The discovery of Australia antigen (2) and its relationship to Type B hepatitis (3), together with development of specific tests for the antibody (4-6), has paved the way for new studies on jungle hepatitis. This article reports on one such study based upon examination of human sera from inhabitants of the eastern jungles and northern coast of Peru. It describes the incidence of the antigen (HB Ag), as determined by single radial immune-diffusion and counter-electrophoresis, and discusses the association between HB Ag and jungle hepatitis in Peru.

Introduction

Shortly after the discovery of Australia antigen (hepatitis B antigen, HB Ag), seroepidemiologic studies in various parts of the world showed that its prevalence among tropical African and Southeast Asian populations was generally between 5 and 20 per cent, whereas in Europeans and North Americans it was between 0.1 and 0.6 per cent. A higher incidence has been reported among males than among females in all regions, and in tropical countries the antigen is found more frequently in children between five and 15 years of age (1).

Countries like Peru, with sharply contrasting geographic areas, offer a unique opportunity to explore this matter of differing prevalences. For this reason a serologic survey was recently carried out with sera collected in 1965, 1967, 1970, and 1972 from residents of eastern Peru, and with sera collected in 1969 from residents of the Department of Tumbes, a region along the country’s northern coast.

Hepatitis cases in the jungle areas of Peru,
including cases with severe clinical pictures, have presented a health problem of mounting concern over the past decade. In the jungle department of Loreto, for example, hepatitis morbidity per 100,000 inhabitants rose from 55.67 in 1961 to 118.05 in 1970.7

In the past, since specific laboratory tests for hepatitis were not available, and since yellow fever and leptospirosis were also endemic in these areas, serologic tests for these diseases were carried out and a serologic diagnosis could not be established. Instead, hepatitis was diagnosed by epidemiologic and clinical methods, by serologic verification that other liver infections were not present, and in fatal cases by histopathologic examination.

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7Data provided by Dr. C. Silva, epidemiologist, Ministry of Public Health, Lima, Peru.

**Materials and Methods**

**Geographic Considerations**

Sera for the study came from residents of the north coast department of Tumbes and from a variety of areas east of the Andes Mountains (see Figure 1). Most of eastern Peru is a dense tropical rain-forest area that runs north to south along the eastern fringe of the Andes. In contrast, the Department of Tumbes is semi-desert, a transitional area between coastal rain forests near the Equator and Peru’s coastal desert.

**Collection of Sera**

A total of 2,593 sera from apparently healthy men, women, and children from one through 85 years of age were used for the study. These were collected at the following times and places:

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**TABLE 1—Peruvian human sera tested for hepatitis B antigen, listed by the year and area of collection of the specimens and the ethnic group, area of residence, age, and sex of the test subjects.**

<table>
<thead>
<tr>
<th>Geographic area and year of collection</th>
<th>Ethnic origin of subjects and area of residence</th>
<th>No. of sera tested over</th>
<th>No. positive for HB Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Children ≤15 years</td>
<td>Males &gt;15 years</td>
</tr>
<tr>
<td>Eastern Peru 1965</td>
<td>Mestizos: north, central, south</td>
<td>145 (3)</td>
<td>378 (4)</td>
</tr>
<tr>
<td></td>
<td>Indians: north, central, south</td>
<td>60 (0)</td>
<td>201 (2)</td>
</tr>
<tr>
<td>Eastern Peru 1967</td>
<td>Mestizos: Curaray River</td>
<td>– (0)</td>
<td>– (0)</td>
</tr>
<tr>
<td>Northern Peru 1969</td>
<td>Mestizos: Department of Tumbes</td>
<td>240 (0)</td>
<td>322 (3)</td>
</tr>
<tr>
<td>Eastern Peru 1970</td>
<td>Mestizos: Iquitos</td>
<td>23 (0)</td>
<td>70 (3)</td>
</tr>
<tr>
<td>Eastern Peru 1972</td>
<td>Mestizos: Tigre and Cenepa rivers</td>
<td>21 (3)</td>
<td>119 (5)</td>
</tr>
<tr>
<td></td>
<td>Mestizos: Iquitos</td>
<td>8 (0)</td>
<td>158 (3)</td>
</tr>
<tr>
<td></td>
<td>Mestizos and Indians: departments of Junín and Cerro de Pasco</td>
<td>– (0)</td>
<td>– (0)</td>
</tr>
</tbody>
</table>

