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AN EPIDEMIC OF SYLVATIC YELLOW FEVER IN THE SOUTHEAST REGION OF MARANHAO STATE, BRAZIL, 1993–1994: EPIDEMIOLOGIC AND ENTOMOLOGIC FINDINGS

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Abstract. Yellow fever virus transmission was very active in Maranhao State in Brazil in 1993 and 1994. An investigation was carried out to evaluate the magnitude of the epidemic. In 1993, a total of 932 people was examined for yellow fever from Maranhao: 70 were positive serologically, histopathologically, and/or by virus isolation, and another four cases were diagnosed clinically and epidemiologically. In Mirador (17,565 inhabitants), the incidence was 3.5 per 1,000 people (case fatality rate [number of deaths/number of cases diagnosed] = 16.4%), while in a rural yellow fever risk area (14,659 inhabitants), the incidence was 4.2 and the case-fatality rate was 16.1% (10 of 62). A total of 45.2% (28 of 62) asymptomatic infections were registered. In 1994, 49 serum samples were obtained and 16 cases were confirmed (two by virus isolation, two by seroconversion, and 12 by serology). No fatal cases were reported. In 1993, 936 potential yellow fever vectors were captured in Mirador and a single strain was isolated from a pool of *Haemagogus janthinomys* (infection rate = 0.16%). In 1994, 16 strains were isolated from 1,318 *Hg. janthinomys* (infection rate = 1.34%) and one *Sabethes chloropterus* (infection rate = 1.67%). Our results suggest that this was the most extensive outbreak of yellow fever in the last 20 years in Brazil. It is also clear that the lack of vaccination was the principal reason for the epidemic, which occurred between April and June, during the rainy season, a period in which the mosquito population in the forest increases.

Yellow fever is an important cause of severe illness and mortality in Latin America, where sporadic cases and/or periodic epidemics of the rural form (transmission occurs near the forest) of infection have been diagnosed almost every year. In Brazil, almost two-thirds of the territory is considered an enzootic area, involving all of Amazonia and the midwestern part of Maranhao State in the northeast and Minas Gerais State in the southeast (Figure 1). From 1930 to 1992, 898 cases of yellow fever were reported, and 720 of these (80.1%) were registered in the States of Para, Goias, and Mato Grosso. In the same period, only 15 confirmed, sporadic cases were recorded in the State of Maranhao (1.6% of all Brazilian cases). The last case previously reported in Maranhao was in 1990.[1]

During 1993–1994), yellow fever was largely distributed in Barra do Corda, Esperantinopolis, Mirador (1993), and Pastos Bons (1994), all municipalities of Maranhao State. An exhaustive entomologic and serologic survey was done by the Instituto Evandro Chagas in Mirador (6°27′S, 44°32′W), and an entomologic survey was done in Pastos Bons (6°38′S, 44°55′W) to determine the magnitude of the epidemic and possible vector populations. In Pastos Bons, blood samples were collected only from febrile patients and people living in the same dwelling place, or the same village, and exposed, therefore, to the same risk of infection (Figure 1).

MATERIALS AND METHODS

Clinical observations and epidemiology. Human serum samples were obtained from blood collected by venipuncture. All people with fever or other symptoms suggestive of yellow fever (n = 34) were included in this survey. Two blood samples were collected, one for virus isolation efforts and one for serology. During the serosurvey at different places in the municipality of Mirador, blood was taken prior to yellow fever vaccination. Participants were informed about the objectives of the study and they gave their consent. People providing blood specimens were administered a standardized questionnaire and the data concerning previous anti–yellow fever vaccination(s), previous disease(s) during the past two months (including all symptoms noted), name, and address were entered on the questionnaire form. The number of people examined (n = 874) was considered to be an appropriate sample considering the estimated total population of the rural area of Mirador (14,659).

