

## AN OUTBREAK OF MAYARO VIRUS DISEASE IN BELTERRA, BRAZIL

### II. EPIDEMIOLOGY\*

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**Abstract.** Epidemiological investigations of an outbreak of Mayaro (MAY) virus which occurred in the rural village of Belterra, Pará, Brazil are reported. Human cases were first recorded in December 1977 and continued through June 1978. Approximately 20% of the more than 4,000 persons resident in Belterra were infected, and a very high proportion of those infected suffered overt clinical illness. Cases were identified in all areas of Belterra and among all age groups; however, the greatest number of cases was seen among those who resided in close proximity to the forests. Yellow fever (YF) virus was also active in Belterra concurrently with the MAY virus outbreak. Six human cases of YF were identified, of which five were fatal. The YF outbreak ended following YF vaccination of the human population.

Simultaneous outbreaks of Mayaro (MAY) and yellow fever (YF) viruses identified in Belterra during 1978 provided a unique opportunity to investigate the epidemiology of MAY virus in detail. Mayaro virus has been recorded from many areas in northern South America in the past, but previous reports have dealt with this virus primarily as a clinical entity, and have generally not addressed the question of the impact of epidemic MAY virus in the affected population.<sup>1,2</sup> Exceptions are the report by Schaeffer et al.,<sup>3</sup> which discussed MAY virus among Okinawan settlers in Bolivia, and that of Karbaat et al.,<sup>4</sup> which described MAY virus infections among Dutch military personnel stationed in Surinam. Results of Shaeffer et al.,<sup>3</sup> based on a serological survey, in-

dicated that from 10-15% of the jungle fevers seen among the settlers could be attributed to MAY virus infection. Karbaat et al.<sup>4</sup> reported that six of eight members of a military patrol in eastern Surinam suffered overt clinical illness ascribed to MAY virus infection. Serological surveys of various other populations in northern South America have found MAY virus antibody prevalence rates which range from 1-60%.<sup>5-7</sup>

In this report we describe the impact of the MAY virus outbreak on the population at Belterra, both in terms of which segments of the population were infected and also what proportion of those infected actually suffered overt clinical illness. We also briefly summarize the results of our studies on the concurrent YF virus activity. A subsequent report details our investigation of the vectors and vertebrate hosts of MAY and YF viruses at Belterra.<sup>8</sup>

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#### MATERIALS AND METHODS

Beltterra is located in the Brazilian state of Pará near the junction of the Tapajos and Amazon rivers, approximately 40 km south of Santarém, the nearest large city (Fig. 1). The village lies on a plateau about 6 km from the eastern bank of the Tapajos River. The plateau is 175 m above sea level and is separated from the river by a lowland plain that is 5 m above sea level.

The climate in Belterra is classified as humid tropical according to the Holdridge life-zone classification system.<sup>9</sup> A local meteorological station



FIGURE 1. Map of the northern Brazilian State of Pará, showing the locality of Belterra.

reported that the average annual rainfall during the previous 7 years was 2.1 m. The quantity of rainfall changes considerably between the 4-month dry season, August through November,

and the 8-month wet season, December through July. Average monthly temperature fluctuates only slightly throughout the year.

The predominant characteristic of Belterra is the rubber plantation located there. The plantation was established by the Ford Motor Company of Brazil in 1934, but is now owned and operated by the Brazilian government. An area of over 7,000 hectares was originally cleared and planted for the plantation; however, during the last 20 years trees and shrubs have been allowed to invade until now the plantation has a continuous secondary scrub undergrowth which reaches to approximately 15 m in height. This secondary growth has encroached to within 2–10 m of many houses. The plantation remains operational and access trails are maintained throughout the plantation to facilitate rubber latex collection and removal.

Figure 2 presents a map of Belterra in which the main roads and residential areas are shown. The greatest concentration of housing and administration buildings is found in the northwest corner of Belterra, with additional housing inter-