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In 1965 serum samples were obtained from mestizos living in Iquitos, Tingo María, Pucallpa, Quincemil, Iberia, and rural areas along the middle portion of the Huallaga River, and from 20 Indian tribes living along the eastern jungle region's major rivers and tributaries.

In 1967 samples were collected along the Curaray River, a tributary of the Napo River in the eastern department of Loreto.

In 1969 samples were obtained from inhabitants of the three provinces of the northern department of Tumbes.

In 1970 further samples were procured from outpatients at the Iquitos General Hospital. Others were also collected in 1972 at Iquitos, along the Tigre and Cenepa rivers (both tributaries of the Marañón), and among rural inhabitants of Chanchamayo and Oxapampa in the respective departments of Junín and Cerro de Pasco.

All of these sera, classified as to age and sex of donor, year of collection, and geographic location of the collection site, are listed in Table 1.

Also, tests were performed on sera from 43 male hepatitis patients between 20 and 24 years of age. These were collected at a local Iquitos hospital in 1972 and early 1973 during a hepatitis outbreak in the northeastern jungle region.

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8As used here, the term "Indian" refers to native people with characteristic customs and features living within a tribal system; they may or may not be racially mixed. "Mestizo," on the other hand, refers to residents of mixed white and Indian heritage who are not living inside a tribal system.
Nine of the 43 patients were from Iquitos proper, while the other 34 came from rural areas along nearby tributaries of the Amazon River. Single serum specimens, obtained between 21 and 118 days after the onset of illness, were procured from 27 patients, and a series of two or more specimens were procured from the other 16.

All the blood samples were obtained by venipuncture, using disposable syringes or vacutainers. After clotting, if local facilities permitted, the sera were separated in the field, transported under refrigeration to a local center, and frozen with dry ice or liquid nitrogen. Otherwise, the whole blood was transported under refrigeration to Lima for serum separation, and the sera were then placed in frozen storage until testing.

Test Procedures

All specimens were tested by single radial immune diffusion (SRID) using Esaï plates, by counter-electrophoresis (CEP) using the Abbott AUS-tect™ CEP test system, or by both methods. In general these two tests produced results that were in close agreement. All positive results were confirmed by double agar gel diffusion and by complement-fixation, using Abbot AUS-tect™ test systems. Occasionally, when the results were not consistent, the solid-phase radioimmune assay developed by Ling and Overby (7) as the “AUS-RIA-125” kit was also used.

Results

The results are shown in Table 1, where the total number of sera positive for HB Ag are indicated in parentheses, underneath the total number of sera tested in each group.

Geographic Distribution of HB Ag

Thirty-three (1.8 per cent) of the 1,803 sera collected from eastern Peru were positive for HB Ag, while only four (0.5 per cent) of the 790 specimens collected along the north coast were positive. Scattered rural dwellers in eastern Peru showed roughly the same proportion of positive responses as town and city dwellers in this area; that is, twenty-four of 1,363 sera from rural subjects (1.7 per cent) were positive for HB Ag, and seven of 440 sera from town and city dwellers (1.5 per cent) were also positive. However, there seemed to be “hot spots” of HB Ag incidence in northeastern rural populations, since three (5.0 per cent) of the specimens collected on the Curaray River and ten (6.4 per cent) of the sera collected along the Tigre and Cenepa rivers were positive. Although these rural localities are far from one another, they are all in the same watershed area and experience similar climatic conditions. There is no indication that the five-year time difference between the taking of samples along the Curaray (1967) and along the Tigre and Cenepa (1972) had a significant impact on the percentages of inhabitants positive for HB Ag.