Diagnosis was made by virus isolation and/or serology. Isolation attempts were made by the intracerebral inoculation of a 0.02 ml suspension of blood (1:10 dilution in a solution of 0.75% bovine albumin in phosphate buffer solution) into suckling white Swiss mice and into cultures of C6/36 cells. Identification of viral isolates was made by immunofluorescent antibody (IFA) assay and the complement fixation test (CF).[2] Serologic diagnosis was made using the hemagglutination inhibition test.[3] This method has been used elsewhere to detect antibodies to arboviruses in seroprevalence studies. All positive samples were later tested for IgM antibodies using an enzyme-linked immunosorbent assay (MAC-ELISA).[4]

Liver biopsies were obtained from fatal cases and histologic sections, stained with hematoxylin and eosin, and examined by light microscopy. Detection of specific antigen in paraffin-embedded liver samples of fatal cases was done by means of an immunohistochemistry (IHC) technique.[5] All cases positive by hematoxylin and eosin–stained sections were confirmed by IHC.

Convalescent samples were obtained three weeks after the first collection. In patients from whom paired sera were not

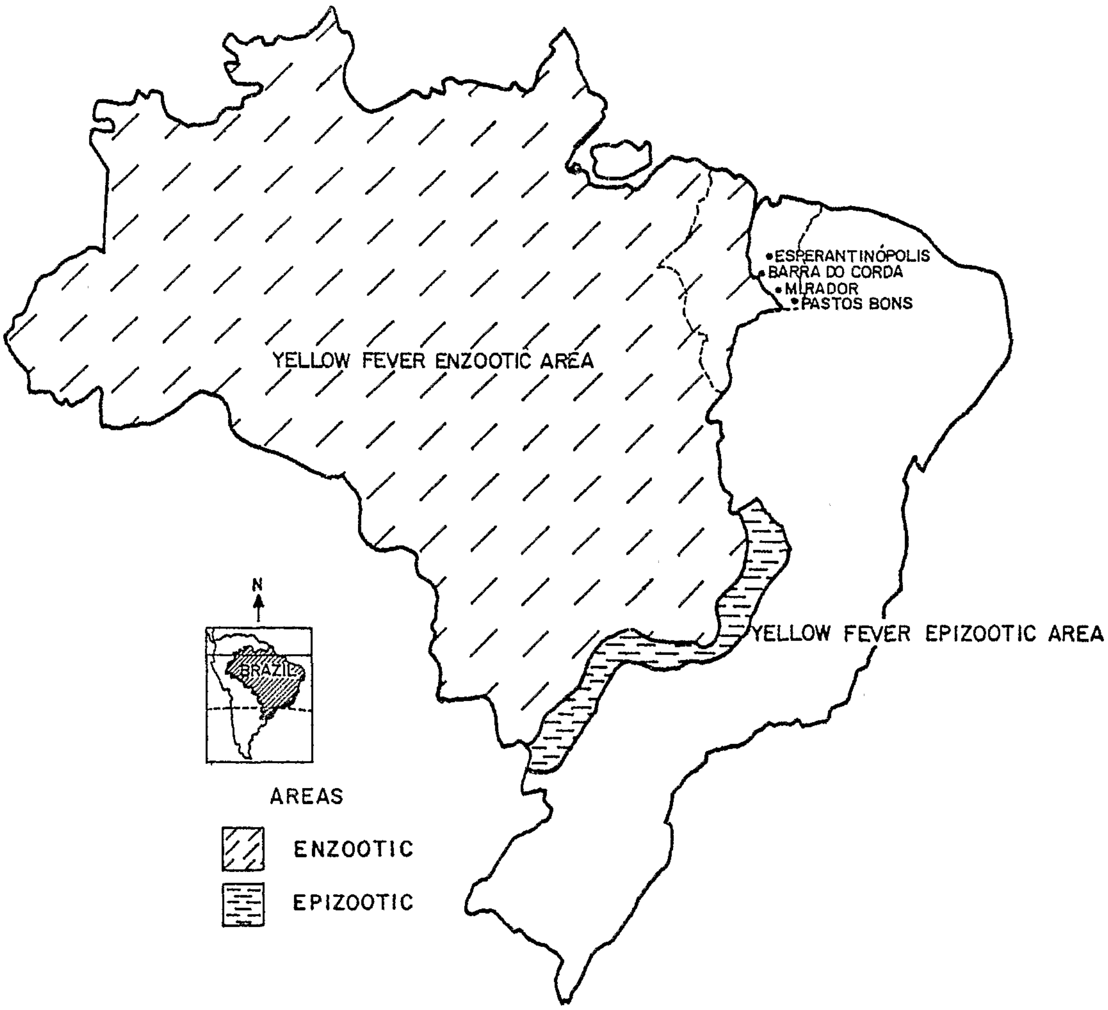

FIGURE 1. Origin of human and mosquito strains of yellow fever virus isolated in Maranhao State, Brazil, 1993–1994.

available, the presence of specific yellow fever IgM without a previous history of vaccination was used as the positive diagnostic criterion.

