Epidemic of Jungle Yellow Fever in Brazil, 2000: Implications of Climatic Alterations in Disease Spread

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Seventy-seven human cases of sylvatic yellow fever were reported in Brazil during the period January–June 2000. The first cases were reported 1 week after New Year’s day and originated at Chapada dos Veadeiros, a tourist canyon site in Goiás state, near Brasília, the Brazilian capital. The laboratory procedures used for diagnoses included serology with an IgM capture assay and plaque reduction neutralization test, virus isolation in suckling mice and C6/36 cells, and immunohistochemistry. All cases were diagnosed by at least two different laboratory procedures, with the exception of the first three fatal cases, which were diagnosed on the basis of clinical and epidemiological information. The cases were reported in eight Brazilian states as follows: Goiás with 64.9% (50 cases); Amazonas (1); Bahia (10); Distrito Federal (1); Mato Grosso (4); Minas Gerais (2); Pará (1); São Paulo (2); and Tocantins (6). Patient ages were within the following ranges: 13–74 years old (mean 34.3), 64 (84.4%) were male, especially agricultural workers (n = 30), but tourists (n = 11), carpenters (n = 4), fishermen (n = 4), students (n = 3), truck drivers (n = 3), and other people (n = 22) were also sickened. The case fatality rate was 50.6% (39/77). In Bahia state, a serologic survey that was carried out has suggested a symptomatic/asymptomatic coefficient of 1:4. Field studies developed in Distrito Federal, Goiás, and São Paulo states showed that Haemagogus janthinomys was the mosquito species associated with the transmission. A single strain was also obtained from Aedes scapularis in Bahia. Epizootic occurrence (monkey mortality) was observed in 49 municipalities mainly in Goiás state, where 40 municipalities made reports, 21 of which also diagnosed human cases. Data obtained by the National Institute of Meteorology in Brazil showed an increase in temperature and rain in December 1999 and the first 3 months of 2000 in Goiás and surrounding states, which perhaps has contributed to the intense and widespread transmission of the yellow fever virus. The relatively small number of cases probably reflects the extensive use of yellow fever 17D-vaccine during the last 3 years, in which about 45 million doses were used. During the last months of 1999, 16 and 11 yellow fever cases were reported in Tocantins and Goiás states, respectively. It is noteworthy that the last reported autochthonous cases of sylvatic yellow fever in São Paulo and Bahia, both states outside the endemic/enzootic area, had occurred in 1953 and 1948, respectively. J. Med. Virol. 65:598–604, 2001.

KEY WORDS: yellow fever; epidemic; Flavivirus; IgM-ELISA; immunohistochemistry; climatic changes; Brazil

INTRODUCTION

Yellow fever virus, the prototype in the genus Flavivirus of the family Flaviviridae, is currently endemic in northern South America and in sub-Saharan Africa, where it causes sporadic cases, outbreaks and epidemics. In South America, only the sylvan cycle is recognized and had been detected in the

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Accepted 16 March 2001
Amazon and Araguaia river basins. In this region, yellow fever virus has been reported in Bolivia, Brazil, Colombia, Peru, Venezuela, and the Guyanas [WHO, 1985; Robertson et al., 1996].

In Brazil, cases have been reported in the Amazon and Central West regions, as well as in the western areas of Maranhão and Minas Gerais states, which together constitute the epizootic area [Vasconcelos et al., 1997].

Transmission occurs when an infected mosquitoes bite humans. The yellow fever virus main vector in Brazil is the *Haemagogus janthinomys*, but other mosquito species of this genus and also of the genus *Sabethes* play a role in the maintenance cycle of YFV, acting as secondary vectors. Monkeys are the main hosts and the source of amplification of virus, since a viremic monkey can infect several mosquitoes [Dégallier et al., 1992; Mondet et al., 1996].

Humans are infected incidentally when he intrudes into ecological sites where infected mosquitoes are circulating. The disease affects especially young men who carry out various activities in the forest, mainly agricultural, as well as hunting, fishing, extracting minerals and wood, and so on [Pinheiro et al., 1978; Vasconcelos et al., 1997].

This article reports the occurrence of yellow fever in Brazil during the first half of 2000, especially the outbreaks reported in Goiás and Bahia states, where most of the cases were diagnosed.

**MATERIALS AND METHODS**

**Human Samples**

Serum samples were taken from suspected cases, relatives, and contacts; 5–10 ml was collected by venous puncture. Liver and other viscera fragments were obtained from fatal cases by autopsy.