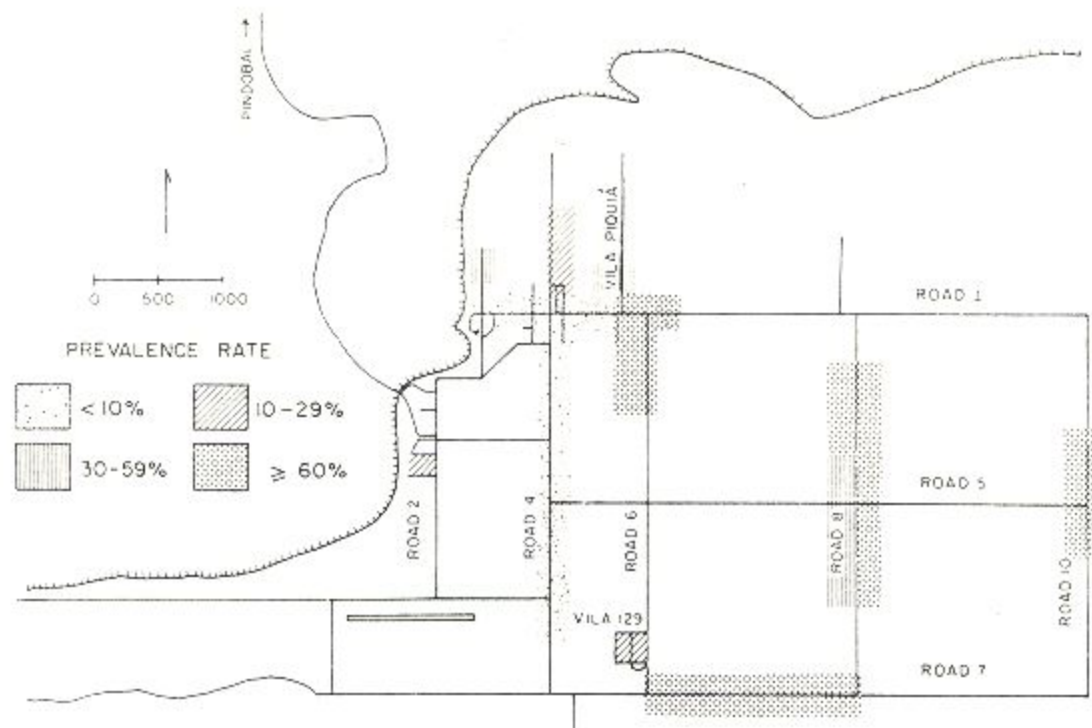


FIGURE 2. Map of Belterra, Pará, Brazil, showing principal roads and residential areas. Residential areas are divided into four groups corresponding to antibody prevalence rate to Mayaro virus based on results of a 10% stratified random sample of all occupied houses made in July 1978. Scale is in meters.

dispersed throughout the plantation. Few houses anywhere in Belterra have screening, and mosquito netting is generally not used.

The lowland area between the river and the plateau is covered with essentially undisturbed forest. The forests on the plateau which surrounds the plantation have been burned in some areas, but not recently, and are now old secondary growth forests. Forests in both the lowlands and on the plateau have had selected trees removed for lumber, but emergent trees from 25-35 m in height are still common.

Most residents of Belterra are associated with some aspect of the plantation. Many people, both men and women, are employed to collect latex from the rubber trees and are required to enter the forests almost daily. Latex is generally collected in the mornings, and many workers maintain their trails in the afternoon or tend to private gardens on the plantation periphery. Frequently the entire family goes to the garden to work. As a result, many residents of Belterra are potentially exposed to infected sylvatic vectors.

The administration of the plantation conducted a census of Belterra in December, 1977 and found a total of 4,083 people resident there. All age adjustments made in this study used this census as the standard population.

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. No information is available as to the sampling frame used or criteria for selection of people into this survey. In April 1978, during the peak of the MAY virus outbreak, another serological survey was made. This sample contained 327 sera which represented all age groups and all residential areas. While no formal sampling frame was established, a random sample was attempted. The final survey was made during July 1978, after the end of the MAY virus outbreak. This was a stratified random sample of 10% of all occupied households. During this survey all occupants of selected houses were bled and questioned for a history of illness compatible with MAY virus infection. People not at home during the initial visit were actively sought, and with repeated visits virtually all residents were sampled.

Sera collected in each survey were tested for the presence of hemagglutination inhibiting (HI) antibody to MAY virus using a reference strain of MAY virus isolated from northern Brazil and fol-

lowing standard procedures.<sup>10,11</sup> Many samples were later confirmed by neutralization tests.<sup>12</sup>

Information was gathered to construct an epidemic curve to depict the outbreak of MAY virus. Cases were identified based on clinical symptoms characteristic of MAY virus infection, namely sudden onset of fever, headache and arthralgia, with or without rash.<sup>13</sup> In order to acquire this information, a house-to-house survey of every occupied household was conducted during the last week of May and people were questioned for a history of illness compatible with MAY virus infection. An estimate of the number of cases with onset in June was extrapolated from results of the clinical questionnaires given during the survey made in July.