Distribution of HB Ag among Different Population Groups

Tests performed on serum samples collected from 20 Indian tribes and from mestizos of eastern Peru in 1965 indicate that both groups had about the same incidence of HB Ag (1.1 per cent).

Based on results from more than 2,300 tests, the antigen was found to be more prevalent in males (1.6 per cent) than in females (0.9 per cent).

In areas with a high incidence of HB Ag, this high incidence tended to be especially pronounced among children. Thus three sera out of 21 (14.3 per cent) from children of the Tigre and Cenepa river areas were positive.

Association of HB Ag with Hepatitis in Eastern Peru

Five (18.6 per cent) of the 27 single serum specimens taken from hepatitis patients during the 1972-1973 hepatitis outbreak were positive for HB Ag. However, of the 16 persons from whom multiple specimens were obtained, 13 (81.2 per cent) provided at least one specimen
that was positive for HB Ag. There was thus a strong correlation between hepatitis infection and the presence of HB Ag in this group of hepatitis patients from eastern jungle areas. (All the serum specimens, both single and multiple, were tested by SRID, CEP, double agar gel diffusion, and complement-fixation.)

Discussion

The overall HB Ag incidence found in this survey (1.4 per cent) is lower than that usually found in Southeast Asian countries (5 to 20 per cent) and higher than that generally found in European and North American countries (0.1 to 0.6 per cent). Also, the survey incidence of HB Ag in males was higher than in females, a finding similar to that reported by many other authors (7). The results also suggest a higher incidence of HB Ag in eastern Peru than along the north coast in the Department of Tumbes.

Regarding sera collected in 1965 from Indians and mestizos in jungle localities, the tests showed sera from each ethnic group to yield a positive result about 1.1 per cent of the time. This is far below the frequency of positive reactions reported for the Peruvian Cashinahuas by Blumberg, et al. (8). It should be noted, however, that the Cashinahuas were not among the 20 Indian tribes included in the present study.

The tests of sera obtained from patients during the 1972-1973 hepatitis outbreak in the northeastern jungle area indicated that HB Ag was associated with 81.2 per cent of those from whom multiple blood samples were obtained. All positive specimens, from apparently healthy carriers of HB Ag as well as from this group of hepatitis patients, have been classed as involving surface antigen subtype adw (9). Taken together, these observations indicate that development and application of a vaccine against viral hepatitis, type B, could significantly reduce the incidence of human hepatitis cases in eastern Peru.

SUMMARY

Single radial immune diffusion and counter-electrophoresis tests were used to examine 2,593 serum specimens from apparently healthy men, women, and children of Peru for the presence of hepatitis B antigen (Australia antigen). The object was to estimate the prevalence of the antigen in two contrasting geographic regions and to investigate the relationship between presence of the antigen and jungle hepatitis in eastern Peru. In connection with the latter goal, both single and serial serum samples collected from hepatitis cases during an epidemic in the northeast were also examined.

Of the 2,593 apparently healthy subjects, the tests showed 1.4 per cent were carrying the antigen. However, when the data were broken down by geographic region it was found that 1.8 per cent of the subjects from eastern Peru were carriers, as compared to only 0.5 per cent of those from the northern coast. Moreover, incidences as high as 5 and 6.4 per cent were found in selected eastern areas, and a peak figure of 14.3 per cent was found in sera from children living in some of these areas.

Comparison of the proportions of male and female sera positive for the antigen indicated that the proportion of males with HB Ag was nearly twice as high. However, sera collected
from Indians of 20 different eastern tribes and from mestizos in the eastern region showed roughly the same proportion of samples positive for HB Ag in each ethnic group.

The study also showed a close correlation between presence of the antigen and hepatitis infection during a 1972-1973 epidemic in eastern Peru. Testing of sera taken from hepatitis patients at that time showed many patients to be carrying HB Ag, specially in cases where serial blood samples were available. In all, positive test results were obtained for 81.2 per cent of the patients from whom two or more samples had been obtained.

REFERENCES