**Monkey Samples**

Liver fragments and blood of nonhuman primates were taken from animals that were killed randomly in areas where people had reported monkey and or human disease. They were killed and specimens were collected. These animals, generally, were found and killed in the neighborhood (200–500 m) of human dwellings.

**Serology**

Serum samples were tested by an IgM capture enzyme immunosorbent assay (IgM-ELISA) as described by Kuno et al. [1987]. Briefly, plates were coated with an anti-IgM preparation for capture of antibody. Plates were washed 5 times in a phosphate-buffered solution (PBS) pH 7.2, and incubated overnight. Serum samples diluted 1:100 in PBS solution, pH 7.2, were put into the wells, as well as two positive and four negative controls, and were incubated for 1 hour at 37°C. The plates were then washed 5 times in the same solution. Antigen was added, plates were washed 5 times as above and incubated for the same time and temperature. The conjugate (antibody labeled with peroxidase) was used. Plates were washed as described previously and incubated for 1 hour at 37°C. Substrate (ABTS system) solutions were used. The plates were incubated at room temperature, and the reaction was stopped after 2 hours and immediately the absorbance was measured in a spectrophotometer using a 405-nm filter. Positive samples were those that showed reactions (green color) with absorbance higher than the cutoff (0.2).

**Plaque Reduction Neutralization Test**

All serum samples positive by IgM-enzyme-linked immunosorbent assay (ELISA) were confirmed by plaque reduction neutralization tests as described elsewhere [Beaty et al., 1989]. Briefly, tests were carried out in microplate culture of Vero cells. A fixed virus inoculum (40–150 plaque-forming units [pfu] ) was used against serial sample dilutions. After heat inactivation (56°C/30 min), human and monkey samples were prepared in twofold serial dilutions. Beginning at 1:10 in PBS, pH 7.2. An appropriate quantity of virus was then added to each dilution. The serum–virus mixture was incubated overnight at 5°C before inoculation. Two microplate wells were inoculated with each serum dilution. The highest serum dilution producing (90% plaque inhibition was recorded as the endpoint. Samples showing titers of ≥1:10 were considered positive.

**Virus Isolation**

Acute-phase serum samples and liver fragments were inoculated into suckling mice and C6/36 cells as previously described [Beaty et al., 1989]. Briefly, sera and visceral samples were harvested and diluted in a solution of bovine serum albumin (BSA) 0.4% in PBS pH 7.4 containing 100 μg/ml of streptomycin and 100 IU/ml of penicillin. Samples were then centrifuged at 2,000g. Supernatant aliquots of 0.02 ml and 0.1 ml were inoculated into mice intracerebrally and cells, respectively.

**Detection of Antigens**

Liver samples were fixed in a 10% buffered formalin solution and labeled in paraffin blocks. Slides from tissues were prepared and were examined using an immunohistochemistry assay, by the method of Hall et al. [1991]. Briefly, 5-μm sections of liver were mounted on glue-coated slides and hydrated. Acid hematin and malaria pigments were removed from the sections by immersing them in a dilute ammonium hydroxide/ethanol solution for 15 minutes. The slides were then neutralized by washing in PBS, pH 7.2, before staining. The slides were immersed in a 0.05% solution of protease VIII, pH 7.8, at 37°C for 3 minutes and washed in PBS. The tissues were then stained with the avidin-biotin complex procedure, using a 0.2% hydrogen peroxide/methanol block for endogenous peroxidase activity. The antibody for yellow fever and
a negative control were added to replicate sections and incubated in a moist chamber for one hour at room temperature. The sections were incubated with biotinylated secondary antibody and avidin-biotin complex reagent with appropriate washes in PBS. Slides were placed in a 0.05% diaminobenzidine containing hydrogen peroxidase and the color reaction was allowed to develop. The slides were washed in water and counterstained with potassium ferrocyanide solution. Slides were further counterstained with Mayer's hematoxylin, dehydrated, and mounted in Permount. Finally, slides were examined by light microscopy.

**Rainfall and Temperature**

Data on rainfall and temperature (min and max) were obtained from the Instituto Nacional de Meteorologia that reports monthly from its meteorology stations located at several sites in each state of Brazil.

**Mosquito Capture**

Mosquitoes were collected between 9:00 AM and 3:00 PM, both at ground and canopy levels, and identification of all species, including *Haemagogus janthinomys*, was made using an entomological microscope.

**RESULTS**

During the first half of 2000, a total of 405 serum samples from suspected patients and people living or working with them were examined by IgM-ELISA and plaque reduction neutralization tests and by virus isolation. Liver samples from 61 patients were also studied. Of the total, 73 (18%) were positive for yellow fever virus. Suspect cases of yellow fever were defined as all unvaccinated people exposed to sylvan mosquito bites in areas reporting cases presenting with signs and symptoms compatible with yellow fever.

