

ECOLOGICAL OBSERVATIONS ON ILHÉUS VIRUS IN THE VICINITY OF
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In 1961 the authors reported the first isolations of Illéus virus in Panama.^{1, 2} Two isolations were obtained from mosquitoes and two from birds. The first recovery was made in 1958 from a pool of *Trichoprosopon* spp. captured in Darien, close to the Colombian border. The second came from a lot of *Haemagogus spegazzinii falco* taken at Cerro Azul, near the Panama Canal, during June 1958. The third and fourth were obtained from sera of the Little Blue Heron (*Florida caerulea*) and the Keel-billed Toucan (*Ramphastos sulfuratus*) captured during March 1960 in a lowland swamp close to the Costa Rican border. The sera of humans and birds from the study area in Darien and of birds from Almirante also possessed antibodies against Illéus virus.^{2, 3} It is thus apparent that Illéus infection has occurred recently from one border of the Republic to the other. The ecological studies herein reported cover the period from April 1960 to April 1961 and were conducted in the vicinity of Almirante.

Description of the study area. The study area covers a portion of 25 square miles of swampy land surrounding the town of Almirante, province of Bocas del Toro, in extreme northwestern Panama. It is bounded on the south and on the west by a series of complex forested ridges forming the Continental Divide, which at this point rises 9,000 feet above sea level. This high mountain barrier produces an extreme uplifting of the moist air masses brought in by the northeasterly trade winds, thus producing heavy rainfall throughout the year. Table 1 gives the monthly rainfall record for the years 1951 to 1961 from 11 rain-gauge stations operated by the Chiriquí Land Co. near Almirante. These figures place the study area almost within the category of "tropical rain forest climate" as defined in the Köppen system of climatic classification.

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The orographic, hydrographic and climatic conditions of the region have contributed to the development of a variety of ecological associations within the study area. Since the region has a tropical rain forest climate, it is mostly covered by evergreen broad-leaf forest broken up only by special hydrographic and edaphic conditions or by human activity. The four main categories of ecological associations recognized in the area are 1) tropical rain forest; 2) specialized forested swamps produced by edaphic conditions, such as mangrove and Silica palm (*Raphia taedigera*) swamps; 3) specialized habitats developed by hydrographic action, such as open fresh water swamps and marshes, in a highly dynamic stage of evolution; and 4) habitats produced by human activity, including banana and cacao plantations, cattle pastures and domestic environments.

The town of Almirante is a deep-sea port used almost exclusively by the United Fruit Co. for the shipment of bananas and cacao. There are 1,188 human dwellings with a population of 4,920 persons. Most of the dwellings are grouped in an urban community about 1 mile long and a quarter of a mile wide. The town is bounded on the north by large stands of palm, freshwater and mangrove swamps, on the south by Almirante Bay, on the east by the Chiriquí Lagoon and on the west by the lower ridges of the Continental Divide. Most of the native population live under very unsanitary conditions. The slum sectors of town, with some 160 houses and a population of 720 persons, are built against the swamps and many "wild" species of mosquitoes may be collected in the act of attacking man in the general neighborhood of the human dwellings.

MATERIALS AND METHODS

Material collected for study consisted of mosquitoes, sandflies and vertebrate blood (human, mammalian, avian and reptilian). The insects and bloods were transported to Panama City by air and if not processed immediately, stored in dry ice. When large numbers of arthropods were obtained, they were pooled in groups of approximately 200, triturated with serum-