Entomology. Mosquitoes were captured by aspiration using human bait (from 9:00 AM to 3:00 PM) on the ground and at an elevation of approximately 10 meters in the forest canopy. In Mirador, the collections were made from May 18 to June 5, 1993 in the Araponga, Caicarinha, and Canabrava sites, and from April 5 to April 30, 1994 only in the Araponga site. In Pastos Bons, the collections were made between April 5 and April 30, 1994 in the Saco Seco and Porcao sites. The parous state of all potential yellow fever vector female mosquitoes collected was determined before attempts at virus isolation. Minimum infections rates (MIRs) were calculated by dividing the total of positive pools by the total number of specimens processed.[6]

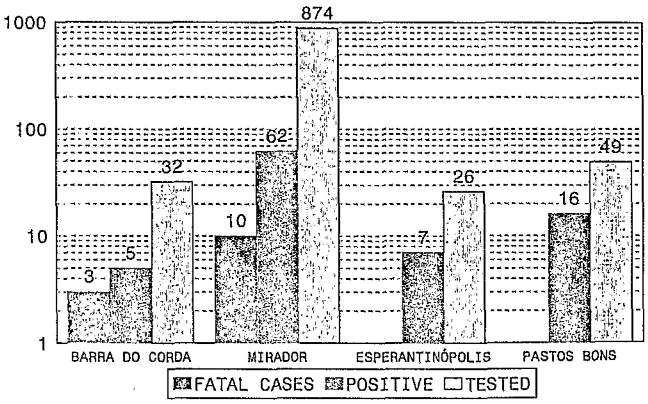

FIGURE 2. Yellow fever in Maranhao State, 1993–1994. Positive serum samples including those of fatal cases and the total collected by municipality.

RESULTS

Human cases—1993. A total of 932 human samples of blood was obtained (Barra do Corda = 32, Esperantinopolis = 26, Mirador = 874), of which 70 were positive for yellow fever by the following methods: histopathology alone: two; histopathology plus virus isolation: one; histopathology plus serology: three; virus isolation alone: two; virus isolation plus serology: three; and serology alone: 59. In addition, four other cases from Mirador were diagnosed by clinical and epidemiologic criteria. This brought the total number of cases of yellow fever diagnosed to 74: five in Barra do Corda, seven in Esperantinopolis, and 62 in Mirador (Figure 2).

TABLE 1
Age, sex, and municipality distribution of yellow fever cases in the Maranhao outbreak. Brazil, 1993*

Age (years/sex)	Mirador		Barra do Corda		Esperantinopolis		Total		Grand total
	M	F	M	F	M	F	M	F	
0–4	2	–	1	–	–	2	3	2	5
5–9	6	3	–	–	2	–	8	3	11
10–14	5	4	–	–	1	–	6	5	11
15–24	10	6	1	–	–	–	11	6	17
25–34	4	6	–	–	–	–	4	6	10
35–44	5	5	1	–	–	–	6	6	12
45–54	1	2	2	–	–	–	3	2	5
≥55	1	2	–	–	–	–	1	2	3
Total	34	28	5	–	3	4	42	32	74
Age (mean)	22	28	33	–	8	14	22	27	24

* – = absence of cases.

The positive cases ranged in age from two to 71 years (mean = 24). Of these, 32 (43.2%) were female patients and 42 (56.8%) were male (Table 1). Thirteen fatal cases were reported, 10 in Mirador and three in Barra do Corda (Table 2). Among these, the age of the patients ranged in age from seven to 71 years: four were female and nine were male. The total mortality rate was 17.5% (13 of 74) and that of Mirador alone was 16.1% (10 of 62).

Seven strains of yellow fever virus were isolated. Of these, only one was from a fatal case, and the patients from whom the other strains were isolated were all hospitalized. The first blood sample from patient number H521937 was taken in his house, after which he was immediately hospitalized. The age of the seven patients ranged from four to 52 years, and six were males. Two strains were isolated from Barra do Corda municipality and the other five from the Mirador patients (Table 3). All those patients from whom yellow fever virus strains was isolated were living at the same municipality as that in which their blood specimens were collected.

