

**A recent outbreak of Yellow fever in south of Maranhão State,
Brazil: epidemiological, clinical and entomological findings.**

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SUMMARY

An outbreak of Yellow fever virus (YF) occurred in Maranhão State in 1993 (Barra do Corda, Esperantinópolis and Mirador) and 1994 (Pastos Bons), where several cases were reported.

In 1993 a total of 932 people was examined for YF distributed as follows: 32 from Barra do Corda, 26 from Esperantinópolis and 874 from Mirador. From these, 70 cases were YF confirmed serologically (IgM capture MAC ELISA), histopathologically and/or by virus isolation. Another 4 cases diagnosed clinically and epidemiologically, brought the total to 74 (Barra do Corda:5; Esperantinópolis:7 and Mirador:62). The age of patients ranged from 2 to 71 years old (mean:24): 32 (43.2%) were female and 42 (56.8%) male and the total lethality-rate was 17.5% (13/74). In Mirador (17,565 inhabitants), where an intensive survey was carried out, the incidence was 3.5 per 1,000 people, but in the rural YF risk area (14,659 inhabitants) the incidence was 4.2, and the mortality 16.1% (10/62). A total of 45.2% (28/62) asymptomatic infections were registered. In 1994, a total of 49 serum samples was obtained from Pastos Bons. Of these, 16 cases, 8 females and 8 males, were YF confirmed as follows: 2 by virus isolation, 2 by seroconversion and 12 by serology. On the other hand, no fatal cases were reported in Pastos Bons.

In 1993, 936 potential YF vectors were captured in Mirador and a single strain was isolated from a pool of *Haemagogus janthinomys* (infection rate(IR):0.16%). In 1994, 16 strains were isolated from 1,318 *Hg. janthinomys* (IR:1.34%) and one *Sabethes chloropterus* (IR:1.67%).

Our results suggest that this was the most extensive outbreak of YF in the last 20 years in Brazil.

RESUMO

Uma recente epidemia de febre amarela silvestre ocorreu no sul do Estado do Maranhão nos anos de 1993 e 1994. Em 1993 os municípios de Barra do Corda, Esperantinópolis e Mirador foram acometidos enquanto em 1994 a epidemia se restringiu a Pastos Bons.

Foram obtidas 932 amostras em 1993, tendo sido diagnosticados 70 casos de febre amarela confirmados por isolamento viral, sorologia e/ou histopatologia. Quatro outros casos diagnosticados clínica e epidemiologicamente elevaram o total para 74 casos, distribuídos como segue: 5 em Barra do Corda, 7 em Esperantinópolis e 62 em Mirador.

A idade dos pacientes variou de 2 a 71 anos (média de 24): 32 (43.2%) eram fêmeas e 42 (56.8%) machos e a letalidade foi de 17.5% (13/74). Em Mirador (17,56 habitantes), onde 874 amostras de soro foram colhidas, a incidência foi de 3,5 por 1,000 habitantes e a mortalidade 16.1% (10/62). Um total de 45.2% (28/62) dos casos deveram-se a infecções assintomáticas. Em 1994, um total de 49 amostras de soro foi obtido em Pastos Bons. Destas, 16 casos, 8 fêmeas e 8 machos foram confirmados, como segue: 2 por isolamento viral, 2 por soroconversão e 12 por sorologia. Nenhum caso fatal foi reportado em Pastos Bons.

Em 1993, 936 vetores potenciais de FA foram capturados em Mirador tendo sido isolada uma amostra do vírus amarelo de um lote de *Haemagogus janthinomys* (taxa de infecção (TI):0.16%). Em 1994, 16 amostras foram isoladas a partir de 1,318 *Hg. janthinomys* (TI:1.34%) e uma de *Sabethes chloropterus* de 73 (TI:1.67%) colhidos em Pastos Bons.

Nossos resultados indicam que esta foi a maior epidemia registrada no Brasil nos últimos 20 anos.

INTRODUCTION

Yellow fever (YF) is an important cause of severe illness and mortality in Latin America, where sporadic cases and/or periodic epidemics of the rural form of infection have been diagnosed almost every year. In Brazil, almost two thirds of the territory is considered an enzootic area, involving all Amazon and the Middle-West regions, west of Maranhão State in the Northeast and Minas Gerais State in the Southeast (Figure 1). From 1930 to 1992, 898 cases of YF were notified, and 720 of them (80.1%) were registered in the States of Pará, Goiás and Mato Grosso. In the same period, only 15 confirmed sporadic cases were recorded in the State of Maranhão (1.6% of all Brazilian cases). The last case previously reported in Maranhão was in 1990 (Nobre *et al.*, 1994).

