AN EPIDEMIC OF YELLOW FEVER IN CENTRAL BRAZIL, 1972–1973

I. EPIDEMIOLOGICAL STUDIES*

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Abstract. An epidemic of jungle yellow fever occurred in Goiás State, Brazil, between December 1972 and March 1973. Laboratory confirmed cases were observed in 36 counties located in the central and southern parts of the State. Seventy-one cases were proved, of which 44 were fatal. The diagnosis was made on the basis of pathology, serology, and virus isolation. Besides yellow fever, malaria and viral hepatitis were present, and in two fatal cases there was malarial pigment in the liver in addition to the specific lesions associated with yellow fever virus infection. The fact that male patients strikingly outnumbered females (9:1) and that young adults were predominantly affected indicates that transmission occurred mainly inside or adjacent to the forests. The lack of cases in urban areas can be attributed to the absence of Aedes aegypti in these areas. Yellow fever complement-fixing antibody in high titers was found in 18 of 1,201 (1.4%) persons living in eight counties of the affected area. This finding suggests that at least 21,000 persons out of the 1.5 million rural inhabitants of the three districts where the epidemic occurred had been infected by the virus. The epidemic subsided following an intensive vaccination campaign, and the last four cases were observed in March 1973.

In December 1972 three persons from Silvania County, Goiás State, died of yellow fever (YF); the diagnosis was made by histopathologic examination. During the following month more cases of the disease were suspected or recognized in other counties of the same State. These events prompted us to carry out epidemiological and ecological investigations in the affected areas from 25 January 1973 until the end of March. As a result of these investigations it was learned that the epidemic involved more than 30 counties of Goiás State. Most of the patients were studied at the Oswaldo Cruz Hospital in Goiânia; however, a smaller number was seen in other hospitals in this city or in the interior of the State, as well as in outpatient clinics. In addition, an attempt was made to assess the extent of the epidemic by means of a survey for detection of YF antibody in normal people from eight counties, in four of which proved cases of the disease had occurred.

MATERIALS AND METHODS

Virus isolation and identification
Blood or viscera were placed in liquid nitrogen immediately after collection. At the Institute Evandro Chagas they were transferred to a −70°C mechanical freezer until the time of the inoculations. Serial 10-fold dilutions of the specimens varying from 10⁻⁴ to 10⁻⁶ were inoculated by the intracerebral route into 1- to 2-day-old white Swiss mice and, in some cases, into Vero cell cultures. Virus identification was performed by the complement fixation (CF) test, according to a technique described earlier. Mouse immune ascitic fluid prepared against the Be H111 strain of YF virus isolated in the Amazon region was employed in the identification.

Antibody determinations
Complement-fixing antibodies were determined by the method referred to above. Methods for

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performing the hemagglutination-inhibition (HI) test, for preparing the antigen, and for treating the serum have been described previously. Sera of patients were tested by HI and CF against YF, St. Louis encephalitis (SLE), Ilheus and Bussaquara viruses, the only flaviviruses known to exist in Brazil. These virus strains had been isolated in the Amazon region. In the neutralization (NT) tests the constant virus, serum dilution method was adopted. The tests were carried out in microplates using Vero cell cultures according to a technique employed in this laboratory. Briefly, serial 4-fold dilutions of the sera, previously inactivated at 56°C for 30 min, were made with loops in microplates, using four wells per dilution, then about 100 TCD₅₀ of virus was added to each well. The microplates were incubated at 37°C for 1 h, followed by addition of 30,000 cells per well. The serum titer was expressed as the highest dilution inhibiting the appearance of the cytopathic effect in 50% of the wells into which it was inoculated.

RESULTS

Diagnosis

A total of 71 cases were diagnosed, 1 by isolation of the virus, 26 by serology, and 44 by histopathology.

Virus isolation

The virus was isolated from a blood sample collected on 16 February from a 7-yr-old boy of Mara Rosa County, who had been taken to a local YF vaccination post. He had developed fever, headache, nausea, and joint pains in the previous 2 days, during which time he was living on a farm 3 km from the city of Mara Rosa. Apparently he recovered uneventfully from his illness. No virus could be isolated from the blood of 37 other suspected cases, although 13 were found later by serology or by pathology to have been infected with YF virus. Two of these persons were in the 4th day of illness and the others in the 6th or later days of illness at the time they were bled for attempted virus isolation. Of the remaining 24 cases, blood smears were obtained from five for malaria examination; all five were positive for *Plasmodium falciparum*. Attempts to recover the virus from tissues of the liver, kidney, lung, spleen, heart, brain, and stomach of four fatal cases were negative. In two instances the autopsy was performed 2 and 4 h after death, and in the other two about 7 to 8 h later.

Serology

Of the 26 cases confirmed by serology, 14 showed a rise in titer of YF HI antibody between paired sera with a corresponding increase in titer of CF antibody, except for two patients whose sera reacted nonspecifically. Another 12 cases were presumptively diagnosed as YF infections on the basis of single serum specimens with YF CF antibody titers equal to or higher than 1:32. These patients also had HI antibodies to YF virus, usually in high titers.