The distribution of cases by month in Goiás state is shown in Figure 1. In this state, the epicenter of the outbreak, 50 cases (64.9%) were diagnosed in people living in 21 municipalities (Fig. 2). Of these, 11 municipalities also reported epizootic circulation of yellow fever virus. It is noteworthy that 19 other municipalities reported only epizootic circulation of the virus, totaling 40 municipalities with yellow fever virus activity. Yellow fever cases were also diagnosed in Bahia state, 10 (13.7%) from Coribe municipality; in Tocantins, 6 (8.2%) from four municipalities; in Mato Grosso, 4 (5.5%) from four municipalities; in Minas Gerais, 2 (2.7%) from Natalândia and Planura municipalities in the Unai region; in São Paulo, 2 (2.7%) from Santa Albertina municipality; in Amazonas, 1 (1.4%) from Manacapuru; in Distrito Federal, 1 (1.4%) from Planaltina; and in Pará, 1 (1.4%) from Anajás. Figure 3 shows the approximate sites where yellow fever cases were reported in Bahia, Goiás, Minas Gerais and São Paulo states.

Sixty-five patients (84.4%) were male. The case-fatality rate was 50.6% (39/77). Patient ages ranged from 13 to 74 years, and 97.4% (75/77) were > 15 years old (Table I).

Of the 77 cases, 3 with fatal outcome were diagnosed on the basis of clinical and epidemiological information,

![Fig. 1. Association of rainfall, temperature and monthly distribution of yellow fever cases in Goiás State, Brasil, 1980–2000. For the 1980–1999 period, all cases reported are shown.](image-url)
since no specimens were collected from these patients. Of the 74 with laboratory diagnosis, 50 (64.9%) were made exclusively by serology, 8 (10.4%) by immunohistochemistry, 3 (4.0%) by virus isolation and serology, 2 (2.7%) by virus isolation and immunohistochemistry, and 11 (14.9%) by immunohistochemistry and serology.

A sero-survey was carried out in relatives and people (n = 30) working together with patients of the two first cases in Bahia state, and showed a symptomatic/asymptomatic coefficient of $\frac{1}{4}$. Six more infections of people who were apparently neither ill nor vaccinated were confirmed by IgM–ELISA and plaque reduction neutralization tests.

Agricultural workers, tourists, carpenters, fishermen, and truck drivers were mostly affected with 30, 11, 4, 4, and 3 cases, respectively. In 9 patients, no information was obtained.

*Haemagogus janthinomys* was the main vector. Strains were obtained from pooled groups of this species. A single strain was also obtained from a pool of *Aedes scapularis*. Virus was not isolated from pooled groups of other mosquito species. Epizootics were reported in 40 municipalities of Goiás state, 10 of Minas Gerais state (near the São Paulo state border and close to the municipality of Santa Albertina, where cases were reported), seven of Tocantins state and two of Bahia state. Five yellow fever virus strains were isolated from blood and liver specimens of howler monkeys (*Alouatta caraya*). Specific yellow fever antigens were detected in the liver of nine out fourteen (64.3%) monkeys by immunohistochemistry.

Climatic changes with increase in rain were associated with epidemic/epizootic episodes. Rain was particularly intense in November and December 1999, allowing a large number of mosquitoes to breed in several municipalities where yellow fever was diagnosed both in humans and monkeys. Table II shows the comparative data of rainfall and temperature in Goiás state between 1931 and 2000 (January–June), and Figure 1, compares these data with monthly occurrence of yellow fever cases.

### DISCUSSION

Yellow fever has been diagnosed year after year in Brazil. The cases have been reported in the Amazon and central western regions that represent more than 90% of the endemic area of disease [Mondet et al., 1996; Vasconcelos et al., 1997]. The virus is reported sporadically outside this region, especially near an area considered epidemic or transitional for yellow fever. The occurrence of cases in states that were
considered outside of endemic and epidemic areas is noteworthy. The last cases reported in São Paulo and considered outside of endemic and epidemic areas is noteworthy. The last cases reported in São Paulo and considered outside of endemic and epidemic areas is noteworthy. The last cases reported in São Paulo and considered outside of endemic and epidemic areas is noteworthy. The last cases reported in São Paulo and

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Bahia state is located in the Northern region and is one of the driest Brazilian states. The annual rainfall in the area in which the cases were reported ranges from very low in the winter to moderate in summer. Normally, the rain ranges within an average of 0–100 mm, respectively in the winter (dry) and summer (wet). This year the rainfall reported in the summer was considered higher for all regions and particularly in the areas where cases were reported, where this high precipitation was followed by an increase in the numbers of the mosquito population, near areas in which yellow fever virus was circulating in Goiás, Tocantins, and Minas Gerais states.