Comparing the data from the questionnaires with positive serology, the proportion of asymptomatic/symptomatic cases was 1:2, while that of severe disease/mild or asymptomatic cases was 1:7. All cases with hemorrhagic symptoms, renal failure, or jaundice were considered severe forms of yellow fever.

At the time of the study, Mirador had an estimated population of 17,565 inhabitants (urban area = 2,906; rural area = 14,659). The serologic survey showed that the incidence per 1,000 inhabitants in the municipality was 3.5, but in rural areas (the higher risk area for sylvatic yellow fever) it was 4.2. Based on the survey carried out in Mirador, 45.2% (28 of 62) of the cases had an asymptomatic infection.

Entomology—1993. Of a total of 936 mosquitoes captured (five different species), there were 602 (64.3%) *Haemagogus janthinomys* (the main vector in Brazil) and 158 *Sabethes chloropterus:* these were divided into 26 and 11 pooled groups of insects, respectively. One strain of yellow fever virus was obtained from baby mice inoculated intracerebrally with a pool of 25 *Hg. janthinomys.* The MIR in this species was calculated to be 0.16%.

Human cases—1994. No human cases were reported in Mirador. A total of 49 serum samples were obtained in Pastos Bons, including 12 paired samples. A total of 16 infections was detected as follows: two strains were isolated, two seroconversions were obtained, and 12 other infections were diagnosed by MAC-ELISA. In addition, another 14 cases were confirmed in the rural area of the Pastos Bons municipality, about 70 km southeast of Mirador and near the Piaui State border (Figures 1 and 2). All were diagnosed by serology (three seroconversions, with yellow fever virus also isolated from one of them). No deaths occurred as a result of these infections and, consequently, no cases were diag-

TABLE 2
Distribution by sex, age, municipality, and diagnostic procedures of fatal yellow fever cases reported in the Maranhao outbreak, Brazil, 1993

Case	Sex	Age (years)	Municipality	Method of diagnosis*
H 520961	F	42	Mirador	MAC ELISA
H 520964	F	71	Mirador	MAC ELISA
H 520965	M	7	Mirador	MAC ELISA
H 521021	F	45	Mirador	MAC ELISA and histopathology
H 521022	M	38	Mirador	Histopathology
H 521073	M	40	Barra do Corda	MAC ELISA and histopathology
H 521088	M	17	Mirador	MAC ELISA and histopathology
H 521089	M	18	Barra do Corda	Histopathology
H 521171	M	52	Barra do Corda	Viral isolation and histopathology
N 1†	F	74	Mirador	Clinical and epidemiologic
N 2†	M	20	Mirador	Clinical and epidemiologic
N 3†	M	20	Mirador	Clinical and epidemiologic
N 4†	M	63	Mirador	Clinical and epidemiologic

* MAC-ELISA = detection of IgM by enzyme-linked immunosorbent assay.
† Clinical specimens were not obtained.

TABLE 3
Distribution by sex, age, municipality, evolution, and methods of diagnosis of yellow fever virus isolations in the Maranhao outbreak, Brazil, 1993

Case	Sex	Age (years)	Locality/county	Death	Other procedures*
H 520933	M	28	Caicarinha/Mirador	No	Seroconversion
H 520988	M	4	Cinturao/Barra do Corda	No	–
H 521041	F	12	Coquinho/Mirador	No	–
H 521171	M	52	Cinturao/Barra do Corda	Yes	Histopathology
H 521244	M	42	Canabrava/Mirador†	No	Seroconversion
H 521706	M	15	Papagaio/Mirador	No	ELISA (IgM)
H 521937	M	42	Canabrava/Mirador†	No	Seroconversion

* ELISA = enzyme-linked immunosorbent assay. – = not available.
† Same patient with two strains isolated (samples were collected on different days).

nosed by histopathology or immunohistochemistry. Eight of the positive patients were male and eight were female.

