ARBOVIRUS ANTIBODY SURVEY OF SERA FROM RESIDENTS OF EASTERN PERU¹, ²

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A serosurvey by hemagglutination-inhibition (HI) test has provided abundant evidence of arbovirus activity among Indian and mestizo residents of tropical foothill and lowland areas in eastern Peru. The data for the two regions suggest that endemicity is greater in the lowlands and that arbovirus infections there tend to be acquired earlier in life.

Introduction

Yearly recurrences of yellow fever have been reported on the slopes of the Andes Mountains of eastern Peru for many years. For example, a total of 262 cases involving 253 deaths were reported from 1960 to 1966.⁶ These cases all occurred in tropical foothill areas, with the largest number occurring in the central Departments of Huánuco and Junín and the more northern Department of San Martín. Specimens were not available for virologic studies, and the diagnoses were all made on epidemiologic, clinical, and histologic grounds.

Although many arboviruses besides yellow fever virus (YF) have been found in other tropical regions of South America (₁), their possible presence in eastern Peru has never been investigated. In the study reported here, sera were collected from residents of various localities and were examined by the hemagglutination-inhibition (HI) test with antigens for arboviruses belonging to groups A, B, C, Anopheles A, Bunyamwera, California, Phlebotomus, and Simbu.

Materials and Methods

Region of Study

About 60 per cent of Peru's total land surface lies east of the crest of the Andes. The study reported here was carried out in portions of this region extending from latitudes 4° to 14° South and from longitudes 70° to 79° West (see Figure 1). Dense tropical rain forest prevails throughout the area studied, and there are only slight deviations from the average annual temperature of 25°C.

The annual rainfall varies with longitude. Thus the eastern slopes and foothills of the Andes receive a lot of rain (there is an annual average of 3.3 meters at Tingo María in the central foothills, for example); whereas less rainfall occurs further east before the rain clouds reach the Andes (Iquitos has an annual average of 2.5 meters). The rainy season lasts from November to April.

Sampling was carried out in two main
areas: the Andean foothills and the eastern lowlands. Partly on the basis of local ecological circumstances, each of the two areas was subdivided into northern, central, and southern sectors, and an effort was made to obtain sera from persons representative of each natural habitat. Donors were chosen from among two groups: twenty-five of the 36 Indian tribes that live under generally primitive conditions and are heavily exposed to the bite of mosquitoes; and populations of mestizos closely involved with the forest, such as woodcutters, rubber plantation workers, farmers, and road workers.

Each of the mestizo populations sampled was pretty well centered around some particular town or settlement. The Indians, however, were not localized in towns, their habitations extending along the rivers which are their means of transportation. The fol-
lowing reference points identify the local-

**Northern foothills.** The mestizos sampled in this area were from Lamas and the central Huallaga River area in the Department of San Martín (latitude 6°50'South, longitude 76°15'West). Indians representative of those sampled in this area are the Aguarunas, whose territory extends along the upper Marañón River and its tributaries (latitude 5°10'South, longitude 78°20'West).

**Northern lowlands.** Samples were obtained from mestizos in the City of Iquitos and nearby settlements (latitude 3°45'South, longitude 73°15'West). The Ticuna Indian Tribe, located along the Amazon River and around the lakes Caballococha and Cushillococha (latitude 3°50'South, longitude 70°30'West) was representative of the 11 Indian tribes sampled in the region.

**Central foothills.** Samples were obtained from mestizos in the City of Tingo María in the Department of Huánuco (latitude 9°15'South, longitude 76°West). The Amuesha Indians, who live in an area southeast of Tingo María along the upper Pachitea River (latitude 9°50'South, longitude 74°55'West) are representative of the four tribes sampled in the region.

**Central lowlands.** Samples were obtained from mestizos in Aguaytia and Pucallpa (latitude 8°20'South, longitude 74°35'West). The Shipibo Indians, who live in a large area along the Ucayali River and its tributaries, are representative of the six tribes sampled in the region.

