

SURVEILLANCE FOR SYLVAN YELLOW FEVER ACTIVITY IN
PANAMA (1957-1961)

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Members of the staff of Gorgas Memorial Laboratory initiated in 1949 a long-range study on the ecology of yellow fever in Panama after the recognition of a sylvan outbreak of the disease. Clark undertook a survey for protective antibodies in the blood of wild animals shot in various parts of the country during 1949 and 1950.¹ Entomological and virological investigations were also begun in 1949 and have been continued without interruption to date. Work completed before 1957 has already been published.²⁻⁴ The present report covers results obtained from 1957 to 1961. Investigations during this period were aimed primarily at resolving the question of the possible endemism or enzootism of yellow fever in Panama and were conducted along the following lines:

- 1) Attempts to isolate yellow fever virus from blood-sucking diptera, especially mosquitoes.
- 2) Attempts to isolate virus from the blood of vertebrates, including birds, a few reptiles, lower mammals and a few human beings with fever of undetermined origin.
- 3) Mouse protection tests with the sera of vertebrates, with special emphasis on monkeys. Results with human sera are not included as they have dubious value due to the fact that it has not been possible to obtain reliable vaccination records. Findings with the sera of nonprimate vertebrates will require a considerable amount of additional field and laboratory experimentation before their significance can be interpreted accurately.

MATERIALS AND METHODS

Techniques employed have been described in previous publications.^{4, 5, 7} Mice were inoculated intracerebrally in attempts to isolate viruses from mosquitoes and from the blood of vertebrates. Suckling mice were used almost exclusively, the exception being mosquitoes taken at Buena Vista which were inoculated into adult mice due to

insufficient quantity of sucklings at the time. Protection tests were performed in young adult mice by the intracerebral route. For testing monkey bloods 100 to 200 LD₅₀ of French neuro-adapted virus previously desiccated in vacuum from the frozen state were combined with aliquots of serum. A lower titer, 25 to 60 LD₅₀, was employed for non-primate blood. A titration of the test was repeated with higher concentrations of virus and/or against two other locally isolated group B viruses, Ilhéus and St. Louis. Unsatisfactory results have been excluded from consideration.

Arthropod collections were all made in rural or forested areas. A brief description of the collecting stations may be found in previous publications.^{5, 7, 8} The following stations were selected (Fig. 1):

a) *Cerro Azul station*. Located at a site where yellow fever activity was detected in 1948 and again in 1957. This station was operated continuously from January 1957 to March 1961.

b) *Buena Vista station*. In June 1957 yellow fever was clinically diagnosed in two male agricultural workers engaged in clearing a forested slope in the vicinity of Buena Vista. Human cases from this same area were positively diagnosed post-mortem during the 1949 episode. A field station was established there soon after the appearance of clinical cases and capture of mosquitoes took place between July 11 and July 23, 1957.

c) *Orchid Island and Piña stations*. Five months after the 1949 outbreak of sylvan yellow fever in the area of Buena Vista, virus activity was detected at Piña, across the Canal from Buena Vista. In June 1957, expecting a possible repetition of the 1949 occurrence, field stations were set up at Orchid Island, in the Panama Canal, and at Piña, on the western shore of the Canal. Orchid Island station was operated until September 1957, while mosquito collections in the Piña station continued until February 1958.

d) *Stations at Pintupo, Paya and Tacarcuna*. Serological, histopathological and virological evidence has been published pointing to the periodic occurrence of sylvan yellow fever in