## RESULTS

### *Epidemic curve*

Figure 3 presents a diagram of the epidemic curve for the outbreak of MAY virus in Belterra. Results presented here are based on the 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 outbreak began in December 1977, reached a peak in April, and the last cases were detected during June, 1978. Active transmission spanned a period of approximately 6 months. The first cases identified lived at Road 8, and a total of 807 (20.5%) clinically suspect cases were recorded.

### *Attack rate*

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 years, and results of this survey may serve as an estimate of the pre-existing MAY virus antibody prevalence rate. The survey made in April, at the peak of the outbreak, found an age-adjusted HI antibody prevalence rate of about 22%, in which males outnumbered females 2:1. The July survey, made after the outbreak had subsided, showed that 29.7% of the population possessed antibody to MAY virus, and the male to female ratio was nearly equal. Consequently an estimate for the attack rate for MAY virus based on serology of 19.4% can be made.

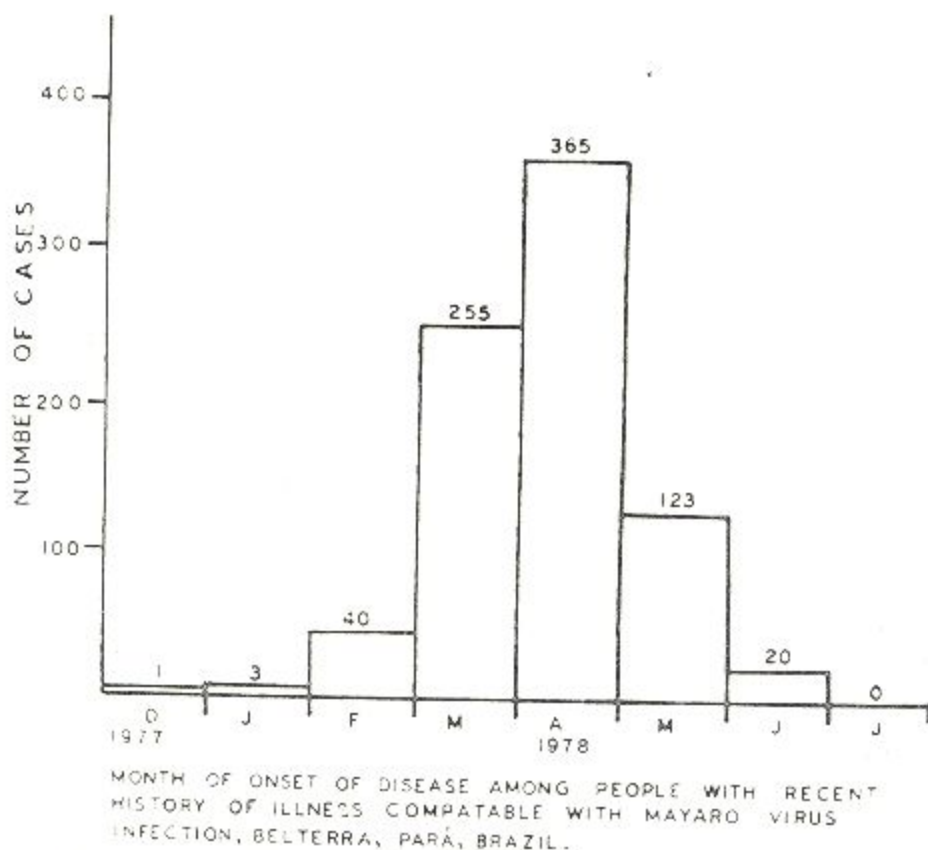


FIGURE 3. Diagram of the epidemic curve for an outbreak of Mayaro virus which occurred in Belterra, Pará, Brazil, 1978.

