Table 28.

As an interesting observation, of the 69 marmosets tested, only 4 (6%) had HI antibody to YF virus, and only 2 (3%) had HI antibody to ORO virus. Of the two howler monkeys tested, one had HI antibody to YF virus, but neither had antibody to ORO. Table 28 shows antibody prevalence rates for MAY, YF and ORO viruses by collection site in and around Belterra.

COMMENTS: These preliminary results indicate that primates may play a role in the amplification of MAY virus in nature. To further clarify this point, experimental infections are needed to demonstrate that primates circulate virus at a sufficient titer to infect feeding, uninfected vectors. This is attempted in a subsequent section of this report.

Of the two species of primates tested from Belterra, the marmosets are by far the more common. It would be reasonable to assume that they stand the greater chance of contributing to an amplification cycle, simply due to their numerical abundance. Certainly the isolation of MAY virus from a feral marmoset adds much support to such a hypothesis.

The low antibody prevalence rate to ORO virus among primates is especially noteworthy, since Belterra experienced an outbreak of ORO virus during 1975. Previous serological surveys have detected antibody to ORO virus among primates, and rarely among other animal species. As a result, an endemic cycle which involves primates as amplifying host has been proposed. However, the results presented here indicate that primates, at least within the areas sampled in Belterra, do not have a high antibody prevalence rate to ORO virus. Either marmosets are relatively short lived, and most exposed during the epidemic of 1975 have already died, or marmosets were not significantly involved in the amplification of ORO virus in the 1975 outbreak. The latter would then support the theory that man is the principal amplifying host in the epidemic cycle of ORO virus as suggested earlier. These results would also suggest that ORO virus is not currently endemic in Belterra.

TABLE 23. Birds collected in mist nets at the Belterra study area, Pará, Brazil, 15 March through 21 July 1978.

Family	Rubber Plantation Sites A,B,C,F	Lowland Forest Site D	Upland Forest Site E	Total
Accipitridae	1	-	-	1
Columbidae	8	3	4	15
Caprimulgidae	2	-	-	2
Trochilidae	1	-	-	1
Momotidae	-	-	1	1
Bucconidae	-	3	-	3
Dendrocolaptidae	8	8	61	77
Furnariidae	3	1	1	5
Formacariidae	136	36	105	277
Conopophadidae	-	-	5	5
Cotingidae	4	4	2	10
Pipridae	110	28	30	168
Tyrannidae	8	9	14	31
Troglodytidae	9	1	14	24
Turdidae	-	3	3	6
Virionidae	2	-	5	7
Icteridae	-	1	-	1
Parulidae	1	-	-	1
Thraupidae	-	1	3	4
Fringillidae	62	7	13	82
Not Identified	2	-	-	2
TOTAL	357	105	261	723
Mornings netted	35	1	13	53

TABLE 24 Total mammals collected in the Belterra study area, Pará, Brazil, 15 March through 25 August, 1978

Species	Total collected	Sera tested
Marsupialia		
Caluromys philander	3	3
Monodelphis brevicaudata	2	2
Marmosa parvidens	1	1
Metachirus nudicaudatus	4	3
Didelphis marsupialis	21	20
Chiroptera		
Micronycteris brachyotis	1	0
Tonatia carrikeri	2	0
T. silvicola	1	0
Phyllostomus latifolius	4	4
Glossophaga soricina	58	5
Linchonycteris obscura	1	1
Carollia brevicauda	81	48
Rhinophylla fischerae	9	2
R. pumilio	1	0
Sturnira lilium	4	3
Uroderma bilobatum	13	4
U. magnirostrum	1	0
Vampyrops helleri	2	0
Vampyressa bidens	1	0
V. pusilla	2	0
Artibeus cinereus	4	1
A. concolor	5	3
A. fuliginosis	19	16
A. jamaicensis	34	29
A. literatus	48	39
A. sp.	2	0
Desmodus rotundus	1	1
Molossus sp.	2	1
Primates		
Alouatta belzebul	2	1
Callithrix argentata	89	69
Edentata		
Bradypus variegatus	12	11
Cabassus unicinctus	1	0
Dasypus novemcinctus	2	1
Rodentia		
Sciurus gilvigularis	2	2
Oryzomys bicolor	4	1
O. concolor	5	0
O. macconnelli	1	0
Zygodontomys lasiurus	128	28
Oxymycteris sp.	25	2
Rodentia		
Proechimys longicaudata	41	24
P. guyannensis	10	5
Mesomys hispidus	7	2
Carnivora		
Nasua nasua	2	1
Mustela africana	1	1
Eira barbara	2	0
45 species	661	332

