

THE INVESTIGATION OF A SYLVAN YELLOW FEVER EPIZOOTIC ON THE NORTH COAST OF HONDURAS, 1954

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In late December, 1953, and in January, 1954, reports were received at the Gorgas Memorial Laboratory of monkey mortality presumed to be caused by yellow fever in the upper watershed of the Rio Patuca in the vicinity of Catacamas, Honduras (see Figure 1). Later in 1954 additional reports came from further north in the vicinity of Olanchito in the drainage of the Rio Aguan. In June of 1954 local authorities reported monkeys dying near Yaruca and La Masica (see Figure 2). Yaruca is about 20 kilometers south southeast of the Caribbean port town of La Ceiba, and La Masica is about 30 kilometers west southwest from La Ceiba. Sr. Pablo Orjuela, field technician of the Pan American Sanitary Bureau, was sent into the area by his organization to investigate and if possible confirm these reports. On July 16th he located a recently dead howling monkey at a point about two hours by foot from La Masica, and preserved liver tissue from it and several other howling monkeys collected in the immediate vicinity. These liver specimens were sent to the Carlos Finlay Institute, Bogota, Colombia, and we were notified of the incident on the 26th of July. On receiving this information confirming the presence of a current epizootic, we proceeded from Panama to Honduras, arriving at Tela on the 30th of July. The tentative working premise was that these incidents were a further expression of the activity of yellow fever virus, in continuation of the episode which was recognized in Panama in December, 1948, (Herrera, Elton and Nicosia, 1949), and which since that time had progressed west and north through Panama, Costa Rica and Nicaragua (Elton, 1952).

The most recent reports of monkey mortality at the time of our arrival at Tela had come from La Masica. This village is at approximately the midpoint on the rail line of the Standard Fruit Company between the two port towns of La Ceiba and Tela. A reconnaissance was made at once of the region between these two towns, and it was found that while there were repeated accounts by the local people of howling monkeys dying within the preceding two months at localities east of La Masica toward La Ceiba, no one interviewed west of La Masica toward Tela had either noticed any reduction in the monkey population or encountered any dead monkeys in the forest.

The air line distance between La Ceiba and Tela is 65 kilometers but the rail distance is about 100 kilometers. There are no roads in the area, and travel between the two ports is possible only by the rail line which passes along the coastal plain skirting the mountains which are five to 25 kilometers from the sea. The coastal plain is at or near sea level, but with a few low hills rising out of it as one approaches Tela. It is in part planted to bananas, cleared as pasture for



FIG. 1. Sketch map of Honduras showing area in which yellow fever epizootic in monkeys occurred.

cattle, or supports the cultivation of corn, rice, plantain and other locally consumed crops. But much of the area between the sea and the bordering mountains is a swampy forest periodically inundated in rainy season. The mountain ridges which rise to the south of the plain reach an altitude as high as 2,451 meters at Pico Bonito, and their seaward slopes are clothed in tropical rain forest unbroken by cultivation.

In this forest there are three genera of primates: howling monkeys (*Alouatta*) spider monkeys (*Ateles*), and white faced monkeys (*Cebus*). In the broken swampy forest of the coastal plain, howling monkeys are surprisingly abundant and tame. Groups of them were frequently seen in the patches of forest along the railroad right of way, and in trees immediately adjacent to villages. It is thus understandable how the epizootic in these animals was immediately recognized by local residents. As at other places where we have spoken with local persons following an epizootic in monkeys in Nicaragua, the consistent details volunteered are that the monkeys stopped calling at dawn, and the woods, and sometimes the streams, became foul with the odor of dead animals. At the end of a rail spur at San Francisco, some five kilometers to the northeast of La Masica, we were shown a row of wild fig trees bordering a stream no more than 100 meters from a small labor camp. In these trees a group of howling monkeys had fed and slept earlier in the year, but in June their calls stopped and soon the bodies of dead animals were found a short distance from the trees.