During the last two years (1993-94), YF was common in Barra do Corda, Esperantinópolis, Mirador

(1993), and Pastos Bons (1994), all municipalities of Maranhão State, where several cases have been reported (Figure 1). Exhaustive entomological and serological surveys were made by the Instituto Evandro Chagas (IEC), in Mirador (6° 27' S, 44° 32' W), and only an entomological survey only in Pastos Bons (6° 38' S, 44° 55' W), in order to determine the magnitude of the epidemic and the situation regarding the vector population. In Pastos Bons, blood samples were collected only from febrile patients and people living in the same dwelling place, or near to it and exposed, therefore, to the same risk of infection (Figure 1).

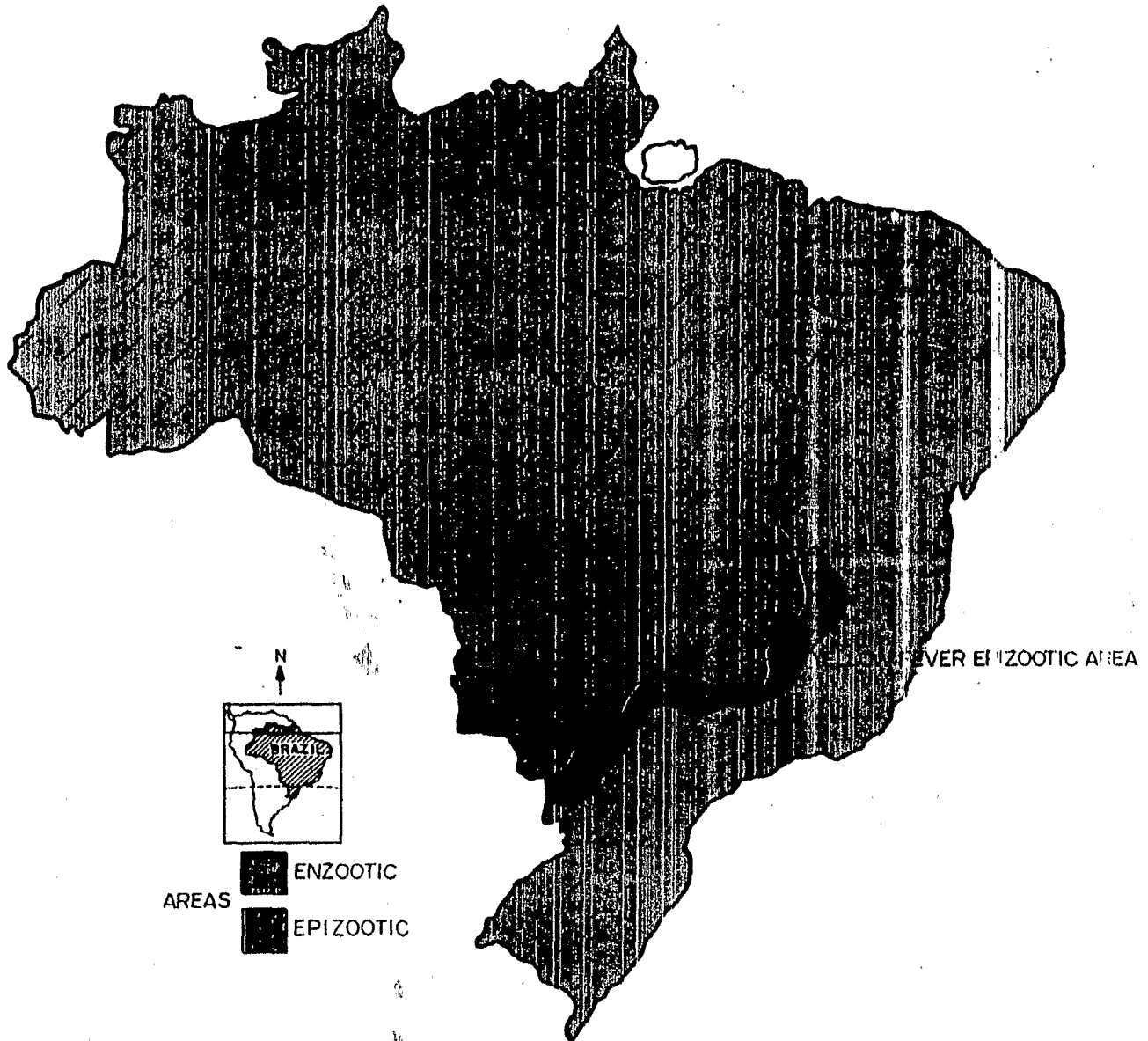


Figure 1. The enzootic area of yellow fever in Brazil and the origin of human and mosquito strains of yellow fever virus isolated in Maranhão State, Brazil, 1993-1994.