It is of interest that 7 of the serologically confirmed cases were among 29 patients hospitalized with a clinical diagnosis of viral hepatitis. Hepatitis B antigen was not found in the serum of these 7 patients, but it was present in 10 of the remaining 22 hepatitis patients who were serologically negative for YF.

The serological pattern developed by the YF patients was always compatible with a primary infection caused by YF virus; YF HI antibody titers in the convalescent patients’ sera were 2- to 16-fold higher than to the other three flaviviruses tested. There was a higher degree of specificity to the YF antigen in the CF test, but still some cross-reactions were observed. In the NT test, however, high YF antibody titers were detected in the convalescent sera and, in contrast, not a single serum specimen neutralized Ilheus virus (Table 1).

Pathology

Forty-four fatal cases were confirmed by the study of liver pathology. The diagnosis was based upon the recognition of classical liver lesions described in human fatalities caused by YF. Most examinations were performed at the Oswaldo Cruz Institute, Rio de Janeiro, but some specimens were also examined at the University of Goiás and five at the Evandro Chagas Institute, in Belém. In two cases, in addition to the typical YF lesions pigments, probably of malarial origin, were also observed in the liver sections. In another three cases only this type of pigment was found in the liver; therefore, a diagnosis of malarial infection was considered. In one of these cases *P. falciparum* was demonstrated in the
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### Table 1

<table>
<thead>
<tr>
<th>Patient code no.</th>
<th>Days after onset</th>
<th>CF test</th>
<th>HI test</th>
<th>NT test</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>YF</td>
<td>BSQ</td>
<td>ILH</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>0†</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>27</td>
<td>512</td>
<td>512</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>53</td>
<td>32</td>
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</tr>
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<td>272</td>
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<td>0</td>
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<tr>
<td>350</td>
<td>32</td>
<td>32</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* YF, yellow fever; BSQ, Bussuquara; ILH, Ilheus; SLE, St. Louis encephalitis.
† Reciprocal of serum dilution.

### General

Confirmed cases of YF occurred in at least 36 counties of Goiás State (Fig. 1). Among the cases in which time of onset was known, 12 occurred in December 1972. Ten of these patients died, seven of them in January 1973. The number of cases increased to 29 during January, followed by a small decrease in February and by a marked decline in March (Fig. 2). No additional cases were detected, despite the fact that serum specimens from patients admitted to the Oswaldo Cruz Hospital through May were examined for evidence of YF infection, and routine surveillance through viscerotomy of fatalities in the area was continued. It should be noted that an intensive vaccination campaign against YF was started in the area in January. The sex of 70 patients could be determined but the age was known for only 67 of them. As seen in Table 2, male cases strikingly outnumbered females (9:1). The majority of patients were in the age group between 11 and 30 years, and the youngest cases recorded were in a boy and a girl both aged 4 yr. All cases for which it was possible to obtain information regarding living place or activity came from the rural areas.

The case fatality rate among 40 patients admitted to the Oswaldo Cruz Hospital in Goiania was 40%.

### Serum survey

Between the end of January and the end of March 1973, serum samples were obtained from 1,201 apparently normal persons in eight counties of Goiás State (Fig. 1). Laboratory-proved YF infections were detected in four of these counties. Most of the samples were collected at the time the individual came to be vaccinated against YF. The majority of the people sampled (55%) lived in forested rural areas; however, many of the urban residents reported periodic contact with forested areas. Males slightly outnumbered females. In the HI test, 136 (11.3%) of the 1,201 sera reacted specifically with the YF antigen and/or with one or more of the other three flaviviruses tested. Positive reactions were observed...
TABLE 3

<table>
<thead>
<tr>
<th>County</th>
<th>No. of persons tested</th>
<th>Persons with flavivirus HI antibody*</th>
<th>Persons with specific YF CF antibody†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formosa</td>
<td>173</td>
<td>18 (10.8%)</td>
<td>4 (2.3%)</td>
</tr>
<tr>
<td>Cabeceiras</td>
<td>78</td>
<td>15 (19.2%)</td>
<td>2 (2.5%)</td>
</tr>
<tr>
<td>Mara Rosa</td>
<td>119</td>
<td>18 (15.1%)</td>
<td>2 (1.6%)</td>
</tr>
<tr>
<td>Goiás</td>
<td>19</td>
<td>2 (10.5%)</td>
<td>0</td>
</tr>
<tr>
<td>St. Luís M. Belos</td>
<td>52</td>
<td>6 (11.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Catalão</td>
<td>166</td>
<td>10 (6.0%)</td>
<td>3 (1.8%)</td>
</tr>
<tr>
<td>Aporé</td>
<td>269</td>
<td>30 (11.1%)</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>Itajá</td>
<td>325</td>
<td>37 (11.3%)</td>
<td>6 (1.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>1,201</td>
<td>136 (11.3%)</td>
<td>18 (1.4%)</td>
</tr>
</tbody>
</table>

* Yellow fever, Saint Louis encephalitis, ilheus and Bussuquara viruses, the only flaviviruses known in Brazil.
† Specific and cross reactions.
‡ Results referring to 104 out of the 136 sera positive by HI. Only sera with YF antibody equal or higher than 1:32 were recorded.

in all eight counties (Table 3), and the antibody rate was about twice as high in males as in females. Of the 136 HI reactors, 107 (78.7%) were among 1,010 persons who gave a negative history of past YF vaccination, 24 (17.7%) were among 161 who were said to have been vaccinated previously against YF, and 5 (3.7%) were among 30 who were unable to give precise information.