Entomology—1994. A total of 1,196 (48 pools) and 1,245 (55 pools) of *Hg. janthinomys* were collected in Araponga (Mirador) and Pastos Bons, respectively, when these municipalities were visited in 1994. The study area in Mirador was visited because of the extensive outbreak of yellow fever there in 1993, and the visit to Pastos Bons followed the reporting of cases in 1994. In addition, another potential vector of yellow fever virus, *Sa. chloropterus,* was obtained in these municipalities with 267 (13 pools) and 73 (three pools) collected in Mirador and Pastos Bons, respectively.

No viruses were isolated from mosquitoes caught with human bait in Mirador. Sixteen strains of yellow fever virus were obtained from *Hg. janthinomys* and one from *Sa. chloropterus* collected in Pastos Bons. The infection rate for these mosquitoes was 1.34% and 1.67%, respectively.

DISCUSSION

Our results suggest that this was the most extensive epidemic of sylvatic yellow fever in Brazil in the last 20 years, with a total of 90 cases diagnosed in Maranhao State in 1993–1994, 13 with a fatal outcome (Figure 2). The thousands of nonimmune people due to the lack of vaccination was clearly the reason for this epidemic. No vaccination campaign had been carried out in the affected area since 1988. At that time, 3,036 people were vaccinated, or 17.4% of the inhabitants (Raposo L, unpublished data).

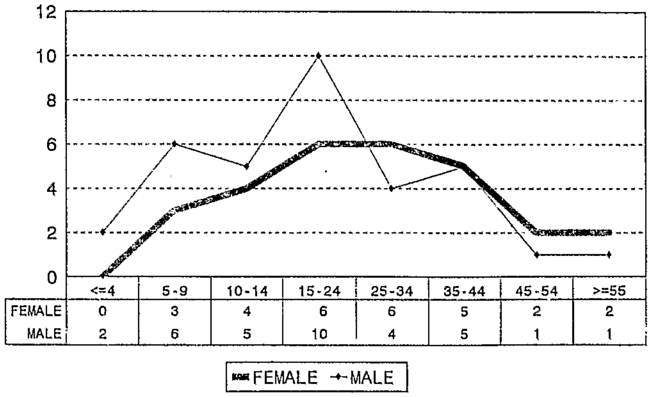

FIGURE 3. Distribution of yellow fever in Mirador, Maranhao State, 1993, by sex and age.

The epidemic areas in Maranhao State were largely those of agricultural, including the production of white sugar cane for alcohol (cachaca) and cattle breeding. It is noteworthy, however, that during the rainy season (December to May) in the affected municipalities, a bean is collected in the forest for sale in the markets and for this reason, women and children frequently enter the forest to collect this vegetable. As a result, the risk of infection was similar for men, women, and children, and there were more cases among women and children than one would have expected (Figure 3) or than have been observed during other epidemics in Brazil.[7,8] It is not clear, however, why 2.25 times more males than females died in Mirador, with a male/female fatality rate of only 1.3 (32:28).

The number of asymptomatic infections in Mirador is probably normal for yellow fever in the Amazon region of Brazil, based on the number of cases in similar situations in Africa,[9] where there is estimated an occurrence of one severe case for each 10 mild or asymptomatic infections. Except during periods in which epidemics are occurring, few cases of asymptomatic or mild infections of yellow fever are diagnosed and consequently reported, and yellow fever is usually not considered in a case of febrile disease. Since our collection of samples was randomized, and people only received the vaccine against yellow fever after blood samples had been collected, the number of nonimmune people (with yellow fever antibodies) was high. Our results indicate a lack of vaccination and consequently, the importance of this procedure for all people living in areas where yellow fever cases have been reported. Asymptomatic patients carrying the virus can be a silent source of yellow fever virus in areas where the urban vector, *Aedes aegypti,* is present in great numbers. Furthermore, urban cases of yellow fever may occur together with cases of dengue and this must be considered when making a diagnosis.

In Mirador in 1993, the MIR of rate for *Hg. janthinomys* (0.16%) was low compared with the estimates made during previous epidemics in Brazil, which were generally greater than 1.0% (Table 4). Data on infectious rates obtained during an epidemic in Mato Grosso do Sul State in 1992[10] showed that the MIR and relative density (number of landing mosquitoes/human bait/hr of *Hg. janthinomys* decreased with the time that had elapsed since the date of the last human cases. Thus, in the three localities where the last human cases had been identified, 17, 41, and 52 days before the current survey, the MIRs were 4.41%, 1.2%, and 0%, respectively.

