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AN OUTBREAK OF MAYARO VIRUS DISEASE IN BELTERRA, BRAZIL

III. Entomological and Ecological Studies*

ALFRED L. HOCH,† NORMAN E. PETERSON,‡ JAMES W. LEDUC,§ AND FRANCISCO P. PINHEIRO

United States Army Medical Research Unit, Belém, Brazil, and Instituto Evandro Chagas, Fundação Serviços de Saúde Pública, Ministerio de Saúde, Belém, Brazil

Abstract. Results of entomological and vertebrate host investigations made during dual outbreaks of Mayaro (MAY) and yellow fever (YF) viruses in Belterra, Pará, Brazil in 1978 are reported. Over 9,000 insects representing 26 species were assayed in 396 pools for the presence of arboviruses. Pools of *Haemagogus janthinomys* Dyar yielded the only isolates of either MAY or YF virus. The minimum field infection rate for nine isolates of MAY virus from *Hg. janthinomys* was 1:82, and for two isolates of YF virus was 1:368. Analysis of collection data showed *Hg. janthinomys* to be attracted to man as a blood source and present in all habitats sampled, although most abundant in the forest canopy. Twelve hundred bird sera and 584 mammal sera were tested by hemagglutination-inhibition (HI) tests for antibody to MAY virus. Highest MAY antibody prevalence rates were found among marmosets (*Callithrix argentata*, 32 positive of 119 tested, 27%). Mayaro virus was also isolated from the blood of a sylvan marmoset captured at the peak of the MAY virus outbreak. Experimental infection of marmosets with MAY virus confirmed that a substantial viremia follows infection with this virus. Marmosets were also found with HI antibody to YF virus (5/119, 4%). The results presented indicate that *Hg. janthinomys* was the principal vector of both MAY and YF viruses and that marmosets were the main amplifying hosts for MAY virus, and perhaps for YF virus as well.

Simultaneous outbreaks of Mayaro (MAY) and yellow fever (YF) viruses occurred in Belterra, Pará, Brazil between January and June, 1978. In the preceding reports we have presented accounts of the clinical disease which results from infection with MAY virus and an analysis of the distribution of cases of MAY and YF viruses in the human population resident in Belterra at the time of these outbreaks.[1,2] In this final report we detail our attempts to identify the insect vector responsible for dissemination of these viruses, and an analysis of the role of sylvatic vertebrates in amplification of both viruses during these outbreaks.

We were especially fortunate to have the opportunity to investigate the transmission of MAY virus in detail. While several reports have described isolated human cases of virus recoveries in the past,[3–6] the results reported here represent the first in-depth analysis of the epidemiology of MAY virus. Since the original description of MAY virus by Anderson et al.[3] in Trinidad in 1957, MAY virus has been reported from several different Central and South American countries, including Colombia, Bolivia, Brazil, Panama and Surinam.[7–11] Several genera of mosquitoes, including *Culex*, *Haemagogus*, *Mansonia*, *Aedes*, *Psorophora*, and *Sabethes*,[7,12] have been the source of MAY virus isolations, with the most frequent isolations being associated with *Haemagogus* species. In 1961, during an apparent syl-

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Address reprint requests to: Reprints Section, Division of Academic Affairs, Walter Reed Army Institute of Research, Washington, D.C. 20012.

† Present address: U.S. Army, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C. 20012.

‡ Present address: Universidade de Brasilia, Faculdade de Ciencias de Saúde, 70.910-Brasilia-D. Federal—Brazil.

§ Present address: Gorgas Memorial Laboratory, APO Miami 34002.

vatic epizootic of MAY virus, MAY virus was recovered 24 times from *Haemagogus* species during a 4-month period at km 87 and 94 of the Belém-Brazilia Highway.[13] However, no clear association has been established to identify a given species as being an important vector to either man or sylvatic vertebrates. Likewise, no vertebrate group has been identified as an important amplifying host of MAY virus, although certain species of primates have high antibody prevalence rates to the virus.[14]

MATERIALS AND METHODS

Details of the environs of Belterra were presented in the preceding report.[2] Briefly, Belterra is a small rural community of approximately 4,000 population located in the northern Brazilian state of Pará near the confluence of the Tapajos and Amazon rivers. The predominant characteristic of the area is the rubber plantation which employs and houses most of the populace. The village and plantation lie on a plateau approximately 5 km east of the Tapajos River and are surrounded by a dense secondary growth forest. Within the boundaries of the rubber plantation a secondary forest is becoming established, so that a continuous scrub undergrowth now exists throughout the area. A slightly disturbed primary forest occupies the lowland area between the plateau and river bank.

