

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 LD₅₀ 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 rep-

tiles. 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.

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 labora-

tory 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 LD₅₀ 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

control titer was 4.5 log LD₅₀ and the titer after exposure to sodium desoxycholate 1:1000 was <3.8 log LD₅₀.

After preliminary testing showed that the new isolate was not neutralized by immune sera for eastern equine encephalitis and rabies viruses, the agent was referred to the Belém Virus Laboratory where further serological studies were carried out.

A hemagglutinin for Itaporanga virus was prepared from baby mouse serum extracted twice with acetone. Goose red blood cells at 1:250 concentration were used to demonstrate hemagglutination over a pH range of 6.0 to 6.5. Optimal agglutination occurred at pH 6.4 at ambient temperature, with a titer of 1:128. An antigen with similar behavior was prepared from mouse brain by sucrose-acetone extraction. Hemagglutination-inhibition (HI) testing was done by the techniques of Clarke and Casals(3).

Complement-fixation (CF) testing was done by a microtechnique in plastic plates with about 4 and 16 units of antigen and 2 units of complement, and with overnight incubation at 4°C. CF antigens for Itaporanga virus were prepared by sucrose-acetone extraction of infected mouse brain or liver. The liver often yielded a higher-titered preparation when mice that sickened during the first 3 days after inoculation were used.

Neutralization testing was done in 3-day-old mice inoculated i.c. with infected baby mouse serum. The serum-virus mixture was incubated for 1 hour at 37°C.

All testing was done with hyperimmune mouse sera.

By HI testing Itaporanga virus is related to Icoaraci virus (Be An 24262), a newly recognized agent from Brazil which in turn is related to Naples phlebotomus fever virus (4). The HI relationship between Itaporanga and Icoaraci is as follows:

Serum	Antigen (8 units)	
	Itaporanga	Icoaraci
Itaporanga	1:320	≤1:20
Icoaraci	1:160	1:320

HI testing at the Rockefeller Foundation Virus Laboratories, New York(5), has shown that Itaporanga is also serologically related to Naples phlebotomus fever virus.

Itaporanga hemagglutinin was not inhibited by hyperimmune sera for arboviruses of groups A, B, C, Bunyamwera and Guamá plus 46 other arboviruses either ungrouped or in groups not yet recognized in formal publication; Sicilian phlebotomus fever virus serum is included in this list. Itaporanga serum (homologous titer 1:320) did not inhibit hemagglutination of antigens for arbovirus groups A, B, C and Guamá.

A CF antigen for Itaporanga virus did not react with hyperimmune sera for arboviruses of groups A, B, C, Bunyamwera or Guamá or for 9 other arboviruses, including Icoaraci. Itaporanga serum (homologous titer 1:64) did not fix complement with antigens for Bunyamwera group viruses, 7 other arboviruses including Icoaraci, or mouse encephalomyelitis virus.

In neutralization testing, the homologous hyperimmune mouse serum neutralized 2.4 log LD₅₀ of Itaporanga virus, but no significant neutralization by Icoaraci serum (homologous neutralizing index 2.8 log LD₅₀) was demonstrated. Conversely, Itaporanga serum failed to neutralize Icoaraci virus.

Summary. Itaporanga virus is a newly recognized agent isolated from sentinel mice near the city of Itaporanga, São Paulo State, Brazil, in April 1962. It is related by hemagglutination-inhibition testing to Icoaraci virus, another newly recognized Brazilian agent.

1. Nilsson, M. R., Sucay, W., *Arquivos do Inst. Biol.*, 1962, v29, 63.
2. Theiler, M., *Proc. Soc. Exp. Biol. and Med.*, 1957, v96, 380.
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5. Whitman, L., personal communication, 1964.

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