TABLE 4
Minimum infection rate (MIR) estimated in pools of *Haemagogus janthinomys, Hg. albomaculatus, Sabethes chloropterus,* and *Sa. soperi* obtained in five outbreaks of sylvatic yellow fever studied in Brazil

Year	Vector	Municipality	State	MIR	Strains obtained*	Human cases
1984	*Hg. albomaculatus*	Faro-Monte Alegre	Para	0.22%	3	31
1988	*Hg. janthinomys*	Breves	Para	1.4%	2	2
1992	*Hg. janthinomys*	Campo Grande†	Mato Grosso do Sul	1.27–4.41%	4	14
1992	*Sa. chloropterus*	Campo Grande†	Mato Grosso do Sul	1.67%	1	14
1992	*Sa. soperi*	Campo Grande†	Mato Grosso do Sul	5.26%	1	14
1993	*Hg. janthinomys*	Mirador	Maranhao	0.16%	1	62
1994	*Hg. janthinomys*	Pastos Bons	Maranhao	1.34%	16	16
1994	*Sa. chloropterus*	Pastos Bons	Maranhao	1.67%	1	16

* Yellow fever virus from pools of mosquitoes.
† Several municipalities in the metropolitan area of Campo Grande.

Therefore, despite having a human population 10 times higher than that in the Mirador region, only 16 human cases have been confirmed in the Mato Grosso site compared with 74 in the Mirador region. In the Pastos Bons area, 16 human cases were confirmed in 1994, and the MIR of the main vector (*Hg. janthinomys*) was 1.28%. However, an extensive serologic survey was not done in this area. Since yellow fever has a sylvatic maintenance cycle in Brazil, it remains very speculative to link the MIR of mosquitoes vectors of this disease to the number of human cases. In fact, human disease depends heavily on the immunization rate and local habits with reference to jungle activity of the people.

At least two hypothesis may be proposed to account for these results. 1) The number of human cases generally reflects the immune level of the population, which itself depends upon the time elapsed since the last vaccination campaign and/or the last epidemic. Thus, the number of human cases may not actually reflect the intensity of the sylvatic transmission of the virus. A much better index of this would be the immune level of the monkey populations, but unfortunately, such data are not available. Differences in people's habits may be important in the intensity of contact with the sylvatic vectors. 2) High infections rates in mosquitoes actually reflect high levels of transmission and amplification in the monkey population. When the level of epizootic transmission is high, virus isolations are made not only from the main vector (*Hg. janthinomys*), but from *Sabethes* mosquitoes as well, which are considered secondary vectors.[10] During the 1994 survey in Pastos Bons, Maranhao State, this scenario seemed to have occurred. There was a comparatively high infection rate of *Hg. janthinomys* (MIR > 1.0%) and there was also virus isolations from naturally infected *Sa. chloropterus*. Such high levels of epizootic transmission are possible only when monkey populations are high. In Mato Grosso do Sul, dead black howler monkeys (*Alouatta caraya*) were found[10] and are assumed to have been the main vertebrate host, but in Mirador, the most common monkey species was the brown capuchin monkey (*Cebus apella*), which unlike the howler monkey, is frequently observed out of the forest foraging in crops.

Finally, it should be emphasized that the situation in Maranhao State was exceptional, with a very large susceptible (nonimmune) population that was under increased risk of infection in an area where human cases had not previously been reported. It is clear that the southeast region of Maranhao State, outside the enzootic yellow fever area, may be considered an emergence zone for the virus, as seen in Africa.[9] Moreover, the number of cases previously reported in that part of Maranhao from 1930 to 1992 was 15 (1.6% of all reported cases in the whole country).[1] On the other hand, in 1993–1994, 90 cases were reported, i.e., six times more than in the 62 previous years. These data are important in measuring the impact of the epidemic. We conclude that whenever possible, yellow fever vaccination in Brazil must be considered a priority because the area with virus circulation is increasing. This control measure is particularly important considering the extant areas of infestation with *Ae. aegypti*, the urban vector of the virus. This area provides an increased risk for the future reurbanization of yellow fever, which would be catastrophic.

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