Entomological survey methods

Human bait or CDC miniature light trap collections of potential insect vectors were made in and near houses in Belterra, throughout the plantation forest and in the lowland forest between the plateau and the river. At three sites within the plantation forest and at two sites in the lowland forest, simultaneous collections were made at both ground and canopy levels by separate two-man collection teams. The height of canopy collections in the plantation and in the lowland forest were 10–12 and 18–20 m, respectively. Collections were conducted in both areas from 0800 to 1800 hours. The capture periods for the taller lowland forest were for 50 min with a 10-min rest period, and the capture times for the plantation forest were 30 min with a 10-min rest period. Peridomestic collections were made by four two-man collection teams. One team was located within 10–20 m of houses (peridomiciliary), while another was located 50–100 m away in the adjacent forest (peridomiciliary-forest). Collections were conducted from 0530 to 2015 hours with 45-min captures. Night-time human bait collections were made from 1800–2400 or 2100–0300 hours in and adjacent to several houses where cases of MAY virus had been reported. All collection teams were rotated frequently between sites to avoid collection biases. Light traps were operated from 1800–0630 hours near selected houses and in the plantation forest. Figure 1 depicts the locations of insect collection points.

Processing of specimens

Captured insects were gathered from all field sites several times a day and transported to a field laboratory, where they were lightly anesthetized with chloroform and separated into general taxonomic groups, then stored in liquid nitrogen. Specimens were transported to the Belém laboratories biweekly, where they were identified to species and pooled in groups of 50 or fewer individuals and assayed for virus by injection into suckling mice following standardized procedures previously described.[15] Insects collected from different areas were mixed only when the collection areas were in close proximity, or when only a few specimens of that species were captured. Blood-engorged insects were identified; however, these were not pooled for virus isolation attempts. Isolated viruses were identified by hemagglutination-inhibition (HI) tests using reference reagents.[16]

Ecological survey methods

Birds and small mammals were collected from various areas throughout Belterra and surrounding forests as shown in Figure 1. Birds were collected in mist nets placed along cleared trails in the forests, and mammals were live-trapped or hunted. Captured animals were bled, with the whole blood being processed for virus isolation attempts in suckling mice and sera tested by HI for antibody to a battery of arboviral antigens which included MAY and YF viruses. Birds were generally bled with a syringe previously moistened with a dilute heparin solution while mammals were bled without heparin. Neutralization tests with Vero cells grown in microplates were used to confirm all positive HI results as well as a sample of the negative results.[17]

Experimental infection of marmosets

Marmosets, especially *Callithrix argentata*, are the most abundant non-human primates found in Belterra, and those collected during the outbreak were found to have a high antibody prevalence rate to MAY virus. Consequently, marmosets were examined further to determine whether they could produce a MAY viremia of sufficient titer to infect blood-feeding mosquitoes. For this study several marmosets were collected or purchased in Belterra and transported alive to Belém, where they were bled to detect pre-existing HI antibody to MAY virus. Those which lacked antibody were inoculated subcutaneously with 0.2 ml of diluted MAY virus which titered between 10^5 and 10^6 $TCID_{50}$/0.1 ml in Vero cells. Each marmoset was then bled daily beginning on day 2 or 3 postinoculation (pi) through day 7 pi. Whole blood was drawn and immediately diluted 1:10 in Medium 199 which contained 5% fetal bovine serum and antibiotics and frozen at −70°C pending assay. Viremia was detected by inoculating 0.1 ml of diluted blood into duplicate tubes of drained Vero cells. Inoculated tubes were incubated for 1 hour at 37°C, then rinsed with 1.0 ml of Medium 199 which contained 1% fetal bovine serum and antibiotics, after which 1.0 ml of the same medium was added to each tube. Tubes were observed daily for at least 7 days for evidence of cytopathic effect.