**Southern foothills.** Samples were obtained from mestizos in Quince Mil in the Department of Cuzco (latitude 13°15'South, longitude 70°40'West). No Indians were sampled in this region.

**Southern lowlands.** Samples were obtained from mestizos in Iberia in the Department of Madre de Dios (latitude 11°20'South, longitude 69°30'West). No Indians were sampled in this region.

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**Collection and Preservation of Sera**

A total of 1,063 blood samples (655 from mestizos, 398 from Indians) were collected during March-July 1965 from apparently healthy men and women between 5 and 75 years of age (see Table 1): Information about prior yellow fever vaccination was requested of each donor.

Blood samples were taken by venipuncture, using either disposable syringes or vacutainers. After clotting, if local facilities permitted, sera were separated in the field and frozen with dry ice. Otherwise the blood samples were transported under refrigeration to Lima for sera separation and storage at -20°C.

**HI Tests**

These sera were then examined at the Yale Arbovirus Research Unit, New Haven, Connecticut, by means of HI tests performed with plastic disposable plates, using a modification of the microtechnique described by Sever (2). The sera were extracted with kaolin, adsorbed on goose red blood cells, and tested in serial twofold dilutions beginning at 1:10.

Hemagglutinating antigens, prepared according to the techniques of Clarke and Casals (3), were adjusted in most cases to a dilution containing 8 units of antigen; however, with some antigens of group C and with Maguari virus antigen, only 4 units, or occasionally only 2 units, were employed.

The sera were tested against the following 27 arbovirus antigens, except as indicated later.

**Group A.** Aura, BeAr 10316; Eastern equine encephalitis (EEE), BeAn 5122; Mayaro, Tr 4675; Mucambo, BeAn 8; Pixuna, BeAr 35645; Una, BeAr 13136; Venezuelan equine encephalitis (VEE), Trinidad donkey; and Western equine encephalitis (WEE), Tr 25717.

**Group B.** U.S. bat salivary gland (Rio Bravo); Bussuquara, BeAn 4073; Dengue 2,
TABLE 1—Sources of 1,063 sera collected in eastern Peru in 1965, and the percentage of these sera giving a positive HI test reaction with at least one of 27 different arbovirus antigens.

<table>
<thead>
<tr>
<th>Origin of donor</th>
<th>Locality (population)*</th>
<th>Number of sera tested</th>
<th>% sera positive for at least one antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Children (≤15 yrs)</td>
<td>Adult males</td>
</tr>
<tr>
<td>Mestizo</td>
<td></td>
<td>20</td>
<td>123</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>44</td>
<td>32</td>
</tr>
<tr>
<td>Indian</td>
<td></td>
<td>7</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>59</td>
</tr>
</tbody>
</table>


Tr 1751; Ilheus, original; Powassan, Byers; St. Louis encephalitis (SLE), BeAr 23379; and Yellow fever (YF), Asibi.

Group C. Caraparu, BeAn 3994; Itaquí, BeAn 12752; Murutucu, BeAn 974; and Oriboca, BeAn 17.

Anopheles A group. TacaiuMa, BeAn 73.

Bunyamwera group. Guaroa, CoH 35211; Maguari, BeAr 7272; and Tensaw, USA A9 171b.

California group. California encephalitis, BFS 283.

Phlebotomus fever group. Chagres, JW 10; and Icoaraci, BeAn 24262.

Simbu group. Manzanilla, Tr 3587.

Interpretation of Test Results

As considerable numbers of positive reactions were obtained with most of the antigens, it was necessary to use three criteria for interpretation of the results. These criteria were as follows:

1) A reaction was called specific or diagnostic for a given antigen when (a) the titer of the serum against that antigen (8 units with groups A and B and some agents of group C; 4 units with the remaining agents) was at least 1:20 and there was no reaction with any other antigen of the same group, or (b) when the titer of the serum against that antigen was four times as great or more than against any other antigen of the same group.