The estimates of a 20.5% attack rate based on clinical histories and 19.4% based on serology suggest that a high proportion of those infected suffered an overt, symptomatic illness. Eighteen individuals were included in both the April and July serological surveys, and were also interviewed in the May house-to-house clinical survey. Analysis of these cases represents an opportunity to link the estimates derived from these two sources. Table 1 presents a summary of the results of both serological testing and clinical questionnaires for these 18 people. Four persons had a distinct seroconversion to MAY virus between the April and July surveys, and of these, three had an associated febrile illness characteristic of MAY virus infection with onset in May. One child was asymptomatic. A fifth person had a 2-fold increase in antibody titer between the serological surveys and a febrile episode with onset in April. We feel confident in including her among those with positive

serology and associated clinical illness since her illness included all the symptoms characteristics of MAY virus infection and the onset of symptoms was prior to the actual survey. Thus, of five persons with positive serology, four (80%) had associated clinical illness. Eleven people had no serological evidence of a current MAY virus infection, either lacking antibody or with antibody titer unchanged between the surveys. Of these, nine (82%) were asymptomatic and two (18%) had clinical symptoms of fever and arthralgia but no rash. Two remaining cases are questionable. One child suffered a febrile episode with onset in March prior to the first survey in April, and had a 1:80 MAY antibody titer at both bleedings. The other, an adult, was asymptomatic with a 2-fold rise in titer between bleedings.

Another estimate of the proportion of those clinically ill among those infected with MAY virus can be made based on the April serological survey.

TABLE 1

Summary of results of serological testing for antibody to Mayaro virus and response to clinical questionnaires for 18 people sampled both in the April and July serological surveys and also questioned in May for prior or current Mayaro-like illness; Belterra, Pará, Brazil, 1978

Group	Age (yrs)	Sex	Mayaro antibody		Onset
			April	July	
<b>With titer rise</b>					
Symptomatic	8	M	0	80*	May
	24	F	0	80	May
	12	F	80	160	April
	10	F	0	80	May
Asymptomatic	10	F	0	320	
<b>Without titer rise</b>					
Symptomatic	11	M	0	0	April
	26	M	0	0	April
Asymptomatic	53	F	0	0	
	14	F	160	160	
	25	F	0	0	
	43	M	0	0	
	14	M	0	0	
	13	M	0	0	
	41	F	0	0	
	17	M	40	40	
12	M	40	40		
<b>Questionable status</b>					
Symptomatic	10	F	80	80	March
Asymptomatic	34	F	40	80	

\* Reciprocal of hemagglutination-inhibition titer.

In this survey, 327 persons were questioned and bled, and 71 were found to have antibody to MAY virus. From the census, 70.3% of the 327, or 230 people, are expected to be above the age of 10 years and would be at risk of pre-existing antibody. At the 1972 estimated rate of 10.3%, 24 of these 230 people would be expected to have antibody prior to the 1978 outbreak. The results of questions directed at past clinical illness made during the April survey are shown in Table 2, and indicate that of the 71 antibody positive persons questioned, 23 were asymptomatic. This is very close to the 24 expected to have preexisting antibody. In addition, the geometric mean titer of antibody to MAY virus among those clinically ill was generally higher than in those asymptomatic. These results also indicate that a high proportion of those infected suffered overt illness.

#### Distribution of cases

Cases of MAY virus infection were observed in all residential areas of Belterra; however, as

TABLE 2

Clinical manifestations and geometric mean titers (GMT) 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	GMT
Fever and arthralgia with or without rash	40	1:124
Fever and rash	4	1:60*
Fever	4	1:170
Asymptomatic	23	1:75
Total	71	

\* Three of 4 were bled from the 1st-2nd week of illness.

shown in Figure 2, the greatest concentration of cases was located in the eastern portions of Belterra where houses were closest to the forest. Mayaro virus antibody prevalence rates decreased from east to west, and were lowest in the north-west 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, 8, and 10 where the population density is low and houses are immediately adjacent to the plantation forests, as compared to Vila 129, also in the southeast part of Belterra, but where houses are clustered together and the forest is not directly adjacent to most homes.

#### Yellow fever

Five fatal cases and one non-fatal case of YF virus were identified in Belterra between January and April, 1978. Diagnosis of these cases was based on virus isolation (2 cases), serology (1) or clinical illness characteristics of YF infection (3). Onset of illness was one in January, one in February, three in March and one in April. All fatal cases were among males with ages ranging from 14-57 yrs. Five resided in Belterra and one lived in a rural area nearby. Of 327 sera drawn during the April serological survey, 59 (18%) had HI antibody to one or more flaviviruses known to exist in Brazil, and of these, 11 (3.3%) had antibody to YF virus which titered  $\geq 1:160$  indicative of recent infection or multiple YF vaccination. Of these 11, four reported an acute febrile episode during the preceding 2 months and two of these required hospitalization. In April an extensive vaccination campaign against YF virus was begun among the

residents of Belterra and thereafter no additional cases of YF were seen.