Follow-up surveys for Hg. janthinomys

Two additional insect collecting trips were made to Belterra following the completion of the initial MAY virus epidemic investigations. Collection techniques were the same as those used during the epidemic investigations and insects were collected at the same forest collection sites, both ground and canopy. No additional collections were made, however, at the peridomiciliary sites used previously.

RESULTS

Virus isolations from arthropods

Approximately 12,000 man-biting insects were captured between 5 April and 5 May 1978, the period which coincided with the peak of MAY virus activity in humans in Belterra, and over 9,000 were processed for virus isolation. The remainder were excluded due to the presence of undigested blood. Table 1 presents a summary of the species collected and tested for virus isolation, and the number of recovered virus strains. Virus isolations were obtained from three species of mosquitoes: *Haemagogus janthinomys*, *Limatus flavisetosus* and *Wyeomyia aporonoma*. Pools of *Hg. janthinomys* yielded nine isolates of MAY virus and two isolates of YF virus, while two Wyeomyia complex viruses were isolated, one each from *Li. flavisetosus* and *Wy. aporonoma*. The minimum field infection rate of *Hg. janthinomys* was 1:82 for MAY virus and 1:368 for YF virus.

Two MAY virus isolates were obtained from *Hg. janthinomys* collected in the peridomiciliary environs of Belterra; one near the junction of Roads 1 and 6, and another at Villa 129 near the junction of Roads 6 and 7 (Fig. 1). An additional two isolates were made from *Hg. janthinomys* collected from the forest canopy at station 1 within the plantation. Collections in the lowland forest areas (stations 4 and 5) yielded five MAY virus isolates, all of which were from mosquitoes captured in the forest canopy. Of the nine MAY virus isolates, seven were from mosquitoes captured in the canopy and two were from ground level collections. Table 2 presents a summary of the virus isolations from *Hg. janthinomys* collections.

Two strains of YF virus were also recovered from the *Hg. janthinomys* tested. One isolate was obtained from canopy collections made in the rubber plantation forest, while the second was recovered from the lowland forest canopy.

Activity patterns of Hg. janthinomys

Figure 2 presents a comparison by collection period of the numbers of *Hg. janthinomys* captured immediately adjacent to houses with those captured 50–100 m away in the plantation forest. The forested areas yielded markedly greater numbers of *Hg. janthinomys* than did the environs nearer the houses, although some specimens were collected from both areas. Temporal activity of *Hg. janthinomys* near houses was low and relatively stable throughout the sampling periods, while activity in the nearby forested areas showed a marked increase from about midday to 1600 hours.

Figure 3 depicts results of paired ground and canopy captures of *Hg. janthinomys* by capture time at two sites in the lowland forest. Canopy

TABLE 1

Summary of insects collected and assayed for virus during field investigations in Belterra, Pará, Brazil, 1978 and 1979

Species	Total tested (pools*)	Virus isolations
Ceratopogonidae (1978)		
Culicoides debilipalpis	758 (24)	
C. insinuatus	123 (4)	
C. paraensis	2,303 (50)	
C. sp.	425 (11)	
Forcipomyia	88 (6)	
Culicidae (1978)		
Aedes fulvithorax	6 (1)	
Ae. septemstriatus	5 (1)	
Culex (Cul.) coronator	378 (12)	
Cx. (Cul.) fatigans	20 ( )	
Cx. (Mel.) sp.	26 (4)	
Haemagogus janthinomys	736 (62)	9 Mayaro: 2 yellow fever
Hg. leucocelaenus	6 (1)	
Limatus flavisetosus	720 (32)	1 Wyeomyia complex
Li. durhamii	1,472 (61)	
Onthopodomyia fascipes	69 (3)	
Psorophora cingulata	77 (6)	
Sabethes belisarioi	174 (9)	
Sa. chloropterus	12 (2)	
Sa. cyaneus	8 (1)	
Sa. glaucodaemon	157 (12)	
Sa. quasicyaneus	102 (14)	
Sa. shannoni	12 (3)	
Trichoprosopon digitatum	244 (14)	
Wyeomyia aporonoma	472 (23)	1 Wyeomyia complex
Wy. sp.	158 (11)	
Psychodidae (1978)		
Lutzomyia sp.	574 (18)	
Culicidae (1979)		
Hg. janthinomys	1,542 (74)	
Total	10,649 (470)	9 MAY; 2 YF; 2 Wyeomyia

* Pool size: 50 or fewer individuals.

collections yielded considerably larger numbers than did the ground collections. Activity at the ground level was relatively constant, while activity in the canopy sharply increased at approximately 1300 hours, followed by a drop in activity around 1600 hours. By nightfall (1800 hours) *Hg. janthinomys* activity had ceased.