The vector once more was the Culicid Haemagogus janthinomys, the main vector in Brazil and in almost all South America Dégallier et al., 1992; Monath 1988. Seven strains of yellow fever virus were isolated from pools of this species. Except for a single strain obtained from a lot of Aedes scapularis captured on human bait in Bahia state, no other yellow fever virus strains were isolated from the other potential yellow fever vectors captured, including Haemagogus leucocelaenus, Haemagogus tropicalis, Aedes fulvus, Sabethes chloropterus, Sabethes soperi, Sabethes cyaneus, and Sabethes quasicyaneus. In a large previous epidemic reported in 1972-73 in the same area [Pinheiro et al., 1978] yellow fever virus was also isolated only from Haemagogus sp.

The occurrence of transmission patterns such as occurred this year, had in the past only been observed during the 1940s, when the time between breakouts was about six years and the movement of virus was considered to be the result of epidemic/epizootic waves [Strode, 1951; Monath, 1988]. This pattern was not observed during this epidemic. In fact, in 2000, the transmission pattern is not compatible with epidemic/epizootic waves, since the occurrence of the cases and epizootics were observed almost simultaneously in areas as far as 2,000 km. Apart the explosive transmission in December 1999–February 2000, the period during which almost 100 yellow fever cases were reported, is typically associated with the presence of the virus in the area, and not as a consequence of transport of the yellow fever virus by the movement of monkeys and vectors.

In December 1999, 16 and 11 yellow fever cases were reported in Tocantins and Goiás states, respectively. This month represented the beginning of the rainy season, that probably were the first cases of this outbreak.

Although the true role that climatic changes play in affecting the life cycle of vertebrates and invertebrates is still unclear, much evidence has been acquired that the increase in rainfall and in temperature may be positively associated with alterations in the productivity of food for animals, consequently favoring biodiversity and the numbers of their populations [Month, 1997; Mills and Childs, 1998; Daszak et al., 2000; Wuehrich, 2000]. On the other hand, it is known that increase in the environmental temperature has been associated with better transmissibility of yellow fever virus [Strode, 1951]. It is reasonable to suppose that these alterations played an important role in the speed with which the mosquitoes could transmit the virus but also the spread of this yellow fever epidemic, because a higher temperature is followed by larger number of infections in the mosquito population [Whitman, 1951]. Table II shows that Brazil is living a period of warming. Effectively, the temperature in Goiás state has increased 2 degrees above average in 2000, and the rains increased 25% in the first two months, in comparison with the means found during the past 20 years. Therefore, it is correct to suppose that the increase in the temperature associated with more intense rain had been responsible, in part, for the dispersion of the virus, which explains the number of yellow fever cases. Of course, this also happened because outbreaks were absent from that region for more than ten years, time enough to renew susceptible populations of both monkeys (amplifier hosts) and humans. This resulted in the epidemic.

Although climatic changes play an important role in transmission, they do not provide a satisfactory explanation for the sudden explosion in transmission of yellow fever virus in that region. It is unclear what other factors were associated in the widespread and

**Table II. Yellow Fever Cases Reported, Rainfall, and Temperature in Goiás State, Brazil, 1980–2000***

<table>
<thead>
<tr>
<th>Months</th>
<th>No. of cases reported*</th>
<th>Temperature</th>
<th>Rain (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>8 (0.4 case/year)</td>
<td>18</td>
<td>22 (±0.93)</td>
</tr>
<tr>
<td>February</td>
<td>4 (0.2)</td>
<td>22</td>
<td>22 (±0.98)</td>
</tr>
<tr>
<td>March</td>
<td>14 (0.7)</td>
<td>9</td>
<td>22 (±0.95)</td>
</tr>
<tr>
<td>April</td>
<td>6 (0.3)</td>
<td>2</td>
<td>21 (±1.25)</td>
</tr>
<tr>
<td>May</td>
<td>4 (0.2)</td>
<td>1</td>
<td>20.5 (±1.0)</td>
</tr>
<tr>
<td>June</td>
<td>2 (0.1)</td>
<td>0</td>
<td>20 (±1.56)</td>
</tr>
<tr>
<td>December</td>
<td>16 (0.8)</td>
<td>0</td>
<td>24.1 (±1.03)</td>
</tr>
</tbody>
</table>

*Yellow fever cases were not reported in Goiás State in the following years: 1982–1985; 1990–1993; 1995–1998.