TABLE 25. Mammals collected by trapping in the Belterra study area, Pará, Brazil, 15 March through 25 August 1978.

Species	Numbers collected per trapping site								
	1	2	3	4	5	6	7	8	Total
Marsupialia									
Caluromys philander	-	1	-	-	-	2	-	-	3
Monodelphis brevicaudata	-	-	-	1	1	-	-	-	3
Marmosa parvidens	-	-	-	-	1	-	-	-	1
Metachirus nudicaudatus	-	1	-	1	2	-	-	-	4
Didelphis marsupialis	-	-	1	5	9	2	3	-	20
Rodentia									
Oryzomys bicolor	-	-	-	-	3	-	-	1	4
O. concolor	-	-	-	-	2	-	-	3	5
O. macconnelli	-	-	-	-	-	-	-	1	1
Zygodontomys lasiurus	-	-	-	-	-	32	95	1	128
Oxymycterus sp.	1	-	-	-	-	1	23	-	25
Proechimys longicaudatus	1	2	1	11	14	-	2	10	41
P. guyannensis	-	-	4	1	-	-	-	5	10
Mesomys hispidus	-	-	-	-	7	-	-	-	7
Carnivora									
Nasua nasua	-	-	-	-	-	-	-	1	1
Mustela africana	-	-	-	-	7	-	-	-	7
TOTAL	2	4	7	19	39	37	123	22	253
Nights trapped	5	4	7	7	21	9	28*	28*	81*
Trap night	335	232	329	1127	1964	774	582	2176	7.519
Mammals"1000 trap nights	1.0	17.2	21.3	16.8	19.8	47.8	211.3	10.1	33.6

* Trapping areas 7 and 8 were trapped during the same nights.

TABLE 26. Bats collected by mist netting in the Belterra study area, Pará, Brazil, 15 March through 25 August 1978.

Species	Number collected per mist net site							
	1	2	3	4	5	6	G	Total
Micronycteris brachyotis	-	-	-	-	1	-	-	1
Tonatia carrikeri	-	-	1	-	1	-	-	2
T. silvicola	-	-	-	-	1	-	-	1
Phyllostomus latifolius	-	1	2	-	1	-	-	4
Glossophaga soricina	15	23	9	3	-	-	2	52
Lichonycteris obscura	-	-	-	-	1	-	-	1
Carollia brevicauda	6	13	3	14	11	6	3	56
Rhinophylla fischerae	-	1	-	2	-	1	-	4
R. pumilio	-	-	-	-	-	-	1	1
Sturnira lilium	1	-	1	1	-	-	-	3
Vampyrodes bilobatum	3	1	6	-	1	-	-	11
V. magnirostrum	1	-	-	-	-	-	-	1
Vampyrops helleri	1	-	-	-	1	-	-	2
Vampyressa bidens	-	-	-	-	1	-	-	1
Artibeus cinereus	1	-	-	1	-	-	-	2
A. concolor	-	-	-	-	5	-	-	5
A. fuliginosus	-	-	-	3	14	-	-	17
A. jamaicensis	5	8	2	-	19	-	-	34
A. literatus	-	2	-	-	46	-	-	48
A. sp.	-	-	-	-	2	-	-	2
Desmodus rotundus	-	-	-	-	1	-	-	1
Molossus sp.	-	-	-	-	2	-	-	2
TOTAL	33	49	24	24	108	7	6	251
Nights netted	2	3	3	2	8	1	1	20
Net hours	12	18	21	5	65.5	9	15	145.5
Bats/net hour	2.75	2.72	1.14	4.80	1.65	0.78	0.40	1.72

TABLE 27. Distribution of hemagglutination inhibiting antibody to Mayaro and Oropouche viruses in birds collected in the Belterra study area, Pará, Brazil, 15 March through 21 July 1978.