Nocturnal insect collections within and around houses in Belterra yielded few insects and resulted in no virus isolations. The most abundant species found in this habitat were *Culex quinquefasciatus* and *Cx. coronator*.

Follow-up surveys for Hg. janthinomys

Return trips to Belterra to collect additional *Haemagogus* mosquitoes yielded 1,524 *Hg. jan-*

TABLE 2

Summary of collection sites and virus isolations from female Haemagogus janthinomys *collected in Belterra, Pará, Brazil, 5 April–5 May 1978*

Locality	Total tested (pools)	Virus isolations	
		Mayaro	Yellow fever
Plantation forest			
Near houses	107 (20)	1 (1:107*)	
Forest floor	34 (6)	1 (1:34)	
Forest canopy	211 (9)	2 (1:105)	1 (1:211)
Lowland forest			
Forest floor	122 (9)		
Forest canopy	262 (18)	5 (1:52)	1 (1:262)
Totals	736 (62)	9 (1:82)	2 (1:368)

* Minimum field infection rate.

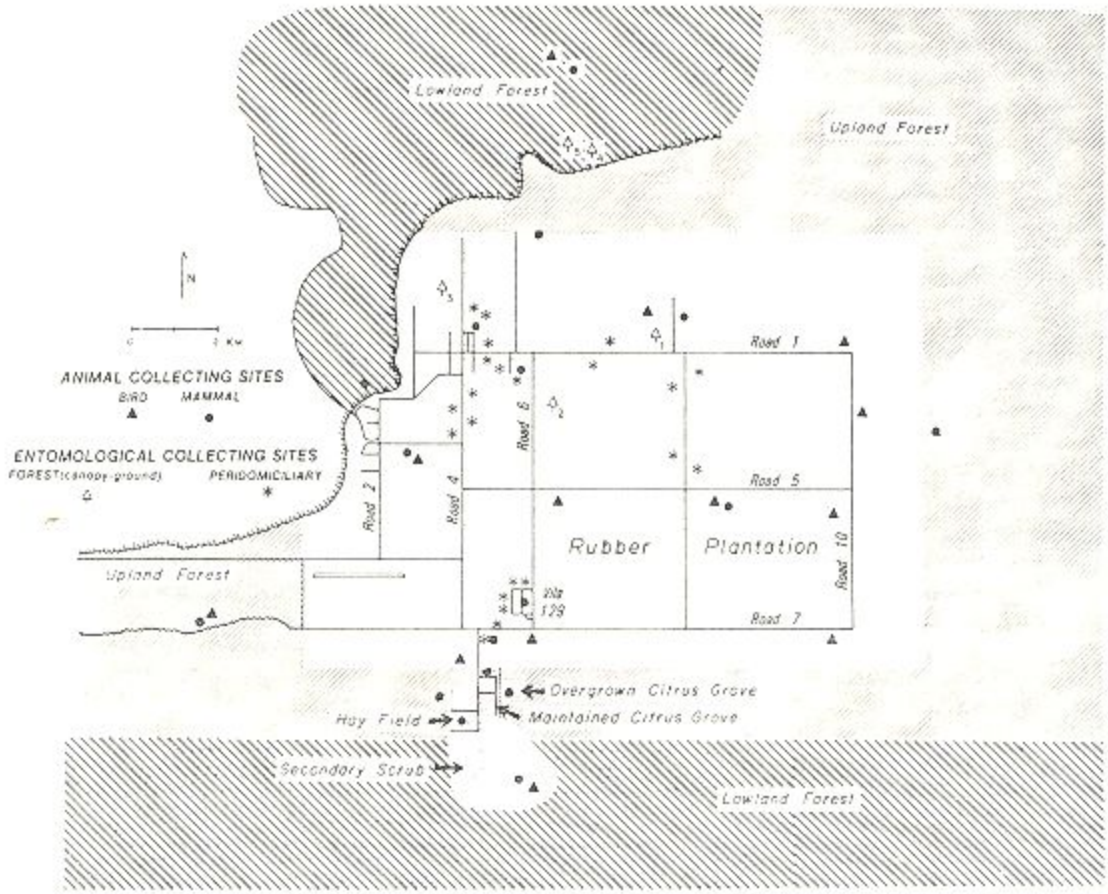

FIGURE 1. Map of Belterra, Pará, Brazil, showing the bird, mammal, and insect collecting sites and ecological habitats.

thinomys. From 14–29 June 1979, 1,484 *Hg. janthinomys* were collected and assayed in 70 pools, and from 7–16 November 1979, 40 *Hg. janthinomys* were collected and assayed in four pools. No virus was isolated from any of these pools.

Vertebrate hosts serology and virus isolations

A total of 1,260 birds were collected between 15 March and 1 November 1978 from 13 sites in and around Belterra. Mist nets accounted for

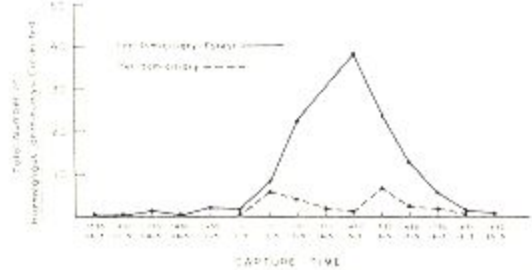

FIGURE 2. Paired captures of *Haemagogus janthinomys* Dyar in two peridomestic habitats (peridomiciliary, peridomiciliary-forest) in the rubber plantation of Belterra, Pará, Brazil, 1978.

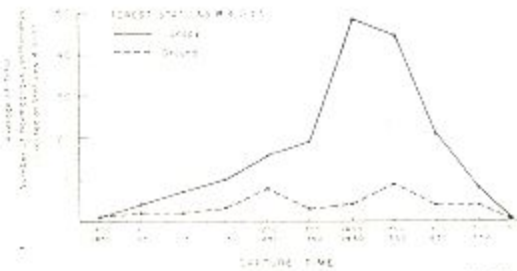