2) A reaction was called a superinfection when the titer of the serum against two or more antigens of a group was 1:80 or higher and the difference in titers was no more than twofold.

3) A reaction was called undiagnosable (a) when the serum reacted with only one antigen and only at a titer of 1:10, or (b) when it reacted with more than one antigen of a group with titer differences less than fourfold and titer levels of 1:40 or lower. The latter type of reaction is similar to that designated superinfection, but it occurs at a lower level.
By way of illustration, the reactions of 12 sera that met these criteria are shown in Table 2. It must be emphasized that the criterion of specificity applies only within the technical limits of the HI test, and is used here solely to facilitate interpretation of the results. Thus a given specific reaction does not necessarily reflect an actual infection with the virus in question, because the possibility remains that the reaction is instead the result of exposure to another, related virus—one not included in this survey and perhaps not yet even discovered.

Results

Percentages of Positive Sera

As shown in Table 1, the percentage of sera positive for at least one antigen was lowest (81 per cent) among the mestizos of the central foothills and highest (97 per cent) among the Indians of the northern foothills, the northern lowlands, and the central lowlands.

**General Distribution of Antibodies for the Main Arbovirus Groups**

Figure 2 shows the percentages of positive sera in both lowlands and foothills according to latitude. The data suggest that viral activity decreases from north to south in the case of arbovirus groups A and C, and perhaps in the case of the Bunyamwera group as well. It should also be noted that the percentages of lowland sera reacting with these antigens are higher than the percentages of foothill sera reacting, especially with respect to group C antigens. In contrast, the percentage of sera reacting with group B antigens was not affected significantly by the latitude or the terrain (lowlands or foothills) in which the sera were collected.

These group B results are difficult to interpret because yellow fever is known to be

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**TABLE 2**—“Specific,” “superinfection,” and “undiagnosable” reactions of 12 Peruvian sera in hemagglutination-inhibition tests with group B antigens.

<table>
<thead>
<tr>
<th>Serum and interpretation of reaction</th>
<th>Reciprocal of serum titer* with antigen for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YF</td>
</tr>
<tr>
<td><strong>Specific:</strong></td>
<td></td>
</tr>
<tr>
<td>Serum 1: YF</td>
<td>640</td>
</tr>
<tr>
<td>Serum 2: YF</td>
<td>20</td>
</tr>
<tr>
<td>Serum 3: Ilheus</td>
<td>160</td>
</tr>
<tr>
<td>Serum 4: Ilheus</td>
<td>0</td>
</tr>
<tr>
<td>Serum 5: SLE</td>
<td>0</td>
</tr>
<tr>
<td>Serum 6: SLE</td>
<td>0</td>
</tr>
<tr>
<td><strong>Superinfection:</strong></td>
<td></td>
</tr>
<tr>
<td>Serum 7</td>
<td>640</td>
</tr>
<tr>
<td>Serum 8</td>
<td>640</td>
</tr>
<tr>
<td>Serum 9</td>
<td>10</td>
</tr>
<tr>
<td><strong>Undiagnosable:</strong></td>
<td></td>
</tr>
<tr>
<td>Serum 10</td>
<td>40</td>
</tr>
<tr>
<td>Serum 11</td>
<td>20</td>
</tr>
<tr>
<td>Serum 12</td>
<td>10</td>
</tr>
</tbody>
</table>

*0 = no inhibition at a serum dilution of 1:10, the lowest serum dilution used.
FIGURE 2—Results of hemagglutination-inhibition tests with sera from Indians and mestizos of eastern Peru, showing the percentages positive for any antigen tested in arbovirus groups A, B, C, and the Bunyamwera group.