Total yellow fever cases reported in period; parentheses (average for the period or standard deviation); bold: figures for 2000. Source: Centro Nacional de Epidemiologia (Cases), Instituto Nacional de Meteorologia, Brazil.
speed of yellow fever virus movement in an area, that is larger than Germany, in a few weeks. Certainly, the infection rate in the Haemagogus population was increased. Based on several observational and field studies, it is believed that yellow fever virus was present in many areas of the region all along. Indeed, yellow fever virus would be transmitted vertically among vectors, especially Haemagogus janthinomys, and that, the increase in the rain and temperature had favored the increase in the mosquito population and a highly susceptible monkey population in the areas. Thus the epidemic wave, following monkey and mosquito movement, is responsible for infection in areas where yellow fever was absent for many years, close to areas with active transmission of yellow fever for years. This probably explain the resurgence of yellow fever in Bahia and São Paulo states, since cases were reported in neighboring areas of Goiás (near Bahia state) and Minas Gerais (near São Paulo state).

This is possible if we consider that vertical transmission of yellow fever virus has been documented under laboratory conditions [Dutary and LeDuc, 1981; Month, 1988]. On the other hand, previous studies carried out in the Amazon region in 1997-2000 in Altamira municipality, has showed the presence of yellow fever virus during the rainy season. Indeed, yellow fever have been isolated every year in the rainy season period from Haemagogus janthinomys mosquitoes and, occasionally also from monkeys, in the same area along the secondary road of Transamazon Highway. Human cases were not reported because people are immunized with 17D vaccine [Vasconcelos et al., 2001].

Further molecular analysis of the isolates from different areas circulating during the epidemic may help in understanding the dynamic circulation of yellow fever virus in large areas, as well as provide information as to whether a single genotype was the common source of the strains, or co-circulation of different genotypes was associated with the transmission. Moreover, if different genotypes were circulating simultaneously in different and widely separated geographical areas. These data could support the hypothesis of the persistence of yellow fever virus in a municipality.

During the 2000 epidemic, excepting a strain obtained from Aedes scapularis, yellow fever virus was isolated exclusively from pooled groups of Haemagogus janthinomys. This mosquito species is especially susceptible to yellow fever virus and is primatophilic, biting human beings when they intrude their ecosystems. In these circumstances, men act as accidental host in substitution of nonhuman primates. The role of men in the maintenance of the jungle yellow fever cycle is not well understood, but it is believed that humans are only dead-end hosts without importance for virus survival [Strode, 1951].

If our hypothesis is correct, we urge that yellow fever vaccination be implemented in all areas where yellow fever virus circulated recently in 2000, because they may be at risk of transmission and, as many of Goiás, São Paulo, Bahia, Pará, and Tocantins states are also Aedes aegypti infested, at risk of the re-urbanization of disease. Regarding this aspect, it is important to emphasize that from 1998 through June 2000, almost 45 million people were vaccinated against yellow fever in Brazil, especially in the central-western and southeast regions.

There has been speculation about re-urbanization of yellow fever in South America, and particularly in Brazil [Mondet et al., 1996; Robertson et al., 1996; Vasconcelos et al., 1997; Van der Stuyft et al., 1999]. In 2000, the first cases were reported in Brasília, Rio de Janeiro, São Paulo, Goiânia, and Campinas (Fig. 3) in people who had been in the Chapada dos Veadeiros National Park during the Christmas and New Year season. All the above cities had, at the time of occurrence of cases, high levels of Aedes aegypti infestation, and dengue transmission had been reported in all except São Paulo City. It is possible that no patient had been bitten by this mosquito, but the risk was very high, and either all people should be vaccinated or, alternatively, an efficient Aedes aegypti control program should be established in the whole country, in order to avoid the re-urbanization of this virus.

The small number of yellow fever cases in people < 15 years of age (n = 2), reinforces that the fact that the rate of vaccination of children is very good. Effectively, yellow fever vaccine was included into the expanded program of immunization for the endemic/epizootic regions in Brazil since 1998, but Goiás state was using yellow fever for vaccination of children since 1991. This is in contrast to the Amazon region, where the occurrence of yellow fever among children under 15 years is high [Vasconcelos et al., 2001, in press].

ACKNOWLEDGMENTS

The authors are grateful to the Instituto Nacional de Meteorologia for meteorological data; Miss Marli Mesquita da Silva of Centro Nacional de Epidemiologia/FUNASA at Brasília for computer support in preparation of the figures, and Dr. John P. Woodall of the Federal University of Rio de Janeiro for the revision of the manuscript.

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