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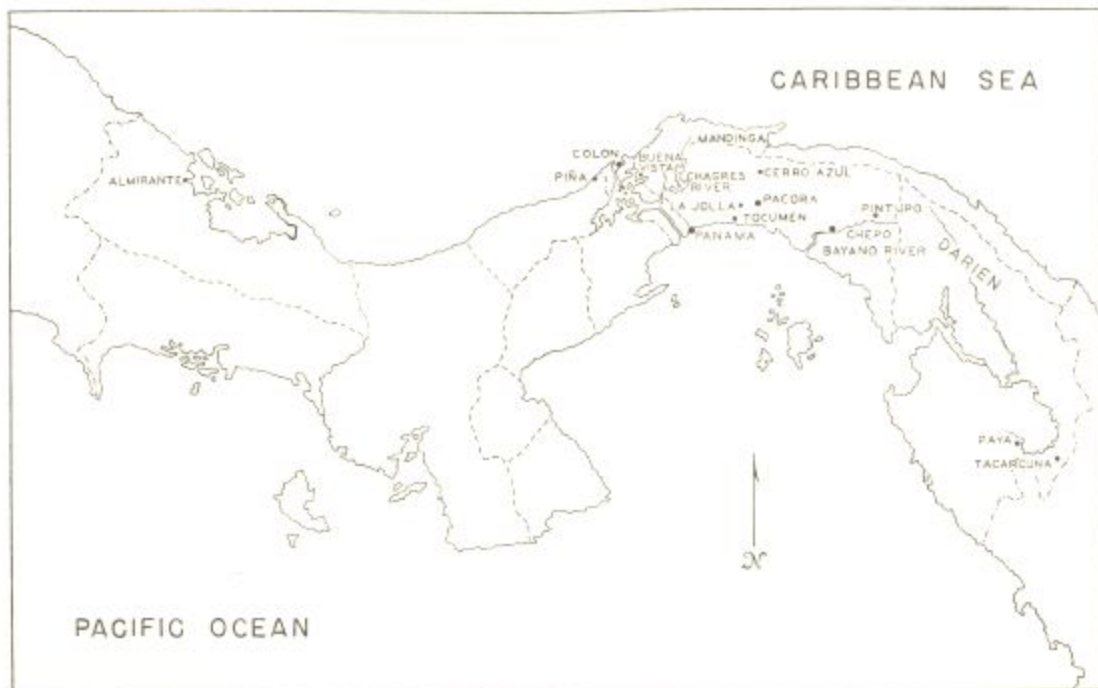


FIGURE 1. Map of Panama, showing location of collecting stations

Panama, east of the Panama Canal.^{1, 2, 4, 5, 9} Speculation has always arisen as to whether these outbreaks have their origin in the endless tracts of tropical rainforest of northern South American or in enzootic foci located within the Republic of Panama. After the disappearance of yellow fever from eastern Panama in 1957, efforts were directed at determining the presence of low-grade active foci of the virus between the Bayano river basin and the Panama-Colombia border. Mosquito collecting stations were established at Pintupo, in the Bayano river basin, and in Tacarcuna and Paya, near the Colombian border. Collection of mosquitoes at Pintupo was carried out between September 18 and November 29, 1957. In Tacarcuna and Paya mosquitoes were captured for virus isolation attempts between February 28, 1958 and March 9, 1959.

e) *Pacora, La Joya and Tocumen stations.* These stations were operated briefly late in the fall of 1958 and again during the summer of 1959. Emphasis was placed on ground catches of mosquitoes in the hope of recovering eastern equine encephalitis virus, then causing illness in horses.

f) *Almirante station.* In September 1959, a field station was established in the vicinity of Almirante, Bocas del Toro province, in extreme north-

western Panama, for a long-term ecological study of arboviruses. Ground and arboreal captures of blood-sucking insects were made from September 1959 to April 1961.

Animals for serological studies were obtained principally by purchase, when their place of origin could be determined accurately. Bleeding of these animals was carried out under aseptic conditions in the laboratory. However, many mammals, birds and reptiles were bled in the field and the blood was flown on ice to the central laboratory. Especially large collections were obtained from Paya and Tacarcuna in 1959 and from Almirante in 1960 and 1961.

RESULTS

Virus Isolations from Mosquitoes

Results of attempted isolations of yellow fever virus from mosquitoes at various stations are detailed in Table 1.

The Cerro Azul station, theater of yellow fever activity on two occasions since 1948, was operated continuously from January 1957 to March 1961. A total of 38,639 arboreal *Culicidae* collected there was inoculated into mice and 12 isolations of arboviruses were obtained. None of these proved to be yellow fever.