Family	Mayaro Pos/tested	Oropouche Pos/collected
Accipitridae	0/1	0/1
Columbidae	0/15	0/15
Caprimulgidae	0/2	0/2
Trochilidae	0/1	0/1
Momotidae	0/1	0/1
Bucconidae	0/3	0/3
Dendrocolaptidae	0/75	5/75
Furnariidae	0/5	1/5
Formicariidae	5/265	52/265
Conopophadidae	0/5	0/5
Cotingidae	0/10	0/10
Pipridae	1/163	5/163
Tyrannidae	1/29	1/29
Troglodytidae	0/23	0/23
Turdidae	0/6	0/6
Vironidae	0/7	0/7
Parulidae	0/1	0/1
Thraupidae	0/4	0/4
Fringillidae	1/77	0/77
Chickens	0/28	0/28
Ducks	0/3	0/3
TOTAL	8/724	64/724

TABLE 28. Summary of antibody prevalence rates to Mayaro, Yellow Fever and Oropouche viruses among primates collected in Belterra, Pará, Brazil, April-July, 1978.

Area	Mayaro	Yellow Fever	Oropouche
Callithrix argentata			
Road 5	1/2 (50%)	0/2	2/2 (100%)
Road 6	1/5 (20%)	0/5	0/5
Vila Piquiá	1/12 (8%)	1/12 (8%)	0/12
Vila Viveiro	1/4 (25%)	1/4 (25%)	0/4
Vila 129	9*/39 (23%)	2/39 (5%)	0/39
Sítio Chagas	0/4	0/4	0/4
Southwest Forests	0/3	0/3	0/3
TOTAL	13/69 (19%)	4/69 (6%)	2/69 (3%)
Alouatta belzebul			
Vila Piquiá	0/1	0/1	0/1
Vila 129	1/1 (100%)	1/1 (100%)	0/1
TOTAL	1/2 (50%)	1/2 (50%)	0/2

* Mayaro virus isolated from one marmoset collected from Vila 129 on 19 April 1978.

TABLE 29. Summary of experimental infections of marmosets collected from Belterra, Pará, Brazil and inoculated with 10^3-10^4 $TCID_{50}$/0.1 ml. Mayaro virus.

Marmoset	Max. viremia on days post-inoculation					
	2	3	4	5	6	7
Callithrix argentata - 1	10^4*	10^2	0	0	0	0
Callithrix argentata - 2	$10^{2.5}$	10^1	0	0	0	0
Callithrix argentata - 3	NT+	0	0	0	0	0
Callithrix argentata - 4	NT	0	dead			
C. humeralifer	10^4	10^2	0	dead		