TABLE 1
Monthly rainfall for the period 1951 to 1961

Month	Rainfall in inches										
	Avg.	51-52	52-53	53-54	54-55	55-56	56-57	57-58	58-59	59-60	60-61
September	4.07	5.05	3.03	2.59	6.36	2.36	5.34	4.70	4.60	3.35	3.34
October	6.26	6.62	13.05	6.24	5.62	9.37	5.81	5.55	4.56	2.85	2.90
November	8.75	8.15	4.23	12.30	9.25	8.54	6.75	17.82	6.07	8.38	6.00
December	12.65	5.67	12.81	9.38	21.40	11.27	20.81	18.90	6.59	7.62	12.01
January	8.65	14.19	9.26	8.12	4.95	12.36	9.25	11.47	3.47	8.31	4.79
February	5.29	4.76	6.32	1.39	4.26	5.04	3.80	16.95	2.74	6.11	1.60
March	5.32	2.34	2.99	6.34	6.03	5.61	1.79	7.60	6.34	6.29	7.86
April	5.71	4.86	4.14	8.99	4.72	5.01	3.60	3.10	12.63	3.79	6.32
May	7.89	5.32	6.97	7.12	9.37	11.51	6.56	8.45	8.96	4.89	9.74
June	7.54	3.77	5.60	6.15	5.73	8.90	6.35	6.21	12.14	8.04	12.55
July	9.77	8.86	9.38	8.34	10.15	17.00	12.12	5.84	8.68	7.04	10.28
August	9.14	3.31	15.47	8.91	1.21	5.39	21.59	10.53	8.03	8.22	8.66

TABLE 2
Sequence of isolations of Ilhéus virus in Bocas del Toro, July 1960 to February 1961

Host	Date of capture	No. specimens in collection	No. isolations
Mosquitoes	1960		
<i>Psorophora lutzii</i>	July 17-21	101	1
<i>Psorophora ferox</i>	Oct. 11-29	117	1
<i>Culex nigripalpus</i>	Nov. 8-12	3,672*	2
<i>Aedes angustivittatus</i>	Nov. 15-19	463†	1
Birds	1961		
<i>Ramphocelus passerinii</i>	Jan. 24	1	1

* Pooled in 18 approximately equal groups.

† Pooled in 3 approximately equal groups.

saline containing penicillin and streptomycin and injected intracerebrally into 2-day-old mice as described previously.⁴ Rarer species were retained in dry ice for a period of 2 months and then injected as described above regardless of quantity. For the isolation of virus from blood, the sera were removed and inoculated into mice individually or in small pools of the same species depending on the quantity available. In the case of badly hemolyzed or toxic blood (e.g., reptilian), dilutions of 1 to 2 or 1 to 4 were made with normal serum-saline with or without anti-

biotics, according to the probability of bacterial contamination.

For identification of the isolates, immunological comparison was made with two strains of Ilhéus virus previously recovered from mosquitoes in Honduras and Guatemala, respectively.¹ Viruses obtained from mosquitoes were compared antigenically by the techniques of complement-fixation with unextracted mouse brain antigens, neutralization and cross-challenge tests. The antibody content of the sera was evaluated by intracerebral neutralization tests in adult mice, using 100 to 200 LD₅₀ per inoculum. Survival rates of 6/6, 5/6 and 5/5 were considered positive, and of 4/6 and 4/5 doubtful. All other results were classed as negative. In a few instances the human bloods were retested against 1000 to 2000 LD₅₀ of virus.

RESULTS

Ilhéus virus was isolated five times from mosquitoes and once from bird blood. The sequence of isolations is shown in Table 2. The first isolation was made from *Psorophora lutzii* captured in July 1960 and the second from *P. ferox* taken in October. The third and fourth were obtained from a lot of 3,672 *Culex nigripalpus* captured in early November and inoculated in groups of approximately 200 mosquitoes each. The fifth isolation was from a pool of 154 *Aedes angustivittatus* also collected in November 1960. The last recovery of the virus was from the blood of the Scarlet-rumped Tanager, *Ramphocelus pas-*

TABLE 3

Vertebrate sera inoculated into mice

Species	No. specimens	Virus isolations	
		Ilhéus	Other
Birds*			
<i>Casmerodius albus</i>	9		
<i>Florida caerulea</i>	8		
<i>Columba nigrirostris</i>	6		
<i>Columba</i> spp.....	24		
<i>Amazona farinosa</i>	12		
<i>Aratinga finschii</i>	7		
<i>Eucinetes haematotis</i>	11		
<i>Pionus menstruus</i>	20		
<i>Cathartes aura</i>	7		
<i>Ramphastos sulfuratus</i>	20		
<i>Centurus pucherani</i>	20		
<i>Crotophaga sulcirostris</i>	9		
<i>Querula purpurata</i>	6		
<i>Hylocichla ustulata</i>	6		
<i>Turdus grayi</i>	7		2
<i>Zarhynchus wagleri</i>	14		
<i>Myiozetetes</i> sp.....	13		
<i>Ramphocelus passerinii</i>	16	1	
Rarer specimens (54 species)	104		
Unidentified specimens.....	11		
Reptiles			
<i>Chelydra serpentina</i>	3		
<i>Iguana iguana</i>	9		
<i>Kinosternon leucostomum</i>	6		
Miscellaneous†.....	5		
Mammals			
Lower mammals‡.....	4		
Human with fever of unknown origin.....	13		2
Total	370	1	4

* Only species of which more than 5 specimens were received are listed by name.