TABLE 1

Results of attempted isolations of yellow fever virus from mosquitoes at various field stations

Collecting station	Province	Period of collection	Total no. mosquitoes tested	Total no. <i>Haemagogus</i>	No. isolations of yellow fever virus	No. isolations of other viruses
Cerro Azul	Panama (east of Canal)	Jan. 1957-March 1961	38,639	14,095	0	12†
Buena Vista	Colón (east of Canal)	July 1957	1,514	953	4	1‡
Orchid Island	Gatun Lake (Panama Canal)	June-Sept. 1957	4,007	2,941	0	1
Pintupo	Panama (east of Canal)	Sept.-Dec. 1957	1,038	650	0	0
Piña	Colón (west of Canal)	Oct.-mid-June 1957	3,402	2,365	0	3
Paya	Darien (east of Canal)	Feb. 1958-early March 1959	16,632	2,439	0	9‡
Tacarcuna	Darien (east of Canal)	Aug.-early Dec. 1958	4,355	439	0	1
Pacora	Panama (east of Canal)*	Oct. 1958	12,088	0	0	0
La Joya	Panama (east of Canal)*	Nov. and Aug. 1959	11,655	0	0	7
Tocumen	Panama (east of Canal)*	Aug. 1959	31,962	0	0	2
Almirante	Bocas del Toro (west of Canal)	Sept. 1958-March 1961	151,588	931	0	24
Total			276,880	24,813	4	60

* Ground catches only; all others arboreal or, at Almirante, both ground and arboreal.

† Includes Ilhéus and St. Louis viruses.

‡ Includes St. Louis virus.

Mosquito captures at Buena Vista were carried out from July 11 to July 23, 1957. Four isolations of yellow fever virus were obtained from 1,514 mosquitoes collected at this station. Two isolations were yielded by the species *Haemagogus spegazzinii falco* and two by *H. lucifer*.

At Orchid Island and Piña collections were carried out between June 1957 and February 1958. A total of 7,409 mosquitoes was captured and inoculated into mice. No yellow fever virus was obtained but four other arboviruses were isolated.

A total of 22,025 mosquitoes, including 3,528 *Haemagogus*, was captured at the Pintupo, Paya and Tacarcuna stations. Inoculation of these mosquitoes into mice produced 10 arboviruses, but none of them was identified as yellow fever.

The stations in Pacora, La Joya and Tocumen were primarily intended for attempts to isolate eastern equine encephalitis virus. Inoculation into mice of the 55,705 mosquitoes captured produced no yellow fever virus, although 9 other arboviral agents (but not eastern equine virus) were isolated.

The station at Almirante yielded the most com-

prehensive collection of blood-sucking insects, including 151,588 mosquitoes, 9,543 *Phlebotomus* sandflies and 176 *Culicoides* sandflies. Inoculation of this material into suckling mice produced 36 viral agents but once again yellow fever virus failed to appear.

To summarize results of virus isolation attempts from arthropods, it may be said that after the 1957 outbreak in eastern Panama and up to 1961 no trace of yellow fever virus was found in blood-sucking insects captured in different areas of Panama, east and west of the Canal.

Attempts to Isolate Yellow Fever Virus from the Blood of Vertebrates

Suckling mice were inoculated with the blood of human beings, wild mammals, birds and reptiles in an effort to recover yellow fever virus. The bloods were obtained principally in Darien in 1959 and Almirante during 1960 and 1961. Results are listed in Table 2. As may be noted, a total of 447 specimens was used for possible virus isolation with a single recovery of yellow fever virus. This isolation was obtained in 1957 from the blood of a mosquito collector in Buena

TABLE 2

Results of attempted isolations of yellow fever virus from the blood of vertebrates