The CF test was performed on 104 of the 136 HI-positive sera and 18 (only 1 with a past history of YF vaccination) had an antibody titer equal to or higher than 1:32, as shown in Table 3. It can be seen in the same table that CF antibodies to YF virus were detected in residents of only 6 of the 8 counties. The CF antibodies were about twice as frequent in males as in females.

Analysis of flavivirus antibodies by age group (Table 4) showed a higher incidence of the HI-positive reactions among persons above 30 yr of age. However, the YF CF antibody rate was not markedly different for any of the age groups, except that children under 10 yr of age were negative for this type of antibody.

Flavivirus CF antibodies were also measured in the sera of 220 non-vaccinated inhabitants of Formosa and Cabeceiras counties; those individuals were asked whether they had a febrile illness within the previous month. As shown in Table 5, 2 of 6 (33.3%) of persons with a yellow fever CF antibody titer equal to or higher than 1:32 gave a history of recent febrile illness, and 2 of
FATAL CASES

NON-FATAL CASES

10 (20%) of those with an antibody titer of 1:8–1:16 also reported fever. In contrast, only 14 of 204 (6.8%) of those whose sera was CF-negative were said to have had fever in the previous month.

**DISCUSSION**

The outbreak of yellow fever described here which involved the central and southern parts of Goiás State demonstrates, as has long been observed, that the infection is still able to reappear in such a zone. The first proved outbreak in Goiás State occurred in 1935; following this outbreak, others have been recognized at intervals of 5 to 9 yr, all in rural areas (Fig. 3). It is logical to assume that these outbreaks reflect YF virus excursions from the enzootic areas of the Amazon region. Nevertheless, its route of invasion is not clear. It has been observed in the past that certain outbreaks in Goiás State are preceded by initial outbreaks in a locality situated in Mato Grosso State, in the watershed between the Amazon and Paraná basins. Such an event, however, was not observed this time, although it is possible that it did occur but escaped the surveillance. The gallery type of forest still existing in the presently affected regions offers favorable ecological conditions to support the spread of the virus.

The much higher proportion of male-over-female cases hospitalized (9:1), and the fact that young adults were more commonly affected,
indicates that transmission occurred inside or adjacent to the forests. The isolation of the virus from Haemagogus mosquitoes collected in the forests of two counties further supports this assumption. It is not improbable, on the other hand, that some transmission may have occurred inside or near the dwellings located close to the forest. In fact, a few Haemagogus were captured inside or around some such houses, but at a much lower rate than inside the forest. However, if domiciliary or peridomiciliary transmission to man did occur, it probably was a rare event. The absence of urban cases can be explained by the absence of Aedes aegypti mosquitoes in the cities of the area.

The isolation of the virus from the blood of only a single patient, and the failure to recover it from the blood of 13 cases of YF infection confirmed by serology or pathology, can be explained by the fact that the latter patients were bled in the non-viremic stage of the disease. Serial dilutions of the blood of these patients were inoculated into newborn mice and sometimes into Vero cells, but this approach was unrewarding.

Besides YF infection, malaria and viral hepatitis were present among the cases admitted to the Oswaldo Cruz hospital. Although blood smear examination of malaria cases usually reveals the malarial parasites, they were not detected by two
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The virus, Reliability of the CF test is indicated by the good degree of correlation found between CF positive cases and recent occurrence of fever; among the 220 persons of Formosa and Cabaceiras counties examined by the CF test against flavivi-rus- virus the incidence of a febrile episode in the month previous to the bleeding was five times higher in those who reacted in high titer with the YF antigen than in those who did not (Table 5). On the basis that the serological survey showed a rate of 1.4% of recent YF infections, and considering that about 1.5 million people live in the rural areas of the three districts affected, it can be estimated that 21,000 people were infected by the virus.

The intensive vaccination campaign in the area with the 17D strain of yellow fever was almost certainly responsible for the interruption of the epidemic. The campaign was started by the middle of January 1973, and within 60 days around 80% of the population of the area had received the vaccine. During this period the climatic conditions remained the same.

Spread of this epidemic focus to neighboring states, as was seen in the years 1934–1940,7 was not observed. The reasons for this lack of spread are not clear, but it can be postulated that effective vaccination against yellow fever in the neighboring states, and the extensive deforestation which has occurred in those states, acted as controlling factors deterring the dissemination of the virus. It should be noted, however, that 11 fatal cases of yellow fever were diagnosed in 1973–1974 in Minas Gerais and in Mato Grosso states,8 which border Goiás State, and that 9 cases were also recognized in Paraguay during 1974.9 Those outbreaks show that the infection can still spill over further to the south and west. These facts demonstrate the need for a continuing YF program for vaccination of the susceptible generations, especially of people who live in rural areas.

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REFERENCES