† Includes 2 *Ameiva* sp., 2 *Basiliscus* sp. and 1 *Geomyda funerea*.

‡ Includes 1 *Dasyprocta punctata*, 1 *Desmodus rotundus* and 2 *Sciurus granatensis*.

serinii, captured in January 1961. In view of previous isolations from the blood of two birds in March 1960,² it is obvious that the virus was continuously present in Almirante from March 1960 to at least February 1961. There can be little doubt of the importance of birds in the ecology of this virus. It is of interest that species of mosquitoes found infected were observed to feed frequently on birds.

TABLE 4

Results of neutralization tests against Ilhéus virus with sera of lower vertebrates captured in Bocas del Toro

Species	Total no. specimens tested	No. pos.
Birds*		
<i>Tinamus major</i>	3	1
<i>Florida caerulea</i>	6	1
<i>Leucophoyx thula</i>	3	1
<i>Herpetotheres cachinans</i>	3	1
<i>Jacana spinosa</i>	1	1
<i>Claravis pretiosa</i>	3	2
<i>Columba nigrirostris</i>	21	3
<i>Amazona farinosa</i>	8	1
<i>Baryphthengus martii</i>	3	1
<i>Ramphastos swainsonii</i>	9	2
<i>Pteroglossus torquatus</i>	6	1
<i>Centurus pucherani</i>	17	6
<i>Erator inquisitor</i>	8	2
<i>Querula purpurata</i>	3	2
<i>Tyrannidae</i>	11	2
<i>Icterus mesomelas</i>	11	2
<i>Ramphocelus passerinii</i>	7	3
<i>Thraupis virens</i>	2	2
<i>Saltator</i> sp.....	8	1
Birds giving totally negative results†.....	103	0
Total	236	35 (14.8%)
Lower mammals		
<i>Didelphis marsupialis</i>	5	0
<i>Artibeus jamaicensis</i>	98	0
<i>Artibeus lituratus</i>	39	0
<i>Desmodus rotundus</i>	5	0
<i>Phyllostomus discolor</i>	4	0
<i>Vampirodes major</i>	8	0
<i>Nectomys alfaroi</i>	4	2
<i>Proechimys semispinosus</i>	36	0
<i>Sigmodon hispidus</i>	42	1
Miscellaneous mammals‡.....	14	0
Total	255	3 (1.2%)

* Only birds giving one or more positive results are listed by species.

† Includes 9 *Cathartes aura*, 7 *Crotophaga sulcirostris*, 11 *Gallus gallus*, 16 *Pionus menstruus*, 6 unidentified species and several species with 6 or less specimens available.

‡ Includes 3 *Hoplostomys gymnurus*, 2 *Heteromys desmarestianus*, 1 *Oryzomys calliginosus*, 3 *Philander opossum*, 2 *Potos flavus* and 3 *Sciurus granatensis*.

TABLE 5
Results of neutralization tests with human sera

Yrs age	Males					Females				
	Total	Positive		Doubtful		Total	Positive		Doubtful	
		No.	%	No.	%		No.	%	No.	%
0-2	5					2				
3-5	7	1	14.3			10				
6-10	66	4	6.6			35				
11-15	49	4	8.2			24				
16-20	23	3	13.0			30	1	3.3		
21-30	78	10	12.8	5	6.4	54	8	14.8		
31-50	91	16	17.6	6	6.6	70	12	17.1	4	5.7
51-100	63	28	44.4	1	1.6	22	4	18.2	1	4.5
Age unknown	7	5	71.4			7			2	28.6
Total.....	389	71	18.2	12	3.1	254	25	9.8	7	2.7

A total of 370 vertebrate sera were inoculated into mice. These included 330 avian, 23 reptilian, 4 lower mammalian and 13 human specimens. The only isolation of Ilhéus virus obtained was that from the Scarlet-rumped Tanager discussed above. Four other viral agents were recovered, two from birds and two from human sera (Table 3).