Clinical observations and Epidemiology

Human serum samples were obtained by venopuncture. All people with fever or other symptoms suggestive of YF were included and they were further bled for attempts to isolate the virus. During the serosurvey at different places in the municipality of Mirador, blood was taken randomly prior to YF vaccination. All people bled were questioned and the data concerning previous anti-YF vaccination(s), previous disease(s) during the past two months (including all symptoms presented), name, address and family history were entered on a questionnaire form. The number of people examined was considered as statistically significant considering the estimated total population of the rural area of Mirador (14,659).

Diagnosis was made by the isolation of YF virus after the intracerebral inoculation of suckling mice, and in cultures of C6/36 cells. Identification of isolations was made by immunofluorescent antibody assay (IFA) and the complement fixation test (CF) (Shope & Sather, 1979). Serum samples were tested using the hemagglutination-inhibition test (HI) (SHOPE, 1963), as the screening method has been used elsewhere to detect both IgM and IgG antibodies in seroprevalence studies. The MAC ELISA (Kuno *et al.*, 1987) was used as a confirmatory test, once IgM antibodies had been detected in natural infections, whether or not the patient presented symptoms.

Liver samples were obtained from fatal cases and histological sections, stained by haematoxylin and eosin (HE), were examined by light microscopy. Detection of specific antigen in paraffin-embedded liver samples of fatal cases was by means of immunohistochemistry (IHC) technique (Hall *et al.*, 1992). All cases positive by HE stained sections were confirmed by IHC.

In patients from whom paired sera were not available, the presence of specific YF IgM without a previous history of vaccination, was the criteria used to consider a patient as positive.

Entomology

Mosquitoes were captured using human bait (09:00-15:00h) on the ground and in the forest canopy in the following counties: In Mirador, the collections were made during the period of May 18th to June 5th 1993 in the Araponga, Caiçarina and Canabrava sites, and from April 5th to 30th 1994 only in Araponga. In Pastos Bons the collections were made between 5th and 30th April 1994, in the Saco Seco and Porção sites.

RESULTS

1993

Human cases

A total of 932 human samples of blood was obtained (Barra do Corda:32, Esperantinópolis:26, Mirador:874), of which 70 were positive for YF by the following methods: histopathology alone:2; histopathology + virus isolation:1; histopathology + serology:3; virus isolation alone:2; virus isolation + serology:3 and serology alone:59. In addition, 4 other cases of YF from Mirador were diagnosed by clinical and epidemiological criteria. This brought the total number of cases of YF diagnosed to 74: 5 in Barra do Corda, 7 in Esperantinópolis and 62 in Mirador.

Table 1. Age, sex and municipality distribution of yellow fever cases in the Maranhão outbreak, Brazil, 1993.

AGE YEAR\SEX⇒	Mirador		Barra do Corda		Esperantinópolis		TOTAL		
	M	F	M	F	M	F	M	F	G
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

The age of positive cases ranged from 2 to 71 years (mean:24). Of these, 32 (43.2%) were female patients and 42 (56.8%) male. Thirteen fatal cases were reported, ten of them in Mirador and three in Barra do Corda (Table 2). Among these, the age of the patients ranged from 7 to 71 years: four of them were female and nine male. The total mortality rate was 17.5% (13/74) and that of Mirador alone was 16.1%(10/62).

Table 2. Fatal yellow fever cases reported in the Maranhão outbreak, Brazil, 1993. Distribution by sex, age, municipality and diagnostic procedures.

CASE	SEX	AGE	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+Histopathology
H 521022	M	38	Mirador	Histopathology
H 521073	M	40	Barra do Corda	MAC ELISA+Histopathology
H 521088	M	17	Mirador	MAC ELISA+Histopathology
H 521089	M	18	Barra do Corda	Histopathology
H 521171	M	52	Barra do Corda	Viral isolation+Histopathology
N 1 **	F	74	Mirador	Clinic & Epidemiologic
N 2 **	M	20	Mirador	Clinic & Epidemiologic
N 3 **	M	20	Mirador	Clinic & Epidemiologic
N 4 **	M	63	Mirador	Clinic & Epidemiologic

* Detection of IgM by Enzyme immune Assay

** Clinical specimens were not obtained

Seven strains of YF virus were isolated. Of these, only one was from a fatal case of YF, and the patients from whom the other strains were isolated were all hospitalized. The first blood sample from patient H521937 was taken in his house, from where he was immediately hospitalized. The age of the seven patients ranged from 4 to 52 years and, with the exception of one case, all were males. Three strains were isolated from Barra do Corda municipality and the other four from the Mirador patients (Table 3). All those patients from whom YF virus strains was isolated were living at the same municipality as that in which their blood specimens were collected.