#### DISCUSSION

Results presented here indicate that the MAY virus outbreak had a substantial impact on the population resident at Belterra. Approximately 20% of the entire population was infected, and a considerably greater proportion were infected among those who resided near the plantation forests. In addition, a very high proportion of those infected suffered a clinically apparent illness, frequently severe enough to interfere with their normal daily activities.

The distribution of MAY virus antibody among the population at Belterra indicates a strong association between sylvatic exposure and MAY virus infection. This is consistent with the results presented in our subsequent report which clearly suggest that the epidemic vector in this outbreak was *Haemagogus janthinomys*, a mosquito species found most often in the sylvatic environment.<sup>7</sup> The fact that all age groups and both sexes acquired infection is not surprising considering the lifestyle of this population.

Estimates of the percent clinically ill among those infected are generally not available for arboviral illnesses, although some estimates have been made for the more commonly occurring ones. Among the alphaviruses, of which MAY virus is a member, Casals and Clarke referenced an estimate of about one overt encephalitis case per 10 infections among infants, one per 20 among elderly persons, and about one per 50 or more in the middle-age range in their discussion of eastern equine encephalitis virus.<sup>11</sup> Reeves and Hammon estimated the ratio with western equine encephalitis virus in the western United States to be one in 58 among infants and children under 5 years, decreasing with age to about one in 1,150 among persons over 15 years.<sup>12</sup> Martin et al.<sup>16</sup> reported that eight of 13 serologically identified cases of Venezuelan equine encephalomyelitis were clinically apparent during investigations in Costa Rica. Among the flaviviruses, an estimate of one apparent case per 64 inapparent cases of St. Louis encephalitis has been reported,<sup>17</sup> and rates of 1:125 to 1:1,000 have been reported for Japanese encephalitis.<sup>18, 19</sup> Consequently, our estimate of 80% or more of the MAY virus infection as being clinically apparent is extremely high. The fact that these estimates were derived using two separate lines of reasoning, yet both concluded with similar

values, tends to support the validity of these figures. The high proportion of clinically apparent cases may be due to an especially virulent strain of MAY virus or an exceptionally susceptible population. It should be noted that most estimates for the proportion of clinically apparent cases for arboviral diseases have been based only on overt encephalitis, and usually have not included milder clinical manifestations, as were included here for MAY virus. Certainly when milder signs and symptoms are included estimates will be higher.

This outbreak was self-limiting, since no vaccine is available for MAY virus, and any attempt to control a sylvatic insect vector would not have been feasible, either in terms of cost or locally available technology. It is of interest then to note the characteristics of this epidemic in an attempt to identify why transmission ceased. The epidemic began with the onset of the wet season and ended with the onset of the dry season. This corresponds to the rise and fall of abundance of the apparent mosquito vector. With regard to susceptible hosts, about 20% of the human population was infected, which raised the overall MAY virus antibody prevalence rate to nearly 30%. The final MAY virus antibody prevalence rate among the suggested sylvan vertebrate amplifying host, *Callithrix argentata*, closely paralleled that seen among humans, also nearly 30%.<sup>8</sup> It appears then that the combined factors of reduced vector abundance due to the onset of the dry season and fewer susceptible hosts, both human and sylvan, resulted in the cessation of transmission.

The clinical similarities between MAY and chikungunya (CHIK) viruses have been discussed previously.<sup>12</sup> Similarities in the epidemiology of these viruses also exist. Perhaps the most intriguing is their mutual similarity to YF virus. Both MAY and CHIK viruses have been isolated during simultaneous epidemics with YF virus; CHIK and YF viruses together in the Congo,<sup>20, 21</sup> and MAY and YF currently at Belterra and previously in Trinidad in 1954, in separate outbreaks near Belém, Brazil in 1954-55, and near Alenquer, Brazil (Pinheiro, unpublished observations).<sup>22</sup> All three viruses apparently utilize non-human primates as amplifying hosts and the same species of epizootic vectors.

During urban outbreaks, CHIK and YF viruses are both transmitted by *Aedes aegypti* mosquitoes.<sup>22</sup> Although transmission of MAY virus by *Ae. aegypti* has not been documented, it remains a distinct possibility. With the current reinfestation by *Ae. aegypti* in some parts of Central and

northern South America, the opportunity for urban transmission of MAY virus may arise. To assess this possibility, laboratory transmission studies using this species should be initiated.

The experience at Belterra exemplifies the epidemic potential of MAY virus in a susceptible population and clearly establishes MAY virus as a significant human pathogen.

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