Neutralization tests for antibodies against Ilhéus virus were conducted with the sera of 236 birds, 255 lower mammals and 643 human beings. Results are listed in Tables 4 and 5. Thirty-five or 14.8% of the bird bloods gave a positive finding. These included 3 of 7 *Ramphocelus passerinii*, species from which virus was recovered in January 1961, and 1 of 6 *Florida caerulea* from which virus was obtained in March 1960. No *Ramphastos sulfuratus* (source of a previous virus isolation) were tested, but 2 of 9 *R. swainsonii*, a closely related species, were positive. Only 3 or 1.2% of 255 lower mammals possessed neutralizing antibodies against Ilhéus virus. These were Alfaro's Water Rat (*Nectomys alfarí*) with 2 positives among 4 specimens and the Cotton Rat (*Sigmodon hispidus*) with one pool of 3 sera positive out of a total of 42 specimens examined.

The 643 human bloods similarly tested were drawn in December 1960 by Drs. Carl M. Johnson and Alexis Shelokov from residents of Almirante. A positive result against 100 to 200 LD₅₀ was obtained in 96 or 14.9% and a doubtful finding in 19 (Table 5). The percentage of posi-

tive males (18.2%) was significantly higher than that of females (9.8%). Males also became positive at an earlier age, the youngest being 5 years old whereas the youngest female found positive was 20. There were 9 positive males in the age group 0-15 years among 127 tested, while all 71 females in this age group were negative. These results seem to indicate an infection contracted in the field rather than a domestic one. The fact that species of mosquitoes which yielded Ilhéus virus are considered "field" rather than "domestic" species would seem to corroborate this theory. Twenty positive human bloods were retested against 1000 to 2000 LD₅₀ of Ilhéus virus and all were found to neutralize this quantity of virus.

The interpretation of the positive neutralizing capacity of human sera is difficult, especially as other group B viruses occur in the area. It may be noted that a wave of Yellow Fever passed northward through Bocas del Toro province in 1951 but this would not explain the presence of antibodies against Ilhéus virus in children. No records of vaccination against yellow fever are available. Although clinical infection with Ilhéus virus has not yet been diagnosed in Panama, it has been found in other areas (Causey *et al.* 1961⁶). Serological surveys in Brazil,⁵ Colombia,⁷ and Trinidad⁸ indicate a more or less extensive distribution of the infection in those countries. The percentage of positive reactions in Almirante was considerably less than that previously encountered in the Province of Darien (43.7%)⁹

although in the latter province also, males gave a higher proportion of positives than females. The over-all higher infection rate in Darien may be explained by the fact that people tested there were nomadic agriculturists living in close contact with the forest, while the survey in Almirante was carried out among residents of an urban community. Our current findings re-emphasize the importance of members of the genus *Psorophora* as vectors. The virus was originally recovered from *Psorophora ferox* in Brazil in 1944⁹ and again obtained there from this species in 1959.⁵ It also was found for the first time in Trinidad¹⁰ and in Central America¹¹ in this genus of mosquitoes.

The importance of some species of the genus *Aedes* is demonstrated by the isolation reported here and by the work of Causey *et al.*⁵ in Brazil. Moreover, mosquitoes of this genus were shown to be capable of transmitting the infection experimentally by Laemmert and Hughes.⁹

There can be little doubt of the significance of birds in the ecology of this viral infection, although further investigations are needed to assess the importance of the different avian species as natural hosts of the virus.

SUMMARY

Isolation of Ilhéus virus is reported from pools of the mosquitoes *Psorophora lutzii*, *P. ferox*, *Culex nigripalpus* and *Aedes angustivittatus* and from serum of the Scarlet-rumped Tanager, *Ramphocelus passerinii*, all captured in the vicinity of Almirante, Province of Bocas del Toro, in Panama during 1960 and 1961. Also 14.8% of 236 birds, 1.2% of 255 lower mammals and 14.9% of 643 human beings were found to possess neutralizing antibodies against this viral agent. The significance of these results is discussed.

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