Species	No. specimens inoculated	No. arboviruses isolated
Birds		
Cormorant (<i>Phalacrocorax olivaceus</i>)	4	
American Egret (<i>Casmerodius albus</i>)	9	
Little Blue Heron (<i>Florida caerulea</i>)	11	1
Muscovy Duck (<i>Cairina moschata</i>)	4	
Kite (<i>Helicolestes hamatus</i>)	1	1
Turkey Vulture (<i>Cathartes aura</i>)	9	
Chicken (<i>Gallus gallus domesticus</i>)	15	
Pigeon (<i>Columba</i> , 3 species)	30	
Parrot (<i>Amazona farinosa</i> , <i>Aratinga finschii</i> , <i>Eucinetis haematotis</i> , <i>Pionus menstruus</i>)	51	
Groove-billed Ani (<i>Crotophaga sulcirostris</i>)	9	
Toucan (<i>Ramphastos</i> , 2 species)	22	1
Woodpecker (<i>Centurus pucherani</i> , <i>Dryocopus lineatus</i>)	21	1
Cotinga (<i>Querula purpurata</i>)	6	
Flycatcher (<i>Myiozetetes</i> sp.)	13	
Swainson's Thrush (<i>Hylocichla ustulata</i>)	6	
Clay-colored Robin (<i>Turdus grayi</i>)	7	2
Scarlet-rumped Tanager (<i>Ramphocelus passerinii</i>)	16	1
Oropendola (<i>Zarkynchus wagleri</i>)	14	
Rarer specimens (58 species)	119	
Unidentified species	11	
Reptiles		
Turtle (<i>Chelydra acutirostris</i> , <i>Geomyda funerea</i> , <i>Kinosternon leucostomum</i>)	10	
Lizard (<i>Ameiva</i> sp., <i>Basiliscus</i> sp., <i>Iguana iguana</i>)	13	
Mammals		
Opossum (<i>Didelphis marsupialis</i>)	1	
Vampire bat (<i>Desmodus rotundus</i>)	1	
Agouti (<i>Dasyprocta punctata</i>)	1	
Squirrel (<i>Sciurus granatensis</i>)	2	
Monkeys	19	
Human beings	22	4*
Total	447	11*

* One agent identified as yellow fever; Ilhéus virus was isolated three times from birds and St. Louis virus twice from man.

Vista during the early acute phase of the disease. Of the remaining specimens, there were 378 avian samples from Darien and Almirante, 23 reptilian sera from Almirante, 24 mammalian bloods from Almirante and Darien and 21 human specimens

from Almirante, Darien and the provinces of Colón and Panama. Although no yellow fever virus was obtained from these blood samples, 10 other arboviruses were isolated. It may be of interest to note that six strains of *Pasteurella multocida* were recovered from mortally ill marmosets and night monkeys brought in from the vicinity of Pacora under suspicion of yellow fever infections.

Results obtained with the inoculation of vertebrate bloods confirm the consistently negative findings yielded by the inoculation of mosquitoes collected after 1957.

Serological Surveys

Results of protection tests in adult mice with the sera of vertebrates is presented in Tables 3 and 4. The total number of animals tested and the number giving positive results are listed. Tests with human sera have not been included as the interpretation is obscured by lack of accurate data as to previous vaccination.

Tests on primate sera. Panama harbors six genera of monkeys of which five were tested. The Chiriquí marmoset, *Saimiri orstedii*, was not studied as it occurs only in a limited area of the Province of Chiriquí, in extreme Western Panama, where sylvan yellow fever has not been found in recent years. The species tested include: spider monkeys (*Ateles geoffroyi* and *A. fusciceps*), marmosets or squirrel monkeys (*Marikina geoffroyi*), night monkeys (*Aotus trivirgatus*), howler monkeys (*Alouatta palliata*) and white-faced monkeys (*Cebus capucinus*).

Results by year of capture are listed in Table 3. It will be noted that positive results were given by 7.1% of 169 monkeys captured in 1957, 4.5% of 178 tested in 1958, 5.0% of 121 tested in 1959 and 0% of 169 tested in 1960 and 1961. If only spider monkeys are considered, the results are 17.9% positive in 1957, an epizootic year, 7.7% positive in 1958 and 2.2% in 1959.

Further analysis according to the age and place of origin of the positives reveals the following facts. In 1957 three positive infant *Ateles* were received from Darien and seven positive infants or juveniles from Chepo, Pacora and the Bayano River Valley. One positive adult *Marikina* also was received from this latter area and one positive infant *Cebus* from the Chagres River Valley. In 1958 three positive infant and juvenile *Ateles* and two adult marmosets were captured in the