Figure 3 shows percentages of positive sera according to donor origin (Indian or mestizo) and terrain (lowlands or foothills). As indicated, antibodies for arbovirus groups A, C, and Bunyamwera were found to be more prevalent among Indians than among mestizos in both foothill and lowland areas. With respect to group B antibodies, however, the percentage of positive sera was

endemic in all the foothill zones sampled, and therefore yellow fever vaccination is common in these areas. For example, 84 per cent of the donors in the southern foothills said they had been vaccinated against yellow fever, as compared to only 2 per cent of the donors in the northern lowlands and 15 per cent of all the lowland donors.
about the same for Indians and mestizos from the foothills, while in the lowlands the percentage of positive sera was only slightly higher for Indians than for mestizos. Again, these latter observations may be explained on the basis of yellow fever vaccination. In the foothills 48 per cent of the mestizos sampled had reported being vaccinated, as compared to only 17 per cent of the Indians; in the lowlands the respective figures were 23 and 4 per cent.

**Distribution of Antibodies to Single Antigens of the Main Arbovirus Groups**

These results are presented in Figure 4 according to terrain (A) and donor origin (B). Part A of the chart shows that among group A agents, Mayaro antigen most frequently evoked specific or diagnostic reactions from both foothill and lowland sera. Except for one serum considered diagnostic for WEE virus, sera from the foothills did not give specific or diagnostic reactions for any other group A antigens tested.

In the lowlands, however, some specific reactions were obtained with VEE antigen and EEE antigen. In addition, 14 per cent of the lowland sera were considered to represent group A superinfections, these latter probably reflecting exposure to more than one virus in that group.

In group B, serum reactions specific or diagnostic for Ilheus and YF viruses were found in both the foothills and the lowlands. These data suggest that Ilheus is more prevalent in the lowlands and YF is more prevalent in the foothills. The true natural infection rate with YF virus is obscured, however, by the fact that yellow fever vaccination had been carried out in 15 per cent of the lowland donors and 39 per cent of the donors from the foothills. About 1 per cent of the sera in each area gave specific reactions with SLE virus; also, one foothill serum was considered diagnostic for bat salivary gland virus and another was considered diagnostic for Bussuquara virus. Many of the sera (24 per cent in the lowlands, 17 per cent in the foothills) gave re-
actions interpreted as group B superinfections, and an even greater portion (31 per cent in the lowlands, 29 per cent in the foothills) had reactions that were classified as undiagnosable.

In group C, antigen for Caraparu virus was tested against all the survey sera. This was the only group C antigen employed in tests with sera from mestizos living in the northern foothills and in the southern regions. The percentage of sera with specific reactions to Caraparu antigen was consistently higher in the lowlands (19 per cent) than in the foothills (7 per cent). In the lowlands, another 3 per cent of the sera was considered diagnostic for Murutucu virus, and still another 3 per cent was classified as diagnostic for Itaqui virus. Group C superinfections were diagnosed in 18 per cent of the lowland sera and 1 per cent of the foothill sera; virtually all of these sera reacted with Caraparu antigen.

In the case of the Bunyamwera group, large numbers of sera reacted with either Guaroa or Maguari antigen or with both. Since the two agents are distantly related by HI test, whenever a serum reacted with both Guaroa and Maguari antigens it seemed logical to conclude that the donor had been exposed to both of these viruses rather than to any of the other agents in the Bunyamwera group. Specific reactions with Guaroa and Maguari antigens were found in sera from both foothill and lowland areas, reactions with Guaroa being slightly more prevalent in the foothill sera and reactions with Maguari being more prevalent in the lowland sera. Twenty-one percent of the lowland sera and 8 per cent of the foothill sera reacted with both antigens.

Part B of Figure 4 shows that the percentages of sera giving specific reactions for Mayaro, Ilheus, Caraparu, and Guaroa viruses were higher among Indians than among mestizos. Percentages of sera representing superinfections with arbovirus groups A, C, and Bunyamwera were higher for Indians than for mestizos; but the percentages indicating group B superinfections were about the same for both. Nine percent of the Indians sampled and 37 per cent of the mestizos sampled had been vaccinated against yellow fever.

