Icoaraci, a New Virus Related to Naples Phlebotomus Fever Virus.* (29862)

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Icoaraci (prototype strain Be An 24262) is a newly recognized virus, isolated from an unidentified forest rat trapped in the Instituto Agronômico do Norte forest near Belém, Pará, Brazil during October 1960. Seven subsequent isolations of the virus have been made from *Proechimys guyannensis oris,* 1 in May 1962 from pooled viscera of a *Proechimys* captured at kilometer 94 of the Belém-Brasilia highway, and 6 in 1963 during January, July, August, September and November from viscera or sera of *Proechimys* captured in the Utinga forest near Belém.

The prototype strain was isolated from pooled liver, spleen, kidney and heart suspended in 0.75% bovine albumin buffered to pH 7.4 and inoculated intracerebrally (i.c.) in 2-day-old Swiss mice. The mice sickened after 1 week and died after another 2-3 days' illness. By the 3rd mouse brain passage, the titer i.c. in 3-day-old mice was 6.5 log LD_{50} in 0.02 ml. When a 10% brain suspension was inoculated i.c., the average survival time was 7.3 days. Icoaraci did not kill 2-day-old mice inoculated intraperitoneally (i.p.) or adult mice inoculated i.c., i.p. or subcutaneously. The virus was reisolated from a portion of the original organ pool suspension that had been stored for 6 weeks at —60°C in a mechanically refrigerated box.

The prototype strain is inactivated by sodium desoxycholate, as shown by the method of Theiler(1). In a test with 6th mouse passage virus, the control titer was 6.5 log LD_{50} while the titer after incubation with sodium desoxycholate was <2.5 log LD_{50}.

A hemagglutinating antigen was demonstrated by sucrose-acetone extraction of mouse brain; the pH range of activity was 6.0 to 6.6, with a maximum titer of 1:1600 at pH 6.2. Hemagglutination and hemagglutination-inhibition (HI) techniques were those of Clarke and Casals(2); goose red blood cells were used. Complement-fixation (CF) testing was done by a microtechnique on plastic plates with approximately 4 and 16 units of antigen and 2 units of complement, and overnight incubation at 4°C. All sera for testing were prepared by hyperimmunizing mice with infected mouse brain. Testing with Naples and Sicilian phlebotomus fever viruses was done by one of us at the Rockefeller Foundation Virus Laboratories, New York, with strains from their collection which had been supplied originally by Dr. Albert Sabin. The Naples strain had undergone 46 mouse passages and the Sicilian strain 37.

TABLE I. Hemagglutination-Inhibition Testing with Icoaraci and Naples and Sicilian Phlebotomus Fever Viruses.

	Serum		
Antigen	Icoaraci 6 injections	Naples 6 injections	Sicilian 2 injections
Icoaraci	*320**	320	0
Naples	80	*640*	0
Sicilian	10	10	*320*

* Titer expressed as reciprocal of serum dilution completely inhibiting 8 units of antigen.

The Naples phlebotomus fever antiserum inhibited Icoaraci hemagglutinin in the 1:40 dilution but did not fix complement with Icoaraci veronal brain antigen. Sera for viruses isolated in Belém, including members of arbovirus groups A, B, C, Bunyamwera and Guamá and 5 ungrouped viruses, were negative in HI and CF testing. An additional series of 17 hyperimmune sera prepared for viruses exotic to Brazil were also negative in CF testing.

Further HI testing using Naples and Sicilian phlebotomus fever viruses confirmed the relationship between Icoaraci and the Naples virus; results are shown in Table I. Icoaraci serum as well as Naples phlebotomus fever serum inhibited the Sicilian antigen in the

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1:10 dilution. The possible significance of this observation has not been determined.

No CF test relationship among Icoaraci and Naples and Sicilian phlebotomus fever viruses could be shown. The homologous serum titer for each was 1:256.

In neutralization testing done i.c. in 3-day-old mice, the homologous hyperimmune serum neutralized 2.8 log LD_{50} of Icoaraci virus and no significant degree of neutralization was shown by the Naples and Sicilian phlebotomus fever sera. The test appeared relatively insensitive.

A survey of wild animals† trapped near Belém revealed HI antibody to Icoaraci virus in 3 of 19 *Nectomys aquaticus amazonicus,* 31 of 55 *Proechimys guyannensis oris,* 11 of 60 marsupials, 1 of 4 *Bradypus tridactylus,* 1 of 4 *Cyclops didactylus* and 10 of 45 reptiles. No positive reactors by HI testing were found among 608 human inhabitants of Belém. At present, no conclusions can be drawn as to the means of transmission of Icoaraci virus.

Summary. Icoaraci, a newly recognized virus, has been isolated on 8 occasions from forest rodents captured near Belém, Pará, Brazil. At least 7 of the isolations were from *Proechimys guyannensis oris.* The virus is related to Naples phlebotomus fever virus by hemagglutination-inhibition testing. Hemagglutination-inhibiting antibody to Icoaraci virus is prevalent in small forest animals but has not been found in human inhabitants of Belém.

† These animals were trapped and bled by a field team of the Oswaldo Cruz Institute, Rio de Janeiro, under the supervision of the late Dr. Hugo Laemmert.

1. Theiler, M., Proc. Soc. Exp. Biol. and Med., 1957, v96, 380.

2. Clarke, D. H., Casals, J., Am. J. Trop. Med. and Hyg., 1958, v7, 561.

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Itaporanga, a Newly Recognized Arbovirus from Sao Paulo State, Brazil. (29863)

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During April 1962 a virus was isolated from sentinel Swiss mice near the city of Itaporanga in São Paulo State, Brazil. One year before, in the same season, Itaporanga had been the site of an epidemic of eastern encephalitis in horses(1), and surveillance for arboviruses was being continued in the area because of the abundance of the mosquito species *Aedes serratus* and *A. taeniorhynchus.*

Six families of 3-day-old mice were exposed to biting arthropods near ground level on the farm of Dr. Teodoro Pinto, located at the forest edge near a lake formed by the receding waters of the Rio Itararé, about 16 kilometers from Itaporanga. The mice were exposed starting at 5 P.M. and early the next morning were returned to the laboratory of the Instituto Biológico in São Paulo for observation. One family had been destroyed by wasps. Babies in one of the other families sickened during the following week and a virus, which has been designated Itaporanga, was established by serial intracerebral (i.c.) passage of brain suspension in 3-day-old mice.

As first isolated, Itaporanga virus killed 3-day-old mice in 4-6 days, but by the 7th passage it killed in 48 hours when 10% brain suspension was inoculated i.c. The titer was 5.2 log LD_{50} per 0.02 ml. Mice at the limiting dilutions of titrations died up to 7 days after inoculation. Adult mice survived i.c. inoculation and developed antibody. The virus passed a Seitz filter. In sodium desoxycholate testing by the method of Theiler(2), the