ST. LOUIS ENCEPHALITIS IN PANAMA

II. Survey of Human Blood for Antibodies against St. Louis and Two Related Group B Viruses, Ilhéus and Yellow Fever*

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In 1958 a focus of infection with the virus of St. Louis encephalitis (SLE) was discovered in the Republic of Panama in the Province of Darién on the Atlantic slopes of the Continental Divide near the Colombian border. The virus was repeatedly recovered from mosquitoes or human blood over an 8-month period from February, 1958, when stations for the collection of canopy mosquitoes were first established, to September of the same year, when they were discontinued. Further epidemiological studies were planned to determine the immunological status of the local population and possible animal reservoirs. In the present report results of a survey of blood drawn from persons resident in the area for antibodies against SLE as well as against two other group B viruses, yellow fever and Ilhéus, are presented. All three viruses are known to be active at least sporadically in Panama. The virus of Ilhéus encephalitis was isolated from mosquitoes captured at the collecting station at Paya, Darién, in June 1958, and thus was simultaneously present with SLE. No evidence whatsoever of recent activity of yellow fever was obtained. However, jungle yellow fever occurred in this section of the Republic in 1948 to 1949 and again in 1955 to 1957. As several vaccination campaigns have been conducted, of which complete records are not available, no attempt is made to differentiate between a positive serology as a result of artificial immunization to yellow fever and natural infection.

Topographical and other ecological data. The study was conducted among the human population occupying the portion of the Tuira River which extends between the mouths of the Pucro and Paya tributaries as well as in two small communities located well up these last two rivers. The area is mainly covered by forest of an intermediate type between tropical rain forest and tropical deciduous forest, with occasional clearings occupied by human habitations and by small banana and plantain patches. Rainfall is concentrated between the months of May and December, with only intermittent and light precipitation during the dry season which extends from January through April. The human population is made up of a mixture of Colombian Negro immigrants and Choco and Cuna Indians. The Cunas are congregated in two small stable communities located well up the Pucro and Paya Rivers, while the Colombian Negroes and Choco Indians are mostly nomadic, living in temporary houses located along the Paya and Tuira Rivers.

MATERIALS AND METHODS

A total of 195 blood specimens was collected in December, 1958, from all available inhabitants of the small villages and settlements along the Tuira, Pucro and Paya Rivers. They were packed in ice and transported by boat and plane to the laboratory located in Panama City where the sera were removed and stored until tested. Slightly more males (105 or 54%) than females (90 or 46%) were represented in the survey. Ages varied from 1 to 70 years and the length of residence in the area, from 8 days to 70 years.

For the neutralization tests aliquots of pure serum and appropriate viral dilutions were incubated for 2 hours at 37°C, chilled and injected intracerebrally into groups of 6 mice. A virus titration and negative and positive controls were included. In testing for antibodies to SLE virus a preliminary screening test was conducted using two serial dilutions of virus as recommended by Hammon, the first containing 10 to 25 LD50 per inoculum and the second, 10 times this concentration. Sera giving a positive reaction in the screening test were titrated against higher serial concentrations of virus. In the reactions with Ilhéus virus each serum was tested against two serial dilutions of virus also, but in this case the

* This investigation was supported by research grant E-1941 from the National Institutes of Health, U. S. Public Health Service.
first dilution was calculated to contain 50 to 75 \( \text{LD}_{50} \) per inoculum and the second, 10 times this concentration, no further titration being conducted. For determining antibody response to yellow fever, a single virus dilution containing approximately 100 \( \text{LD}_{50} \) of virus was used, a 6/6, 5/6, 5/5, 4/5 or 4/4 survival ratio being interpreted as positive.

Virus strains used as antigens in the neutralization studies were as follows: SLE—a local strain isolated from Sabethes chloropterus captured at Buena Vista in 1957; Ilhéus—a strain isolated from a mixed pool of mosquitoes of the genus Psorophora captured in Honduras in 1954; and yellow fever—the French neuroadapted strain received through the courtesy of Dr. Max Theiler of The Rockefeller Foundation Virus Laboratories.