TABLE 3
Results of protection tests in mice with monkey sera by year

Type of monkey	1957			1958			1959			1960			1961			Total		
	No. exam.		Positive	No. exam.		Positive	No. exam.		Positive	No. exam.		Positive	No. exam.		Positive	No. exam.		Positive
	No.	%		No.	%		No.	%		No.	%		No.	%		No.	%	
Spider monkeys.....	56	10	17.9	52	4	7.7	45	1	2.2	27	0	0	14	0	0	194	15	7.7
Marmosets.....	77	1	1.3	71	2	2.8	45	4	8.9	31	0	0	16	0	0	240	7	2.9
Night monkeys.....	29	0	0	35	0	0	16	0	0	19	0	0	7	0	0	106	0	0
Howler monkeys.....	5	0	0	8	1	12.5	2	0	0	45	0	0	0	0	0	60	1	1.7
White-faced monkeys.....	2	1	50	12	1	8.3	13	1	7.7	8	0	0	2	0	0	37	3	8.0
Total.....	169	12	7.1	178	8	4.5	121	6	5.0	130	0	0	39	0	0	637	26	3.8

Chepo-Pacora-Bayano sectors, one positive juvenile *Ateles* in the Chagres River Valley, and one each positive adult *Alouatta* and *Cebus* were shot at Piña, Colón. The latter animals probably obtained their immunity during the 1948-1951 outbreak. In 1959 all positive monkeys were adults including four marmosets, one white-faced and one black spider monkey, four of these specimens being native to Darien, one to the Bayano River Valley and one to Chilibre near the Transisthmian Highway. The high specificity of the tests in monkeys is indicated by the fact that 21 of the 26 positive sera were tested also against St. Louis virus with negative results.

The fact that no positive juvenile monkeys were found after 1958, one year after the last isolation of yellow fever virus in Panama, appears to give additional support to the concept that yellow fever disappeared from the Republic in late 1957 or early 1958.

Non-primate animals. Many of the epidemiological aspects of sylvan yellow fever are not compatible with the recognition of primates as the only animal hosts. Of great importance is the occurrence of this infection in areas where few or no primates exist.¹⁰ In order to gather more data in relation to this problem a large series of sera from birds, reptiles and nonprimate mammals was tested for protective antibodies. Unfortunately none of these were obtained from Buena Vista in 1957, the only area where sylvan yellow fever was recognized in the present survey. Results are presented in Table 4. The only valid conclusion that can be reached from the analysis of these data is that further tests are required, especially with field material during epizootic periods and in the laboratory under controlled conditions.

Previous studies¹⁰ have strongly incriminated the marsupials as hosts of yellow fever virus. In the present survey 2 of 115 adult *Didelphis marsupialis* (common opossum), both bled at Piña in 1959, were positive but yellow fever has not been active there as far as we know since 1949. Positive also were the sera of 2 of 32 adult *Choloepus hoffmanni* (two-toed sloth) received from Pacora in 1958; 2 of 39 *Proechimys semispinosus* (spiny rat), 1 of 9 *Chironectes minimus* (water opossum), and 4 of 92 birds all captured and bled in Darien in 1959. The sera of the four birds and two spiny rats were also strongly positive against St. Louis virus.¹¹ The serum of the water opossum was negative against St. Louis encephalitis but the habits of this animal do not conform to the known ecology of yellow fever. The positive sloth and opossum sera were not tested against any other viruses. A large proportion of doubtful results was encountered also, especially among the opossums, sloths and birds. We do not attempt to interpret these doubtful findings at this time.

DISCUSSION

Our results indicate that yellow fever is not presently enzootic in Panama and that recent outbreaks during 1948 and 1956 have been due to periodic passage of virus from forested areas in South America. This conclusion is based on our failure to find yellow fever virus in any of 285,088 arthropods including 275,266 mosquitoes, since the small sylvan outbreak in Buena Vista in 1957; by our failure to isolate the virus from the blood or tissues of lower mammals and birds and by the gradual decline in the percentage of monkeys with positive serology against the virus from 1956 to

