

ISOLATION OF THE VIRUS OF ILHÉUS ENCEPHALITIS FROM MOSQUITOES CAPTURED IN PANAMA*

ENID DE RODANICHE AND PEDRO GALINDO

Gorgas Memorial Laboratory, Panama, Republic of Panama

In 1956 isolation of the virus of Ilhéus encephalitis from mosquitoes of the genus *Psorophora* captured in Honduras was reported.¹ This represented the first isolation from Central America. The same virus was subsequently recovered from *Sabethes chloropterus* collected in Guatemala.² In the present publication we wish to report the first isolations from Panama of a virus identical with or very closely related to that of Ilhéus encephalitis. The virus was recovered twice from mosquitoes in 1958, once from *Haemagogus spegazzinii falco* collected at Cerro Azul in the Province of Panama and once from *Trichoprosopon spp.* collected at Paya in the Province of Darién.

MATERIALS AND METHODS

The isolations were effected in suckling mice inoculated intracerebrally with mosquito suspensions in accordance with techniques previously described.³ Mosquitoes tested were sylvan arboreal species captured in glass vials as they alighted to feed on men stationed in platforms in the forest canopy and are not to be considered representative of the local culicine fauna as a whole. They were obtained from the two stations, Cerro Azul and Paya, which are described below.

Cerro Azul is a forested area about 60 km east of the Canal Zone near the headwaters of the Pacora River in the Province of Panama. Collections have been made here almost continuously from September, 1956, to the present time. From the time of initiation of the collections to July 1, 1960, a total of 34,643 mosquitoes have been tested including principally members of the genera *Haemagogus*, *Sabethes* and *Wyeomyia*. Although many other viral agents have been recovered in this location, only the one strain here described corresponds to Ilhéus virus.

Paya is located on the forested slopes at the junction of the Paya and Tuira Rivers near the Panama-Colombia border in the Province of

Darién. Collections were made from February, 1958, to March, 1959, a total of 14,406 mosquitoes being tested. The most abundant genera were *Sabethes*, *Wyeomyia* and *Haemagogus* in that order. Only 278 *Trichoprosopon* were captured in all, from which the virus here reported was recovered.

The viral agents were identified by immunological comparison with the Honduran strain. Reliance was placed principally on neutralization tests using mouse hyperimmune sera and cross-immunity experiments in which mice immunized against the homologous strain were challenged with approximately 10,000 LD₅₀ of the heterologous strain administered intracerebrally.

RESULTS

The strain of Ilhéus virus isolated from *Trichoprosopon spp.* was obtained from a pool of 37 of these mosquitoes collected at Paya over a period extending from April to June, 1958. Included in the pool were the following species: *Trichoprosopon magnum*, *T. fluviatilis*, *T. digitatum* and *T. pallidiventer*. The strain recovered from *H. spegazzinii falco* was obtained from a pool of 89 specimens collected in June of the same year at Cerro Azul. Neither agent corresponded immunologically to yellow fever or St. Louis encephalitis, other group B viruses known to occur locally. Both corresponded very closely to Ilhéus, as may be noted in the results of neutralization and cross-immunity experiments summarized in Table 1. The strain from *H. spegazzinii falco* produced illness in three of seven 2-day-old mice, 8 and 9 days, respectively, after inoculation by the intracerebral route. On third passage all infant mice inoculated intracerebrally sickened or died within 5 days and adults, within 7. Reisolation was effected after 24 days of storage of the original mosquito suspension in CO₂ ice.

The strain of Ilhéus virus recovered from *Trichoprosopon spp.* proved of especial interest because of its slow neuroadaptation. Only 2 of 8 infant mice injected intracerebrally with the original mosquito suspension developed symp-

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TABLE 1

Results of cross-challenge and neutralization tests with isolates of Ilhéus virus

Virus strain	Logs virus neutralized by antisera against		
	Honduran	<i>Haemagogus</i>	<i>Trichoprosopon</i>
Honduran.....	4.0	2.5	3.3
<i>Haemagogus</i>	3.7	2.9	3.2
<i>Trichoprosopon</i>	4.6	3.0	3.5

Challenging strain	Survival rates of challenged mice previously immunized to		
	Honduran	<i>Haemagogus</i>	<i>Trichoprosopon</i>
Honduran.....	20/20*	17/19	17/18
<i>Haemagogus</i>	15/16	19/20	18/18
<i>Trichoprosopon</i>	19/20	19/19	20/20

* First number indicates the number of mice surviving challenge; the second, the number of mice challenged.

toms, which appeared on the 10th and 15th days, respectively, after inoculation. However, all 6 survivors were found immune to the later passage of more virulent material. Capacity to produce death in all adult mice injected was not achieved until the 10th consecutive passage. Second passage brain tissue showed an LD₅₀ of 10^{-2.6} and an ID₅₀ of 10^{-6.0}; 14th passage tissue, an LD₅₀ of 10^{-5.6} and an ID₅₀ of 10^{-6.5}. Thus increased virulence was not associated with great increase in virus titer but rather with gradual acquisition of the capacity to produce fatal central nervous system disease. Reisolation could not be attempted because of insufficiency of material. Both the isolate recovered from *H. spegazzinii* and that from *Trichoprosopon* protected immunized mice from cross-challenge with the other strain and each was neutralized by antisera against the other strain in similar titer.

DISCUSSION

The isolation of Ilhéus virus in Panama is important because it confirms the existence of

this virus there. It seems unlikely, however, that the arboreal mosquitoes from which this virus was recovered play a major role in the epidemiology of the disease. Antibodies against Ilhéus virus have been found to be prevalent in the blood of rural residents of the Paya section of Darién,⁴ but only one virus isolation was made from mosquitoes. Undoubtedly, recovery of the virus would have been more frequent if ground catches had been attempted. Previous isolations of Ilhéus virus have been made principally from mosquitoes of the genus *Psorophora*.^{1, 5, 6} *Psorophora ferox* as well as *Aedes aegypti* and *A. serratus* also have been shown capable of transmitting the infection experimentally by bite.⁵ It is of interest to note that St. Louis encephalitis virus was simultaneously active in this area, being recovered repeatedly from canopy mosquitoes and twice from human blood.³

SUMMARY

The first isolations of the virus of Ilhéus encephalitis in Panama are reported. The virus was recovered once from *Haemagogus spegazzinii falco* and once from mixed species of the genus *Trichoprosopon*.

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