The local civil and police authorities at all points along the rail line were requested to broadcast the fact that we would pay a reward to any person taking us to a freshly dead monkey. A crew of local woodsmen was employed to collect

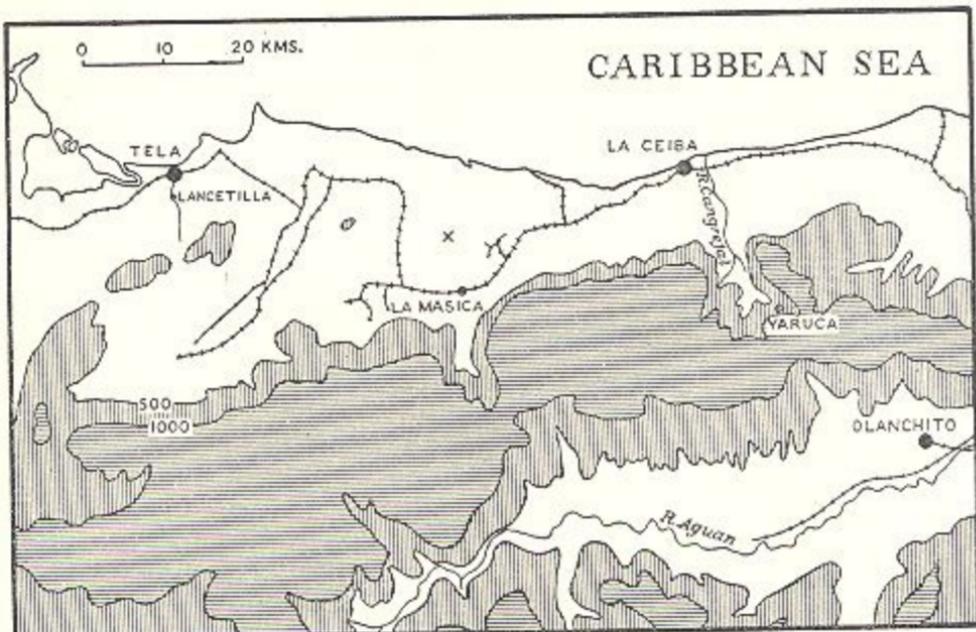


FIG. 2. Sketch map of north coast of Honduras showing detail of area in which yellow fever epizootic in monkeys occurred. The 500 and 1000 meter contours are indicated by hatching. An "X" to the north of La Masica indicates the place where monkeys died of histopathologically proven yellow fever, and where forest canopy mosquitoes were collected.

mosquitoes in the trees at the place where the last dead monkey had been found two weeks previously near La Masica. During the following month these men were under the daily supervision of either Sr. Orjuela or a reliable technician from our laboratory in Panama. On the day that these men were selecting trees for mosquito collecting, August 6th, another dead monkey was obtained near La Masica. Formalin fixed liver tissue from this animal was sent by air to the Gorgas Memorial Laboratory, and on August 14 we received a cable from Dr. Carl M. Johnson, pathologist, reporting a diagnosis of yellow fever. The mosquito collections were thus begun at a time that the presence of the virus in the area was proven. A subsequent report from Dr. Gast Galvis of the Carlos Finlay Institute on the monkey found on July 16th also confirmed the presence of yellow fever. No further reports of monkey mortality were received, but the mosquito collections were continued for one month until the 8th of September, when the work was discontinued after about 20,000 mosquitoes had been collected.

METEOROLOGICAL CONDITIONS

The north coast of Honduras is an area of high but erratic rainfall. We have available 22 years of rainfall data from Lancetilla during which period the total annual figure has been as high as 172 inches or as low as 92 inches (this latter figure, however, being ample to support true tropical rain forest). The rainfall

is particularly erratic with regard to distribution, the "dry season" months of February, March and April having had as little as a fraction of an inch or, as on one occasion, April 1931, as much as 21 inches. Through the courtesy of Mr. K. J. Davidson, of the research station of the United Fruit Company at Lancetilla, we have the data on rainfall for 1954. In this year there was no month with less than five inches, conditions thus being favorable for the maintenance of the forest canopy mosquito fauna, most of the species of which depend for the completion of their larval cycle on accumulation of water in tree holes or water holding plants. The total rainfall for 1954 at Lancetilla was 138.01 inches.

The north coast of Honduras is sufficiently far north so that seasonal differences in temperatures are also significant, as they might limit both the mosquito fauna and the rate of virus multiplication in the insect vector. In three years of data recorded by Stanley (1931) the mean mid-summer temperatures reach 80°F. while in December the mean temperature may be as low as 70°F.

METHODS

Through the courtesy of the Standard Fruit and Railroad Company a laboratory was set up at La Ceiba. The collections of mosquitoes were brought here from La Masica each evening by a special rail car. Mosquitoes were identified alive, made up into pools by species or species groups, sealed in pyrex tubes with an acetylene flame and frozen on dry ice for air shipment to Panama and subsequent inoculation into mice for possible recovery of virus by Dr. Enid de Rodaniche, virologist at the Gorgas Memorial Laboratory.