TABLE 4

Results of yellow fever protection tests in mice
with sera of lower vertebrates

Species	Total exam.	No. pos.	Doubtful
Mammals			
Marsupials			
Woolly opossum (<i>Caluromys derbianus</i>)	11		
Water opossum (<i>Chironectes minimus</i>)	9	1	
Common opossum (<i>Didelphis marsupialis</i>)	115	2	10
Murine opossum (<i>Marmosa robinsoni</i>)	32		
Brown-masked opossum (<i>Metachirus nuda-</i> <i>caudatus</i>)	7		
Four-eyed opossum (<i>Philander opossum</i>)	31		2
Rodents			
Pocket mouse (<i>Heteromys</i> spp.)	2		
Darien gopher (<i>Macrogeomys dariensis</i>)	1		
Rice rat (<i>Oryzomys</i> spp.)	3		1
Spiny rat (<i>Proechimys semispinosus</i>)	65	2	
White-tailed rat (<i>Tylomys panamensis</i>)	6		
Red squirrel (<i>Sciurus granatensis</i>)	36		
Common agouti (<i>Dasyprocta punctata</i>)	23		1
Spotted agouti (<i>Agouti paca</i>)	3		
Forest rabbit (<i>Sylvilagus brasiliensis</i>)	1		
Porcupine (<i>Coendou rothschildi</i>)	3		
Sloths			
Three-toed sloth (<i>Bradypus infuscatus</i>)	6		
Two-toed sloth (<i>Choloepus hoffmanni</i>)	32	2	5
Carnivores			
Olingo (<i>Bassaricyon gabii</i>)	1		
Coati mundi (<i>Nasua narica</i>)	20		2
Kinkajou (<i>Potos flavus</i>)	16		
Crab-eating raccoon (<i>Procyon cancrivorus</i>)	2		
Anteaters			
Pygmy anteater (<i>Cyclopes didactylus</i>)	19		
Tamandua anteater (<i>Tamandua tetradactyla</i>)	7		
Bats			
Vampire bat (<i>Desmodus rotundus</i>)	11		
Mastiff bat (<i>Eumops aereus</i>)	1		
Spear-nosed bat (<i>Phyllotis hastatus</i>)	6		
Miscellaneous mammals (5 spp.)*	16		3
Reptiles			
Common iguana (<i>Iguana iguana</i>)	5		
Birds			
Turkey Vulture (<i>Cathartes aura</i>)	2		1
Domestic Chicken (<i>Gallus gallus domesticus</i>)	22		5
Little Blue Heron (<i>Florida carulea</i>)	6		1
Crested Guan (<i>Penelope purpurascens</i>)	5	1	
Muscovy Duck (<i>Cairina moschata</i>)	7		3
Red-throated Caracara (<i>Daptrius americanus</i>)	1		1
Crested Caracara (<i>Caracara blanda</i>)	1		
Ringed Kingfisher (<i>Megascops torquata</i>)	11		2
Amazon Kingfisher (<i>Chloroceryle amazona</i>)	4	1	1
Toucan (<i>Ramphastos</i> spp.)	5	2	
Miscellaneous birds (17 spp.)	28		
Total	582	11	38

* Includes 6 negative *Dasyprocta* *noveboracensis*, 1 negative *Lutra* *annectens*, 2 negative *Tayassu* *albigularis*, 4 negative *Felis* *paguaroundi* and 3 doubtful *Galictis* *allamandi* sera.

1961. Only adult monkeys were found positive in 1959 and all specimens tested were found negative in 1960 and 1961. Failure of yellow fever virus to cross the Panama Canal from east to west in 1957

seems to be a normal occurrence. Previous serological evidence⁹ points to repeated yellow fever activity east of the Canal from 1925 to 1949 and complete freedom of the disease west and north of the canal during those years. In 1949 yellow fever virus managed to cross the canal and to spread through the forests of Middle America. Conditions which normally prevent passage of virus across the canal are not fully understood at present.

SUMMARY

A surveillance report on sylvan yellow fever in Panama from 1957 to 1961 is presented. The last known outbreak of yellow fever in this country occurred in June 1957 near the town of Buena Vista, located along the Transisthmian Highway a few miles east of the Panama Canal. During the outbreak, yellow fever virus was isolated from the blood of an acutely ill human and from forest, day-flying, arboreal mosquitoes.

Failure to isolate yellow fever virus from forest mosquitoes and vertebrates captured in various areas of Panama and results of neutralization tests on the blood of numerous primates and other vertebrates lead the authors to the conclusion that yellow fever virus has not been active in Panama since 1957.

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