FIGURE 3. Comparison of paired captures of *Haemagogus janthinomys* Dyar conducted in the tree canopy and on the ground of the lowland forest habitat, Belterra, Pará, Brazil, 1978.

1,229 birds of 23 families captured during 106 mornings of netting. Members of the families Formicariidae and Pipridae were the most commonly captured birds. Antibody to MAY virus was detected in the sera of only 16 to 1,200 (1%) birds tested, and no virus was isolated from 1,242 bird bloods assayed. Blood samples from three domestic ducks and 28 chickens which belonged to the local inhabitants all lacked antibody to MAY virus. Table 3 presents a summary of the families of birds which were found to be positive for antibody to MAY virus.

From 15 March to 1 November 1978, 790 mammals of 47 species were collected and sera from 584 were assayed for HI antibody to MAY virus (Table 3). Only primates had demonstrable antibody to MAY virus. Antibody to MAY virus was detected in 32 of 119 (27%) marmoset sera tested. Positive marmosets were found in all parts of the Belterra forest; however, as shown in Figure 4, the highest prevalence rates for MAY virus antibody apparently occurred in the central region of Belterra. Also, MAY virus was isolated from an adult female marmoset collected near Villa 129 in the plantation forest on 29 April 1978. Two howler monkeys (*Alouatta belzebul*) were collected during the MAY virus epidemic; however, only one was tested for HI antibody and it was found to be positive.

Mammalian sera were also assayed for HI antibody to YF virus. Antibody was detected in the single howler monkey serum, in five of 119 (4%) marmosets and in one of three *Caluromys philander* (Marsupialia, Didelphidae).