As part of our continuing study of sylvan yellow fever in Middle America, stations for the year long capture of forest canopy mosquitoes had been established in Nicaragua, Honduras, Guatemala and Mexico in 1953. The Honduras stations, which were operated from June 1953 to June 1954, had been located in the Lancetilla Valley, about seven kilometers inland (south) of Tela and about 35 kilometers west of La Masica. A full report on the collections from these stations will be made at a later date, but reference will be made to certain of the data here.

Collections of mosquitoes were made within a quarter mile radius of the spot at which a howling monkey had died of proven yellow fever, during the month immediately following the fatality. Ten men equipped with aspirating tubes, small hand nets, and vials for individual mosquito captures, collected all mosquitoes which either bit or approached to bite at their stations in the crotches of trees. In all, approximately 20,000 mosquitoes were collected, but there was some mortality in bringing these into the base laboratory. At first all mosquitoes were identified, but when it became evident that the mortality was primarily in such ground-breeding genera as *Psorophora* and *Mansonia*, which were attacking in the trees only by virtue of their high densities, and which are not thought to be involved in yellow fever transmission, only the living mosquitoes to be used in the virus recovery work were identified and recorded.

DISCUSSION OF RESULTS

The total number of mosquitoes identified at the site of the monkey fatalities was 17,420, of which 11,431 were pooled alive and frozen for the attempted virus recovery. The summary of the collections made is given in Table 1.

Of greatest interest are the results with regard to the mosquitoes of the genus *Haemagogus*, which includes several species known to transmit sylvan yellow fever in South America (Whitman, 1951). The only *Haemagogus* taken was *equinus*, which comprised slightly more than three per cent of the mosquitoes taken in the canopy. This species is widely distributed throughout Middle America, and we have taken it as far north as the southwestern corner of Tamaulipas, the Mexican state bordering the United States on the Gulf coast. While this species is known to be able to transmit yellow fever in the laboratory (Waddell and Taylor, 1945; Waddell, 1949), virus has never been recovered with certainty from wild caught individuals, although Boshell and Osorno (1944) infected a monkey with a field-collected group of three species of *Haemagogus* including *equinus*. Also in this group of *Haemagogus*, however, was *spgazzinii falco* from which virus has been repeatedly recovered in Colombia. Thus *equinus* has not thus far gained general acceptance as a natural vector. *Haemagogus spgazzinii falco* was lacking from the present captures, but in the year-long collections (June, 1953, to June, 1954) made in the rain forest at Lancetilla, 35 kilometers to the west, 63 specimens were taken. Sixteen specimens of *falco* were also taken on August 14th, 1954, a day when the field crew collected in the true tropical rain forest on the mountain slope to the south of La Masica, in the direction opposite that where the dead monkeys were found. There were no reports before, during, or after the period of this study of monkey mortality in the rain forest either on the mountain slope behind La Masica, or at Lancetilla where a reliable European observer was stationed. Thus in the area where a known South American vector was present (*H. spgazzinii falco*) the monkeys did not to our knowledge die, while in the coastal forest in which the monkeys did die, the only *Haemagogus* present was *equinus*, which has not been considered a natural vector. It may be added that at first we found it difficult to give credence to the reports of monkey mortality in the swampy forest of the coastal plain, since our experience gained during five years of field work in Middle America was such that in this ecological situation we expected to find only *equinus*, which to this time we had excluded as a probable natural vector. The subsequent collections which produced only *equinus* confirmed this ecological estimate of the situation. Our previous exclusion of *equinus* as a probable vector was based not only on the lack of proof of its natural infection, but also on the fact that in previous episodes of proven monkey or human sylvan yellow fever, which we had investigated in Panama, Costa Rica and Nicaragua, we had in every case been able to demonstrate the presence of *H. spgazzinii falco*. The conservative course had been to accept this association at face value. While *equinus* was also invariably present at the sites of proven yellow fever, by virtue of its less discriminating ecological requirements it was also generally distributed in areas into which yellow fever did not penetrate, such as the Pacific side of western Panama. The

TABLE 1

Mosquitoes taken in the canopy of the forest near La Masica, Honduras 8 August to 8 September, 1954