Table 3. Yellow fever virus isolations in the Maranhão outbreak, Brazil, 1993. Distribution by sex, age, municipality, evolution and methods of diagnosis.

CASE	SEX	AGE	LOCALITY/COUNTY	DEATH	OTHER PROCEDURES
H 520933	M	28	Caiçarina/Mirador	NO	Seroconversion
H 520988	M	4	Cinturão/Barra do Corda	NO	-
H 521041	F	12	Coquinho/Mirador	NO	-
H 521171	M	52	Cinturão/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

* Same patient with 2 strains isolated.

Comparing the data of 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 haemorrhagic symptoms, renal failure or jaundice were considered as severe forms of YF.

At the time of the study, Mirador had an estimated population of 17,565 inhabitants (urban area:2,906; rural area:14,659). The serological 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 YF) it was 4.2. Based on the survey carried out in Mirador, 45.2% (28/62) of the cases were with asymptomatic infection.

Entomology

Of a total of 936 mosquitoes captured (5 different species), there were included 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 YF virus was obtained from baby mice inoculated intracerebrally with a pool of 25 *Hg. janthinomys*. The infection rate in this mosquito species was calculated as 0.35%.

1994

Human cases

No human cases were reported in Mirador.

A total of 49 serum samples was obtained in Pastos Bons, 12 of them were paired, and two strains of YF virus were obtained from the sera. In addition, another 14 cases of infection were confirmed in the rural area of the Pastos Bons municipality, about 70 km Southeast from Mirador and near the Piauí State border. All were diagnosed by serology (three seroconversions, with YF virus also isolated from one of them). No deaths occurred as a result of these infections and, consequently, no cases were diagnosed by histopathology or immunohistochemistry. Eight of the positive patients were male and eight female.

Entomology

A total of 1196 (48 pools) and 1245 (55 pools) of *Hg. janthinomys* was collected in Arapongá, a district of Mirador extending into Pastos Bons, when these municipalities were visited in 1994. The study area in Mirador was visited because of the extensive outbreak of YF there in 1993, and the visit to Pastos Bons followed the registration of cases in 1994. In addition, another potential vector of YF virus *Sabethes chloropterus* was obtained in these municipalities with totals of 267 (13 pools) and 73 (3 pools) collected in Mirador and Pastos Bons, respectively.

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

DISCUSSION

Our results suggest that this was the most extensive epidemic of sylvatic YF in Brazil during the last 20 years, with a total of 90 cases of YF diagnosed in Maranhão State in 1993-94 (Figure 2). The thousands of non-immune people due to the lack of vaccination was clearly the reason for this epidemic.