* $TCID_{50}$/0.1 ml in Vero cells

\+ NT = not tested

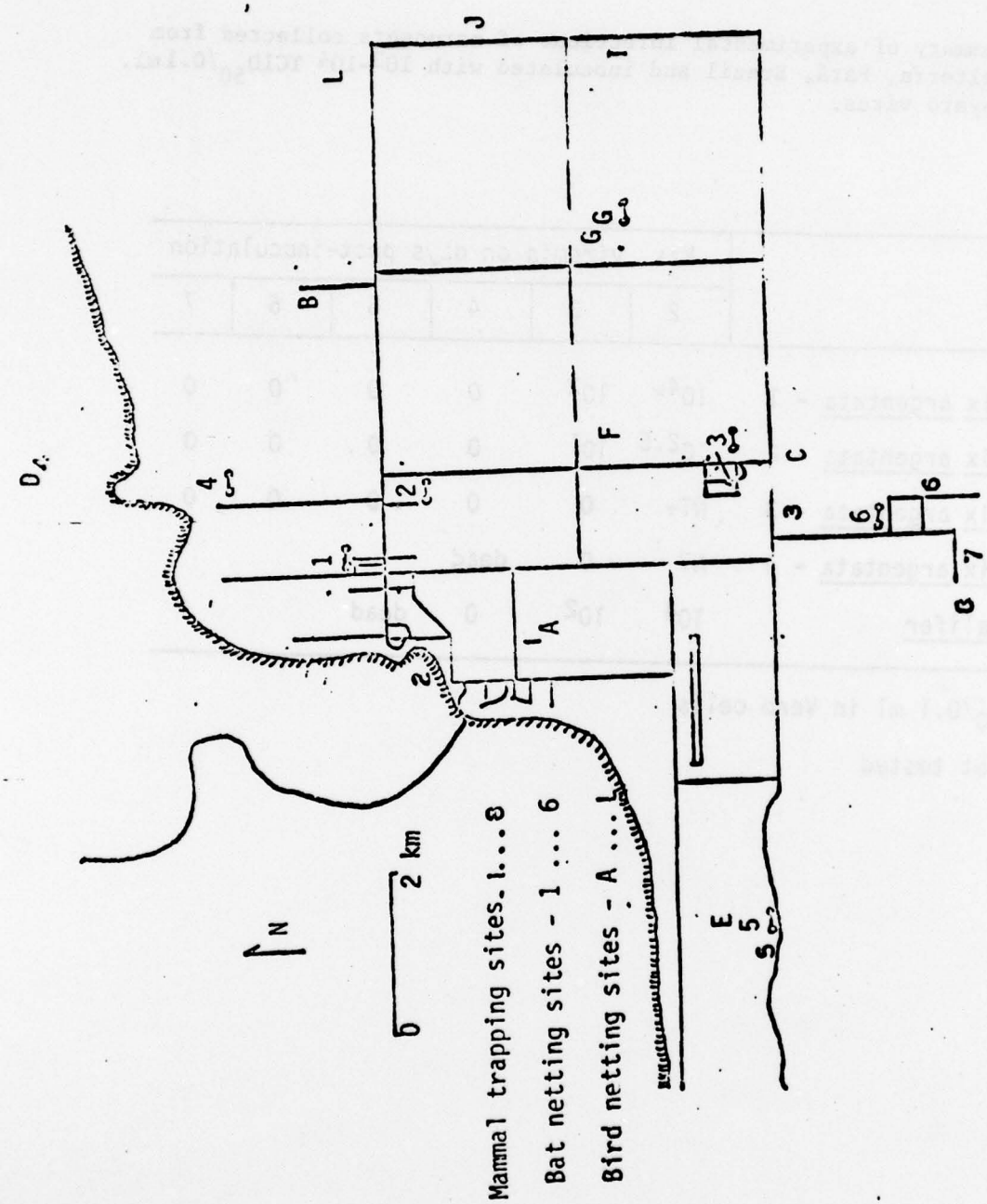


FIG. 13 Map of Belterra, Pará, Brazil, showing the bird and mammal collecting sites.

II. ECOLOGY OF MAYARO VIRUS

B. Studies on the Epidemic Cycle - the Belterra Outbreak

6. Vertebrate hosts in the epidemic cycle

b. Experimental infections of marmosets

OBJECTIVES: The objective of this section is to determine if marmosets found in Belterra, Pará, Brazil, will produce a significant viremia when infected with Mayaro (MAY) virus.

BACKGROUND: Marmosets, especially Callithrix argentata, are the most common non-human primate found in Belterra. Serological studies on feral marmosets collected in Belterra during the MAY virus outbreak have revealed a high (19%) antibody prevalence rate to MAY virus. In addition, MAY virus was isolated from a viremic, feral C. argentata collected during the outbreak.

All MAY virus isolations made from vectors have been from a single species, Haemagogus janthinomys. Mosquitoes of the genus Haemagogus are known to feed readily on primates, thus an amplification cycle involving Haemagogus mosquitos and marmosets seems reasonable. For such a cycle to exist, infected marmosets must produce a viremia of sufficient titer to infect feeding uninfected Haemagogus. The objective of this study is to determine the titer of virus circulated by infected marmosets, and thus determine the likelihood of their involvement in an amplification cycle.