Experimental infections of marmosets

A total of five marmosets (4 *C. argentata* and 1 *C. humeralifer*) were experimentally infected with MAY virus to elucidate their potential to act as amplifying hosts. Viremia was detected in three of the five as shown in Table 4. The two which failed to produce a viremia were not bled until the 3rd day pi, at which time one of these died, apparently as a result of the trauma of blood removal. Of the remaining three animals, two were viremic on day 2 with titers equal to or in excess of 10^4 $TCID_{50}$/0.1 ml when assayed in Vero cells. The remaining marmoset produced a viremia which titered $10^{2.5}$ $TCID_{50}$/0.1 ml. Titers dropped on day 3 to 10^2 $TCID_{50}$/0.1 ml in two marmosets and to 10^1 $TCID_{50}$/0.1 ml in the other. Viremia was not detected on days 4 through 7 pi.

TABLE 3

Summary of bird and mammal families collected and tested by hemagglutination-inhibition for antibody to Mayaro and yellow fever viruses; Belterra, Pará, Brazil, 1978

Family	Total positive/total tested: Mayaro		Yellow fever	
Avian				
Columbiformes				
Columbidae	1/84	(1%)		
Caprimulgiformes				
Caprimulgidae	1/5	(20%)		
Passeriformes				
Dendrocolaptidae	1/97	(1%)		
Formicariidae	5/444	(1%)		
Pipridae	1/229	(0.4%)		
Tyrannidae	1/102	(1%)		
Fringillidae	6/131	(5%)		
Other families*	0/158			
Total Avian	16/1,200	(1.3%)		
Mammalian				
Marsupialia				
Didelphidae	0/37		1/35	(3%)
Chiroptera				
Phyllostomatidae	0/171		0/171	
Desmodontidae	0/1		0/1	
Molossidae	0/2		0/2	
Primates				
Cebidae	1/1	(100%)	1/1	(100%)
Callithricidae	32/119†	(27%)	5/119†	(4%)
Edentata				
Bradypodidae	0/11		0/11	
Dasypodidae	0/1		0/1	
Rodentia				
Sciuridae	0/2		0/2	
Cricetidae	0/150		0/150	
Echimyidae	0/85		0/85	
Carnivora				
Procyonidae	0/4		0/4	
Mustelidae	0/3		0/3	
Total mammalian	33/885	(3.6%)	7/585	(1.2%)

* Other Avian families found negative: Accipitridae, Anatidae, Bucconidae, Conopophagidae, Cotingidae, Cuculidae, Furnariidae, Icteridae, Momotidae, Paridae, Phasianidae, Rallidae, Strigidae, Thraupidae, Trochilidae, Troglodytidae, Turdidae, Vireonidae

† Mayaro virus isolated

DISCUSSION

Results presented strongly indicate that *Hg. janthinomys* was the principal vector involved in the transmission of both MAY and YF viruses. The recovery of YF virus from *Hg. janthinomys* was not unexpected since this species has been