**RESULTS**

Sixty-nine, or 31%, of the sera neutralized 1 log or more of SLE virus; 27, or 13.3%, neutralized 1.5 logs or more; and 16, or 8.4%, 2 or more logs. Seven persons resident in the area for 20 to 70 years had titers of 3 or more logs of virus neutralized. Eighty-five, or 43.7%, of the sera neutralized 2 or more logs of Ilhéus virus and 82, or 42%, gave a positive reaction against 2 or more logs of yellow fever virus. Another 21 (11%) sera showed a doubtful reaction against the latter virus. For purposes of tabulation and comparison (see Table 1) sera neutralizing 2 or more logs of virus have been classified as positive. It is recognized that this figure does not take into account weak antibody responses, falling titers due to the passage of time since infection was incurred, or heterologous reactions. Most of the sera neutralized more than one virus. However, it may be considered significant that 4 were single positives against SLE virus, 28 against Ilhéus and 29 against yellow fever. Seventy-one sera were negative against all three. With all three viruses the proportion of positive reactors among males was higher than among females. Although males constituted 53% of the persons tested, 62.5%, 68.8% and 56.1% of the sera neutralizing at least 2 logs of SLE, Ilhéus and yellow fever viruses, respectively, were from males. Age also seemed to be a significant factor in the incidence of infection. Although 45 children under 10 years of age, or 23% of the total, were included in the survey, only 2, or 4.5%, were positive to SLE and 3, or 6.7%, to Ilhéus. Positives against all three viruses were scattered throughout the area surveyed and not significantly concentrated in any one place. Since the viruses of SLE and Ilhéus encephalitis were isolated from local mosquitoes shortly prior to the collection of blood samples, it is not known whether the presence of neutralizing antibodies was the result of recent or old infection. No data were gathered concerning illnesses suffered prior to the survey.

**DISCUSSION**

Interpretation of results is complicated by the fact that the three viruses are antigenically related and may show varying degrees of cross-reaction. Immunological overlap among group B viruses has been amply demonstrated. Theiler and Casals have recently shown that the production of heterologous antibodies is greatly stimulated by infection with a second group B virus after recovery from a previous infection with another member of the same group. They conclude that no single serological test will permit accurate diagnosis of such secondary infections. Furthermore, although we
have demonstrated the presence, at least sporadically, of three group B viruses in Panama, there is no reason to believe that others may not occur. In fact a number of other as yet unclassified viruses were isolated in the course of this work.

As regards SLE infection in Darién, the picture is relatively clear. Virus was isolated repeatedly from mosquitoes and from the blood of two persons who were especially exposed to their bites. Also about 8% of persons residing in the study area had high titers of antibodies in their blood. In this forested area the disease apparently takes a relatively mild form and is transmitted to man by canopy mosquitoes. Like sylvan yellow fever it is more common in males old enough to engage in field work. No urban outbreaks of the disease have been recognized.

As regards Ilhéus infection much information is still lacking. The virus was isolated once from arboreal mosquitoes in Paya, Darién; and among the local population there is a surprisingly high percentage of persons with neutralizing antibodies in their blood (43.7%). However, the infection has never been recognized clinically nor has the virus been isolated from a human being in Panama. Obviously more extensive investigation is required.

In the case of yellow fever no differentiation can be made between persons immune as a result of vaccination and as a result of natural infection. There were 82, or 42%, positive and an additional 21, or 11%, doubtful reactions against 2 logs of virus. These figures indicate that only about half of the rural population in the area is demonstrably protected against yellow fever although this is the group most exposed to danger from the jungle form of the disease. Results very similar in certain respects to ours have been obtained by Causey and Theiler in Brazil and by Groot and co-workers in Colombia, indicating similar epidemiological patterns for these viruses in tropical America.

SUMMARY

One hundred and ninety-five persons residing in a forested area of the Province of Darién in Panama near the Colombian border were tested for neutralizing antibodies against yellow fever, St. Louis encephalitis (SLE) and Ilhéus viruses. Capacity to neutralize 2 or more logs of virus was observed in 8.4% against SLE, in 43.7% against Ilhéus and in 42% against yellow fever. The interpretation of these results is discussed.

REFERENCES