DESCRIPTION: Marmosets were collected or purchased in Belterra and transported to Belém alive. Once in the laboratory they were bled to detect pre-existing HI antibodies to MAY virus. Those which lacked antibody to MAY virus were inoculated subcutaneously with 0.2 ml of MAY virus which titered between 10^3 and 10^4 $TCID_{50}$/0.1 ml in Vero cells grown in tubes. Each marmoset was then bled daily beginning on day 2 or day 3 post-inoculation (p.i.) through day 7 p.i.

Whole blood was drawn and immediately diluted 1:10 in Vero cell growth medium (Medium 199 with 5% fetal bovines serum and antibiotics) and frozen at -70°C pending assay.

Viremia was detected by further diluting each blood sample to 10^{-4}, then inoculating 0.1 ml of diluted blood into duplicate tubes of drained Vero cells. Tubes were incubated for 1 hr. at 37°C, then rinsed with 1,0 ml of maintenance medium (medium 199 with 1% fetal bovine serum and antibiotics), and 1.0 ml of maintenance medium added to each tube. Tubes were observed daily for at least 7 days for evidence of cytopathic effects caused by virus. Neutralization tests of virus isolated from viremic marmosets are in progress.

PROGRESS: A total of 5 marmosets were experimentally infected, 4 C. argentata and 1 C. humeralifer. Of these, 3 produced a detectable viremia. Of the two marmosets which did not produce a detectable viremia, neither were bled on day 2, but rather daily bleeding began on day 3. One of these died on day 3 as a result of the trauma of bleeding.

Two of the three marmosets viremic on day 2 had titers equal to or in excess of $10^4 TCTD_{50}$/0.I ml in Vero cells. The remaining marmoset produced a viremia which titered about $10^{2.5} TCID_{50}$/0.1 ml in Vero cells. Titers dropped on day 3 to $10^2 TGID_{50}$/0.1 ml in 2 marmosets, and $10^1 TCID_{50}$/0.1 ml in the other. Viremia was not detected on days 4 through 7 p.i. Table 29 presents a summary of the viremia responses of these 5 marmosets experimentally infected.

COMMENT: It appears from these experimental infections that marmosets are capable of producing a high titered viremia following infection with MAY virus. It seems likely that the two marmosets which failed to produce a viremia may have been viremic on day 1 or 2, but went undetected since they were not bled.

The rapid onset of viremia may have been the results of the high titered inoculum administered. The intensity and duration of viremia following infection through the bite of an infected vector are questions which remain to be answered. Likewise, the titer of viremia needed to infect a feeding vector must be established. Nevertheless, the preliminary results presented here strongly suggest that marmosets may play an active role in the amplification of MAY virus in nature.

LITERATURE CITED

References

1. Ridgely, R.S.: A guide to the birds of Panama. Princeton University Press, Princeton, N.J., 394 p., 1976.

2. Calisher, C. H., Gutierrez V., E. Maness, K. S. C. and Lord, R. D.: Isolation of Mayaro virus from a migrating bird captured in Louisiana in 1967. Bull. Pan Am. Hlth. Org. 8: 243-248, 1974.

II. ECOLOGY OF MAYARO VIRUS

C. Studies on the Endemic Cycle

1. Vertebrate host serology from

Cachoeira Porteira

OBJECTIVE: The objective of this section is to identify those feral vertebrate hosts which have been exposed to Mayaro (MAY) virus, as an initial step in identifying the endemic maintenance cycle of this virus.

BACKGROUND: The environs and program of study at Cachoeira Porteira have been presented in detail elsewhere. Briefly, investigations in this area attempt to study the ecology of arboviruses in a tropical forest essentially devoid of human inhabitants.

DESCRIPTION: Collection techniques and processing of serological specimens were described in detail earlier in vertebrate host serology for Oropouche virus. Data included here are from the same sera as described earlier, but for convenience, only those results pertaining to MAY virus have been extracted and presented.