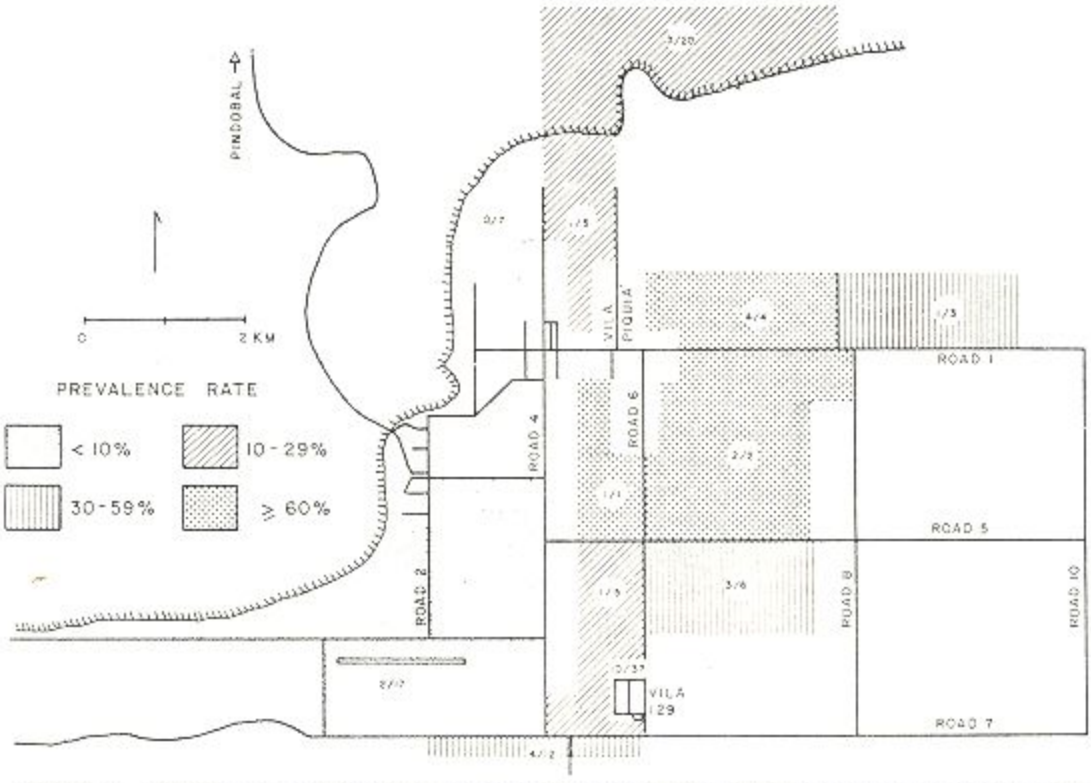

FIGURE 4. Map of the rubber plantation, showing the spatial distribution of hemagglutination-inhibition antibody prevalence rates to Mayaro virus for 119 marmosets examined in Belterra, Pará, Brazil, 1978.

documented to be an important sylvatic vector of YF virus in several localities throughout Central and South America.[18–20] While MAY virus has also been recovered from *Haemagogus* mosquitoes on several occasions in the past,[13] investigations of the outbreak at Belterra represent the first documentation of epidemic MAY virus and identification of the specific insect vector.

The evidence to incriminate *Hg. janthinomys* as the vector of MAY virus is substantial. While over 9,000 insects representing 26 species were assayed, only *Hg. janthinomys* was the source of virus isolations. The success of the human bait teams in capturing this species attests to the attractiveness of man as a blood source, and its diurnal activity pattern coincides with the time when man is most likely to enter the forests. Finally, the distribution of antibody to MAY virus

TABLE 4

Viremia produced following experimental infection of marmosets collected from Belterra, Pará, Brazil and inoculated with 10^5–10^6 $TCID_{50}$/0.1 ml of Mayaro virus

Marmoset	Viremia on days post-inoculation					
	2	3	4	5	6	7
Callithrix argentata	$10^{1.8}$*	10^2	0†	0	0	0
C. argentata	$10^{2.5}$	10^3	0	0	0	0
C. argentata	NT‡	0	0	0	0	0
C. argentata	NT‡	0	Dead			
C. humeralifer	10^3	10^2	0	Dead		

* $TCID_{50}$/0.1 ml in Vero cells
† 0 = less than 10^1 $TCID_{50}$/0.1 ml
‡ NT = not tested

among the human population correlates well with the distribution of *Hg. janthinomys* in and around Belterra.[2] While no *Hg. janthinomys* were captured inside houses, few indoor daytime collections were made and it is conceivable that any endophilic behavior was simply not detected. Others have observed that *Hg. janthinomys* would enter houses which bordered on forests, and this may have been the case in Belterra as well.[21-23]

Clinical studies on MAY virus infection of man indicate that infected persons frequently have a high titered viremia, probably sufficient to infect a portion of feeding vectors and which may at times precede or extend beyond the clinically apparent phase of illness.[1] When infected, however, most patients in Belterra were too ill to continue their daily routine; consequently, it is unlikely that many viremic persons entered the forest where *Hg. janthinomys* is most abundant. In the absence of evidence to support substantial endophilic transmission of MAY virus, it is likely that man was not the only amplifying host in the MAY virus transmission cycle in Belterra. Thus, it seems apparent that a sylvatic vertebrate host provided some of the necessary virus amplification to maintain transmission.