Age Distribution of Antibodies to Selected Antigens of Main Arbovirus Groups

As shown in Figure 5, the percentages of sera giving specific reactions with Mayaro, Ilheus, Caraparu, and Guaroa viruses were higher in adults than in children in both foothill and lowland areas. Among children, the percentages having Mayaro, Ilheus, and Caraparu antibodies were higher in the lowlands than in the foothills, the data thus suggesting that infection with these viruses tends to occur earlier in life in the lowlands.

Sera obtained from children (see Table 1) accounted for a disproportionate share of those sera considered diagnostic for YF virus in both foothill and lowland areas. Since about the same proportion of children and adults sampled had been vaccinated against yellow fever, the higher percentage of diagnostic reactions among sera from children may indicate a lack of experience with other group B viruses.

Distribution of Antibodies to Antigens of Minor Arbovirus Groups

A significant percentage of sera from the foothills (4.8 per cent), and an even greater percentage from the lowlands (12.0 per cent), reacted with Tacaiuma antigen. In tests with California virus, 1.5 per cent of the foothill sera and 3.5 per cent of the lowland sera reacted positively. Reactions with Chagres virus were detected in sera
FIGURE 5—Results of hemagglutination-inhibition tests with sera from eastern Peru, showing percentages of sera giving specific reactions with selected antigens in groups A, B, C, and Bunyamwera group, classified by age of donor and terrain.

Discussion

Interpretation of the data was more difficult for the lowlands than for the foothills, chiefly because a larger proportion of sera with reactions interpreted as superinfections were found in the lowlands. This indicates that the lowlands may be the area of higher endemicity.

In spite of the difficulties of interpretation, however, the following numbers of reactions were obtained that were considered specific or diagnostic for the viruses named: Mayaro (358 sera), VEE (41), EEE (8), Ilheus (186), YF (151), SLE (11), Caraparu (136), Murutucu (16), Guaroa (217), and Maguari (215). Of these totals, the percentages of sera reacting at titers of 1:80 or more were: Mayaro (65 per cent), VEE (59 per cent), Ilheus (70 per cent), YF (25 per cent), SLE (45 per cent), Caraparu (15 per cent), Guaroa (41 per cent), and Maguari (16 per cent). It is quite possible that these high titers actually reflect specific infections and not, in any given instance, infection with a closely related virus.

When selected survey sera were reexamined by means of neutralization tests (4), the results showed a close correlation with the HI test results and suggested that the HI antibodies detected were evoked by Mayaro, VEE, EEE, Ilheus, YF, SLE, Caraparu, and Guaroa viruses.

Final interpretation of these serologic findings must, of course, await virus isolations from patients as well as information about vectors and host reservoirs. Nevertheless, it is apparent from the present survey that the sampled population in eastern Peru has been heavily exposed to a number of arboviruses.

SUMMARY

A total of 1,063 sera were collected in eastern Peru in 1965 from Indian and mestizo residents of tropical foothill and lowland areas, and were subsequently examined by hemagglutination-inhibition tests with 27 different arbovirus antigens. Antibodies were detected, primarily against the following viruses or closely related agents: Mayaro,
Venezuelan equine encephalitis (VEE), Eastern equine encephalitis (EEE), Ilheus, Yellow fever (YF), St. Louis encephalitis (SLE), Caraparu, Murutucu, Guaroa, Maguari, and Tacaiuma.

Antibodies against VEE and EEE viruses were found only in the lowlands. Group B antibodies, mainly against YF, Ilheus, and SLE, were detected in both areas; however, Ilheus antibodies were more prevalent in the lowlands and YF antibodies more prevalent in the foothills. In general, Indians had a higher percentage of sera with antibodies, including sera classified as representing superinfections; mestizos, however, had a higher percentage of sera with yellow fever antibodies.

The results suggest that endemicity is greater in the lowlands, and that arbovirus infections there are acquired earlier in life.

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