PROGRESS: A total of 508 avian sera were tested for HI antibody to MAY virus. Of these, only two individuals were found positive. Both were members of the family Bucconidae, the puffbirds, and both were captured on the Nov-Dec. 1977 trip. Of the 3 puffbirds captured on that trip, 2 were positive for MAY antibody. All 3 puffbirds captured were Monasa morphoeus, the white-fronted nunbird. The two positive birds were a male and a female, both captured together. Both had HI antibody titers of 1:40 to MAY virus, and neither had IH antibody to anyother Group A arbovirus, so it seems apparent that these results do not represent cross reactions. Neither positive sera has yet been confirmed by neutralization tests. Table 30 presents a summary of birds collected from Cachoeira Porteira and tested by HI for antibody to MAY virus.

Similar to the results presented for Oropouche virus, the only mammals which demonstrated a high prevalence rate of antibody to MAY virus were the primates. Of 37 primate sera tested, 22 (59%) had HI antibody to MAY virus. Of the six species of primates collected, 5 had at least one individual positive. The single species which lacked at least one positive individual, Pithecia pithecia, had only one serum tested. All five sera tested of Alouatta seniculus, and all three Ateles paniscus sera tested were positive. Of 15 Cebeus apella sera tested, 9 (60%) were positive, and 2 of 3 (67%) Ateles belzebul were positive, as were 3 of 10 (30%) Chiropotes satanas sera. Positive sera were found among specimens collected on each of the 3 trips made during 1977. All positive and negative sera have been confirmed by neutralization tests.

Of the other groups of mammals tested, only one Dasyprocta aguti rodent sera of a total of 7 (14%) tested, was positive for HI antibody to MAY virus. Of the remaining 126 rodent sera of other species tested, all lacked HI antibody to MAY virus, as did all 74 marsupial sera, 6 carnivore sera, 15 ungulate sera and 8 other sera. A summary of mammalian sera from Cachoeira Porteira tested by HI for antibody to MAY virus is presented in Table 31.

COMMENTS: White-fronted nunbirds are found from southeastern Honduras to northern Bolivia and Southeastern Brazil. They are most often seen in small groups, and are usually noisy. The nest in Costa Rica is reported to be in a burrow in level ground. Puffbirds as a group are exclusively neotropical and found chiefly in forest and woodlands. Nunbirds in Panama are uncommon to locally fairly common in humid forest and forest borders in lowlands and foothills[1]. The finding of 2 of 3 nunbirds collected in Nov.-Dec. positive for HI antibody to MAY virus deserves further investigation.

An association of MAY virus with birds has been reported previously by Calisher et al.2, who isolated MAY virus from an Orchard Oriole, Icterus spurius, migrating into Louisiana in 1967. Serological surveys conducted in the past, however, have very rarely found HI or neutralizing antibody to MAY virus among birds, and the contribution of birds to the maintenance of MAY virus has yet to be resolved.

The results reported here from primates indicate that they are frequently exposed to feeding by the endemic vector of MAY virus. It is possible that the epidemic vector seen in Belterra, Haemagogus janthinomys, is also the principal endemic vector as well, and that primates serve as the main vertebrate host for virus amplification. For such a cycle to be continued indefinitely, some mechanism must be available to the virus for long term maintenance, since primates are relatively few in number and have a low reproduction potential. Potential mechanisms for such maintenance include transovarial transmission by the vector, extreme longevity of the vector, and continual movement of the virus from area to area and population to population. Additional studies are required to determine which of these alternatives, if any, most closely represents the endemic maintenance of MAY virus.

TABLE 30. Distribution of hemagglutination inhibiting antibody to Mayaro virus among birds captured at Cachoeira Porteira, km 71, municipality of Oriximiná, Pará, Brazil, 1977.