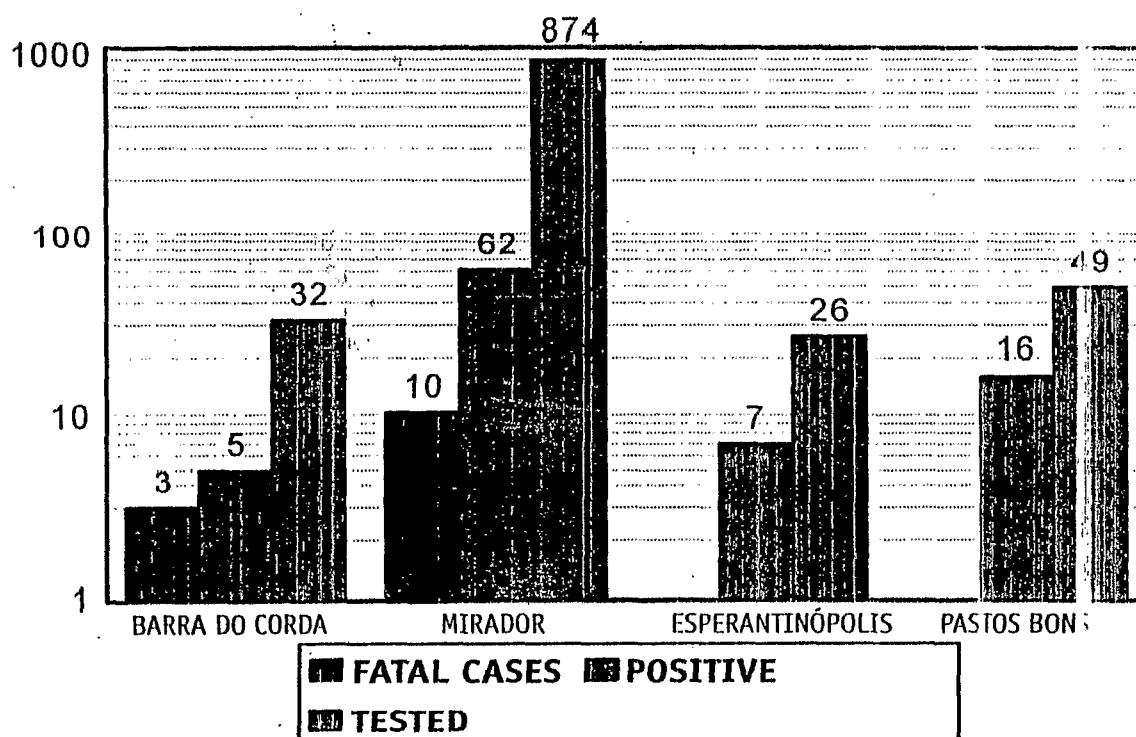


Figure 2. Yellow fever in Maranhão State, 1993-94. Positive serum samples including those of fatal cases and the total collected by municipality.

The epidemic areas in Maranhão State were largely those of agricultural activities, including the production of white sugar-cane for alcohol ("cachaça") and cattle breeding.

It is noteworthy, however, that during the rainy season (December to May), in the affected municipalities a leguminous bean is collected in the forest for sale in the markets and, for this reason, women and children frequently enter the forest at this time. 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 expect, or than have been observed during other epidemics in Brazil (Pinheiro *et al.*, 1978; Travassos da Rosa *et al.*, 1984). It is not clear, however, why 2.25 times more males than females died in Mirador, with a male/female lethality-rate of only 1.3 or 32/28 (Figure 3).

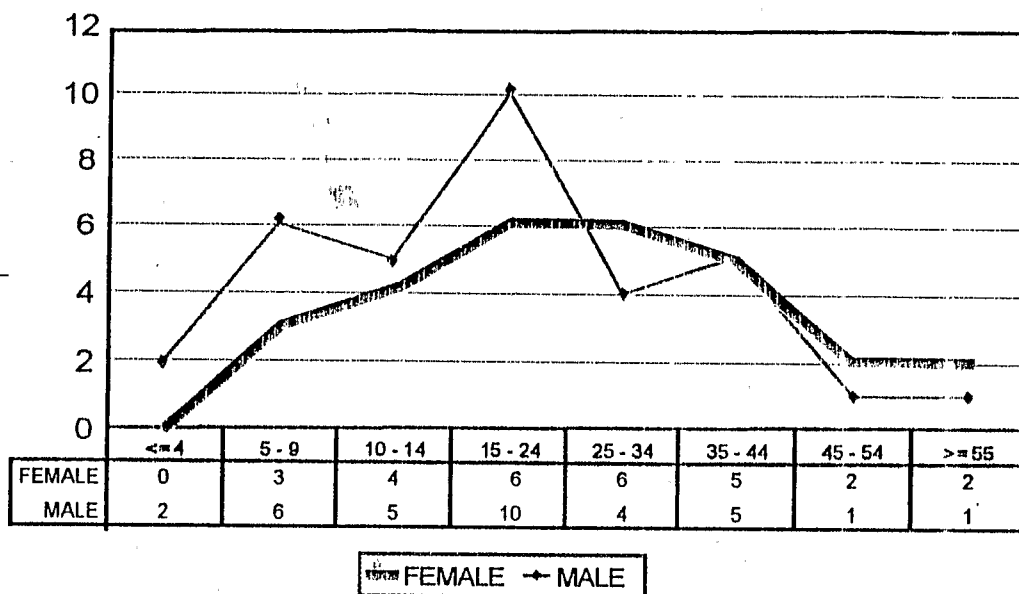


Figure 3. Yellow fever in Mirador, Maranhão State, 1993 Distribution of cases by sex and age.

