

BIRDS AS HOSTS OF ILHÉUS ENCEPHALITIS VIRUS IN PANAMA*

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The importance of birds in the ecology of a number of arthropod-borne viruses is being recognized with increasing frequency. In the present publication, investigation of these vertebrates as hosts of the virus of Ilhéus encephalitis is reported. In the course of studies on the local arbor virus population here, blood specimens were collected from a wide variety of birds both in the Province of Bocas del Toro on the Costa Rican border of Panama and in the Province of Darién at the opposite end of the Republic. These blood specimens were used for possible virus isolation and also for serological studies. Ilhéus virus was recovered from sera of two of the birds, a little blue heron, *Florida caerulea*, and a keel-billed toucan, *Rhamphastos sulfuratus*, respectively. Antibodies against Ilhéus virus also were encountered in several avian sera.

MATERIALS AND METHODS

Birds were obtained both by shooting and trapping and were bled by cardiac puncture. The blood was stored in ice and forwarded by plane to the laboratory in Panama City at weekly or biweekly intervals. Most of the material arrived in satisfactory condition. Badly hemolyzed or contaminated specimens were discarded. For virus isolations the sera were diluted with equal quantities of rabbit serum saline containing 1,000 units of penicillin and 1,000 µg of streptomycin per cmm and injected intracerebrally into 2-day-old suckling mice. The viral isolates were identified by immunological comparison with a strain of the virus of Ilhéus encephalitis recovered from mosquitoes in Honduras.¹ Reciprocal cross-neutralization tests were performed with mouse hyperimmune sera prepared against the known and new strains. Also, cross-challenge experiments were conducted in which mice immunized against the homologous strain were challenged by the intracerebral introduction of 1,000 to 10,000 LD₅₀ of the heterologous strains.

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RESULTS

Altogether 48 sera from birds captured in Darién and 118 sera from birds captured in Bocas del Toro were injected into suckling mice for possible virus isolation. More than 40 different avian species were included, some of which have not yet been completely identified. Two recoveries of the virus of Ilhéus encephalitis were made, one from a little blue heron and one from a toucan, both captured in mid-March of 1960 in a lowland swampy section of the study area located in Bocas del Toro.

No other virus was obtained from birds in Bocas del Toro but two strains of an as yet unidentified virus were isolated in Darién. On primary isolation the heron strain (BT 871) produced illness in two of seven suckling mice on the 7th day after inoculation. By the fourth passage it produced nervous symptoms and death within 5 to 7 days in all infant and adult mice injected by the intracerebral route with 10% virus brain suspensions. The virus was reisolated from this blood after storage for 15 days in CO₂ ice. On primary isolation the viral agent recovered from toucan blood (BT 868) produced death in 1 of 7 suckling mice 13 days after inoculation. This strain also was readily adapted to adult mice, producing coma and death within 6 days of injection on the third successive passage. Reisolation was attempted but was unsuccessful. The two avian strains showed a very close cross-relationship with the type Ilhéus strain as may be noted in Table 1. There was marked immunological difference between the avian strains and St. Louis and yellow fever viruses, other group B viruses known to occur locally.

Neutralization tests also were performed with the Honduran strain of the virus of Ilhéus encephalitis and the blood of 130 birds, 62 from Bocas del Toro (captured in February and March of 1960) and 68 from Darién (captured in February, March and April of 1959).

Seven specimens neutralized 2 or more logs of the virus. These were as follows: 1 of 5 little

TABLE 1

Results of immunological tests with strains of Ilhéus virus

Serum against	Neutralization tests Virus strains, logs neutralized		
	Hon- duran	Heron (871)	Toucan (868)
Honduran strain.....	2.8	3.3	2.5
Heron (871) strain.....	2.8	3.7	2.7
Toucan (868) strain.....	2.4	3.0	3.0
Mice immune to	Cross-neutralization tests Challenged with virus strains		
	Hon- duran	Heron (871)	Toucan (868)
Honduran strain.....	—	12/12*	11/12
Heron (871) strain.....	9/10	—	10/10
Toucan (868) strain.....	9/10	9/9	—

* The first figure refers to the number of mice surviving challenge with the heterologous strains; the second, the number of previously immunized mice challenged.

blue herons (*Florida caerulea*) from Bocas; 1 of 3 snowy egrets (*Leucophoyx thula*) from Bocas; 1 of 3 oropendulas (*Zarhynchus wagleri*) from Bocas; 1 each of the tropical kingbird (*Tyrannus melancholicus*) and the yellow-billed cacique (*Amblycercus holosericeus*) from Bocas; 1 of 15 chickens from Darién; and 1 of 4 keel-billed toucans (*Rhamphastos sulfuratus*) from Darién.

Ilhéus virus was known to be present in mosquitoes in Paya, Darién, shortly prior to the capture of the birds.²

DISCUSSION

The isolations of Ilhéus virus here reported are the first to be made from birds in Central America. Further studies are needed to determine the relative importance of the little blue heron and toucan as well as other birds in the ecology

of this infection in Panama. It is of interest in this connection that another group B virus, Japanese B, was repeatedly isolated from the blood of members of the family Ardeidae in Japan and antibodies to this virus were found in their blood.^{3, 4} The family Ardeidae includes egrets and herons. Kissling and co-workers⁵ found antibodies to equine encephalomyelitis in the heron; and in a closely related species, the white ibis, they found antibodies not only to equine encephalomyelitis but also to St. Louis. Antibodies to St. Louis virus likewise occur in the blood of toucans, herons and other birds in Panama.⁶

SUMMARY

The virus of Ilhéus encephalitis was isolated from the blood of two birds captured in March, 1960, in the Province of Bocas del Toro in Panama. Five of 62 birds obtained in Bocas and 2 of 68 birds obtained in Darién had neutralizing antibodies against this virus in their blood.

REFERENCES

1. RODANICHE, E. DE, 1956. Isolation of the virus of Ilhéus encephalitis from mosquitoes of the genus *Psorophora* captured in Honduras. *Am. J. Trop. Med. & Hyg.*, 5: 797-801.
2. RODANICHE, E. DE, AND GALINDO, P., 1961. Isolation of the virus of Ilhéus encephalitis from mosquitoes captured in Panama. *Am. J. Trop. Med. & Hyg.*, 10: 393-394.
3. BUESCHER, E. L., SCHERER, W. F., MCCLURE, H. E., MOYER, J. T., ROSENBERG, M. Z., YOSHII, M., AND OKADA, Y., 1959. Ecologic studies of Japanese encephalitis virus in Japan. IV. Avian infection. *Am. J. Trop. Med. & Hyg.*, 8: 678-688.
4. HAMMON, W. MCD., SATHER, G. E., AND MCCLURE, H. E., 1958. Serologic survey of Japanese B encephalitis virus infection in birds in Japan. *Am. J. Hyg.*, 67: 118-133.
5. KISSLING, R. E., CHAMBERLAIN, R. W., NELSON, D. B., AND STAMM, D. D., 1955. Studies on the North American arthropod-borne encephalitides. Equine encephalomyelitis studies in Louisiana. *Am. J. Hyg.*, 62: 233-254.
6. RODANICHE, E. DE, AND GALINDO, P., 1961. St. Louis encephalitis in Panama. III. Investigation of local mammals and birds as possible reservoir hosts. *Am. J. Trop. Med. & Hyg.*, 10: 390-392.