Marmosets are most likely the sylvatic hosts which contributed most to amplification of MAY virus. Like man and *Hg. janthinomys*, marmosets are predominantly diurnal in activity, and they are very abundant in the forested areas of Belterra. While no mosquito blood meal identifications were made during the studies at Belterra, the attractiveness of primates as blood sources for *Haemagogus* mosquitoes has been reported previously.[24] Marmosets had by far the highest MAY virus antibody prevalence rate of any sylvatic vertebrate species tested, and the distribution of antibody in the marmoset population throughout Belterra closely paralleled that in humans (see Fig. 2, ref. 2). In addition, MAY virus was isolated from a marmoset captured in Belterra at the height of the outbreak. Our subsequent experimental infections confirm that many marmosets produce a substantial viremia following infection with MAY virus, probably of sufficient titer to infect feeding vectors.

The few isolations of YF virus from mosquitoes, the low antibody prevalence rates among sylvatic vertebrates and the relatively few human cases seen all indicate that YF transmission was not as extensive as that of MAY virus. The isolations of YF virus from *Hg. janthinomys*, and the fact that this species has been incriminated as a vector elsewhere, support the contention that this species was responsible for the dissemination of YF virus during the Belterra outbreak. Identification of the associated vertebrate amplifying host is less obvious. The paucity of howler monkeys and the finding of antibody to YF virus in the single serum tested, suggest that although this species is heavily hunted for food an epizootic may have occurred. We found no evidence of howler monkey deaths; however, it is possible that forest predators removed the remains of dead monkeys before observations by our field teams. Marmosets have been the source of YF virus isolations in the past,[21,25] and it is possible that they played a role in the amplification of YF virus in Belterra as well.

In view of these findings and information reported by others, we can hypothesize that the MAY virus outbreak in Belterra represented an epidemic/epizootic phenomenon rather than an endemic/enzootic sylvatic focus. The epidemic/epizootic occurrence of this disease in the Belterra outbreak has been clearly demonstrated by the number of MAY virus isolations and prevalence of MAY antibodies recorded for both man and sylvatic vertebrates. Supportive evidence for a non-endemic focus of MAY virus is suggested by the lack of human cases following the epidemic and the absence of MAY virus recovery from *Hg. janthinomys* collected from the same area in 1979. The negative results for *Hg. janthinomys* collected in the subsequent surveys also suggests that transovarial transmission is not a significant mechanism of maintenance for MAY virus.

While we did not obtain direct evidence for the enzootic maintenance of MAY virus in our present investigation, we can make a few observations based on the available information. It may be argued that the basic MAY virus cycle is similar to that of sylvatic YF, a constantly moving wave of virus activity among susceptible vertebrates, primarily monkeys, and transmitted by sylvatic culicine mosquitoes, especially those of the genus *Haemagogus*. However, there is available evidence which suggests that birds and rodents may be a fundamental animal reservoir in the enzootic virus cycle. During the Belterra outbreak, antibody to MAY virus was found in seven families of birds, with antibody prevalence rates ranging from 0.4–20%. Earlier antibody studies of birds at the Instituto Evandro Chagas (Pinheiro, un-

published observations) showed that during 1959 and 1960, 34/119 (29%) of doves (*Columbigallina sp.*) in the Belém forest had MAY antibodies. In addition, MAY virus has been isolated from an Orchard Oriole (*Icterus spurious*) migrating into the southern United States.[26]

While the animal surveillance program in Belterra failed to find evidence of MAY antibodies in several groups of nonprimate mammals, rodents have been a source of MAY virus antibodies in separate serological investigations. In 1960 an antibody prevalence rate of 18% (6/34) was found in *Oryzomys* rodents captured at km 92 of the Belém-Brazilia Highway (Belém Virus Laboratory Annual Report, 1960). Theiler and Downs reference the finding of high rates of antibodies in various rodents belonging to the genera *Proechimys*, *Nectomys* and *Oryzomys*, and also suggest that rodents may play an important role in the maintenance of MAY virus in nature.[13]

Although it would be presumptuous to conclude that birds and rodents are involved in the basic maintenance of MAY virus in nature based primarily on the presence of MAY antibodies, it would nevertheless, be worthwhile to further investigate their possible involvement in an enzootic cycle.

In summary, results of entomological and vertebrate host investigations made during the dual outbreaks of MAY and YF viruses indicate that *Hg. janthinomys* was the principal vector for both viruses, while marmosets were the main amplifying host for MAY virus, and perhaps for YF virus as well.

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