The number of asymptomatic infections in Mirador is probably normal for YF. Obviously, few cases are diagnosed and consequently notified. Since, our collection of samples was randomized, and people only received the vaccine against YF after blood samples had been collected, these results indicate the importance of vaccinating all people from an area where YF cases have been recorded. Asymptomatic patients carrying the virus can be a silent source of YF virus in areas where the urban vector, *Aedes aegypti*, is present in great numbers. Furthermore, urban cases of YF may occur together with cases of dengue and this must be considered when making a diagnosis.

In Mirador, in 1993, the entomological data showed a low infection rate (IR) for *Hg. janthinomys*, the main vector of sylvatic YF in Brazil (0.35%), but a high one (1.34%) in Pastos Bons in 1994. The IR in previous outbreaks has generally been higher than 1.0%, as for example in Breves, Pará State (1.4%), in 1988, and in Campo Grande, Mato Grosso do Sul State (1.27-4.1%) in 1992 (Dégalier *et al.*, 1992). It is concluded from these data that, contrary to the situation in Southern Mato Grosso do Sul, the high number of cases was the result of two main factors: i) a high proportion of susceptible people living in a high-risk area for YF virus circulation (emergent zone) - the situation observed in Mirador - and, ii) high densities of infected mosquito vectors (*Hg. janthinomys* and *Sa. chloropterus*), as was observed in Pastos Bons. Table 4 correlates the IR, number of strains obtained from *Hg. janthinomys* and other YF virus vectors, and the number of human cases notified. It shows that in Mirador and Faro/Monte Alegre, Pará State, many cases were diagnosed despite the low IR; while in Campo Grande and Pastos Bons, where there was a high IR and numerous strains were obtained from *Hg. janthinomys*, only a few cases of human infection were confirmed. We are convinced that at least 2 factors are involved in this phenomenon: i) a low rate of immunization in the first area and a high rate in the second one, ii) different levels of vector competence, favouring transmission in Mirador and Faro/Monte Alegre, and not favouring transmission in Pastos Bons and Campo Grande. The quantity of parous female mosquitoes captured in Mirador and Faro/Monte Alegre was higher than in Pasto

Bons and Campo Grande, conducive to a higher infection rate in the mosquitoes and, therefore, a higher transmission rate to man.

Table 4. Infection rate (IR) estimated in pools of *Haemagogus janthinomys*, *Hg. albomaculatus*, *Sabethes chloropterus* and *Sa. soperi* obtained in five outbreaks of sylvatic yellow fever studied by the IEC in Brazil

YEAR	VECTOR	MUNICIPALITY	STATE	IR	STRAINS OBTAINED+	HUMAN CASES
1984	<i>Hg. albomaculatus</i>	Faro-Monte Alegre	Pará	0.22%	3	31
1988	<i>Hg. janthinomys</i>	Breves	Pará	1.4%	2	2
1992	<i>Hg. janthinomys</i>	Campo Grande *	Mato Grosso do Sul	1.27-4.41%	4	14
1992	<i>Sa. chloropterus</i>	Campo Grande *	Mato Grosso do Sul	1.67%	1	14
1992	<i>Sa. soperi</i>	Campo Grande *	Mato Grosso do Sul	5.26%	1	14
1993	<i>Hg. janthinomys</i>	Mirador	Maranhão	0.35%	1	62
1994	<i>Hg. janthinomys</i>	Pastos Bons	Maranhão	1.34%	16	16
1994	<i>Sa. chloropterus</i>	Pastos Bons	Maranhão	1.67%	1	16

* Several municipalities in the metropolitan area of Campo Grande.

+ Of YF virus from pools of mosquitoes

Finally, it should be emphasized that the situation in Maranhão State was exceptional, with a very large receptive (non immune) population that was under increased risk of infection, in an area where human cases had not been notified. It is clear that the Southeast region of Maranhão State, outside the enzootic YF area, may be considered an emergence zone for the virus, as seen in Africa (WHO, 1986). Moreover, the number of cases previously reported in that Maranhão, during the period 1930 to 1992, was 15 (1.6% of all notified cases in the whole country) (Nobre *et al.*, 1994). On the other hand, in 1993-94, 90 were reported, i.e. 6 times more than in the 62 previous years. These data are important in measuring the impact of the epidemic. The authors conclude that, whenever possible, YF vaccination in Brazil must be considered a priority because the area with virus circulation is increasing. This control measure is particularly important considering the extent areas of infestation with *Aedes aegypti*, the urban vector of the virus. These provide an increased risk of the future reurbanization of YF, which would be catastrophic to this country.

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**An overview of
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