Family	Collection period			Total
	March-April	July-August	Nov.-Dec.	
Alcediridae	-	0/2*	-	0/2
Bucconidae	-	0/1	2/3	2/4
Coeribidae	-	-	0/1	0/1
Columbidae	-	0/1	0/4	0/5
Conopophagidae	0/2	0/3	-	0/5
Cotingidae	-	0/1	0/2	0/3
Cracidae	0/1	-	-	0/1
Dendrocolaptidae	0/16	0/21	0/16	0/53
Falconidae	-	0/1	0/1	0/2
Formicariidae	0/78	0/71	0/45	0/194
Fringillidae	0/1	0/6	0/2	0/9
Furnariidae	0/9	0/17	0/12	0/38
Galbulidae	0/1	0/3	-	0/4
Momotidae	0/7	0/3	0/2	0/5
Parulidae	0/3	-	0/2	0/5
Picidae	0/2	0/1	-	0/3
Pipridae	0/35	0/20	0/5	0/60
Ramphastidae	0/1	-	-	0/1
Sylvilidae	0/3	-	-	0/3
Thraupidae	0/6	-	-	0/6
Tyrannidae	0/12	0/9	0/13	0/34
Trochilidae	0/1	-	-	0/1
Troglotididae	0/3	0/20	0/7	0/14
Turdidae	0/13	0/20	0/7	0/40
Vireonidae	-	0/1	-	0/1
	0/201	0/186	2/121	2/501

*Number pos/number tested

TABLE 31. Distribution of hemagglutination inhibiting antibody to Mayaro virus among mammals captured at Cachoeira Porteira, km 71, municipality of Oriximiná, Pará, Brazil, 1977.

Species	March-April	July-August	Nov-Dec.	Total
Marsupials				
Didelphis marsupialis	0/2*	0/6	0/6	0/14
Marmosa cinerea	-	-	0/1	0/1
M. murina	0/4	0/3	0/2	0/9
Monodelphis brevicaudata	0/4	0/3	0/10	0/17
Philander opossum	0/9	0/11	0/13	0/33
Total	0/19	0/23	0/32	0/74
Rodents				
Agouti paca	-	0/1	-	0/1
Dasyprocta aguti	-	1/5	0/2	1/7
Hydrochaeris hydrochaeris	-	-	0/1	0/1
Myoprocta acouchy	0/2	-	0/1	0/3
Neacomys spinosus	-	0/3	0/1	0/4
Nectomys squamipes	0/1	0/7	0/1	0/9
Oryzomys bicolor	-	0/1	-	0/1
O. capito	-	0/15	0/7	0/22
Proechimys guyannensis	0/34	0/28	0/21	0/83
Sciurus gilvigulares	0/1	0/1	-	0/2
Total	0/38	1/61	0/34	1/133
Primates				
Alouatta seniculus	2/2	-	3/3	5/5
Ateles belzebuth	-	2/3	-	2/3
A. paniscus	1/1	-	2/2	3/3
Cebus apella	-	6/11	3/4	9/15
Chiropotes satanas	-	1/6	2/4	3/10
Pithecia pithecia	-	-	0/1	0/1
Total	3/3	9/20	10/14	22/37
Carnivores				
Eira barbara	0/1	-	-	0/1
Felis concolor	-	0/1	-	0/1
F. pardalis	0/1	-	-	0/1
Nasua nasua	-	0/1	0/2	0/3
	0/2	0/2	0/2	0/6

TABLE 31. Distribution of hemagglutination inhibiting antibody to Mayaro virus among mammals captured at Cachoeira Porteira, km 71, municipality of Oriximiná, Pará, Brazil, 1977. (Cont.)

Species	March-April	July-August	Nov-Dec	Total
Ungulates				
Mazama americana	0/1*	0/1	0/3	0/5
Tapirus terrestris	-	-	0/1	0/1
Tayassu pecari	-	0/5	0/3	0/8
Dycotyles tacaju	-	0/1	-	0/1
Total	0/1	0/7	0/7	0/15
Other mammals				
Dasypus novemcinctus	0/1	-	-	0/1
Bats	-	0/5	0/1	0/6
Tamandua tetradactyla	-	-	0/1	0/1
Total	0/1	0/5	0/2	0/8

*Number pos/number tested

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