	Total	Alive and pooled for virus recovery
Tribe Culicini		
<i>Haemagogus equinus</i>	547	495
<i>Aedes (Finlaya) terreus</i>	2	0
<i>Aedes (Ochlerotatus) serratus</i> (group).....	602	562
<i>Aedes (Ochlerotatus) angustivittatus</i>	52	51
<i>Aedes (Ochlerotatus) scapularis</i>	1	1
<i>Aedes (Ochlerotatus) fulvus</i>	17	11
<i>Aedes (Ochlerotatus) sp.</i>	612	409
<i>Mansonia (Mansonia) titillans</i>	23	14
<i>Mansonia (Mansonia) indubitans</i>	1,016	420
<i>Mansonia (Rhynchotaenia) nigricans</i>	34	21
<i>Mansonia (Rhynchotaenia) venezuelensis</i>	334	263
<i>Psorophora (Psorophora) lineata</i>	5	2
<i>Psorophora (Psorophora) cilipes</i>	2	2
<i>Psorophora (Janthinosoma) ferox-lutzi</i>	11,023	7,015
<i>Psorophora (Grabhamia) cingulata</i>	5	3
<i>Culex sp.</i>	8	5
Total Culicini	14,283	9,274
Tribe Sabethini		
<i>Trichoprosopon (Ctenogoeldia) magnus</i>	1,860	1,396
<i>Trichoprosopon (Rhunchomyia) longipes</i>	191	93
<i>Trichoprosopon (Rhunchomyia) leucopus</i>	78	74
<i>Trichoprosopon (Isogoeldia) espini</i>	75	41
<i>Wyeomyia sp.</i>	432	229
<i>Limatus sp.</i>	4	1
<i>Sabethes (Sabethes) cyaneus</i>	111	70
<i>Sabethes (Sabethes) tarsopus</i>	21	9
<i>Sabethes (Sabethoides) chloropterus</i>	352	235
Total Sabethini	3,124	2,148
Tribe Anophelini		
<i>Chagasia bathanus</i>	13	9
Total Anophelini	13	9
Total all species	17,420	11,431

N.B. The mosquito nomenclature follows Lane, 1953.

present incident is the first in which we have not been able to demonstrate the immediate association of a proven natural vector.

There are, however, two alternate hypotheses to that of accepting *equinus* as the vector in the present circumstance:

1. That the monkeys were in fact dying of *falco* transmitted yellow fever in

the rain forest of the mountain slope, but that the mortality was not observed; and that numbers of infected *falco* so small that we failed to find them, made flights of 10 to 20 or more kilometers to the coastal plain where they produced the monkey mortality which was observed. A variation of this hypothesis would be that only sufficient *falco* made flights of this sort to infect a few or even one monkey on the coastal plain, and the epizootic was then carried on by *equinus* or some other as yet unproven secondary vector.

2. That some other as yet unproven vector or vectors were alone involved.

The first alternate hypothesis would have in its favor the fact that the coastal plain is inhabited while the rain forest of the mountain slope is not. Thus there would be a greater possibility of receiving reports of monkey mortality from the coastal plain than from the mountain slope. In opposition to this would be the fact that while the mountain slope forest is not inhabited, natives do hunt there, and the reward offered would have been sufficient inducement for reports of monkey mortality in the forest to have reached us. Additionally, the slopes of the Lancetilla Valley are clothed with rain forest, support a substantial population of howling monkeys, and are subject to continuous surveillance for protection of the forest by personnel of the United Fruit Company research station. Yet, while we had shown *falco* to be present here (though in small numbers), and this area is only 35 kilometers from the place where virus was active, no evidence of monkey mortality could be obtained.

There is still to consider the second alternate possibility that vectors other than those of the genus *Haemagogus* might be involved. The commonest mosquitoes were *Psorophora ferox* and *lutzii*. The large numbers of these mosquitoes taken in trees is a reflection of the overwhelming populations breeding in the swampy forest in which the collections were made, rather than any indication that they are truly arboreal (see Trapido, Galindo and Carpenter, 1955). As the *Psorophora* are not properly arboreal, and as the evidence with regard to their ability to transmit yellow fever is either negative or equivocal (Whitman and Antunes, 1937), we did not consider them of particular significance, although 7,022 were frozen for mouse inoculation. The *Mansonia* which were also present in substantial numbers were not of special interest for the same reasons. The only *Aedes* of the subgenus *Ochlerotatus* taken by us, which has been shown to be capable of transmitting yellow fever virus by bite, is *scapularis* (Davis and Shannon, 1929 and Whitman and Antunes, 1937). We exclude this from consideration as only a single specimen was taken. The only *Aedes* (*Finlaya*) present was *terrens* of which but two specimens were collected. This species is known to retain virus (Davis and Shannon, 1931) but has never been shown to be capable of transmitting virus by bite. Another member of this subgenus, *Aedes leucocelaenus*, which is known to transmit virus by bite, and which has been found infected in nature (Shannon, Whitman, Franca, 1938), had been found by us associated with yellow fever in Panama and Costa Rica, but it is rare north of the Rio San Juan, the boundary between Costa Rica and Nicaragua. We identified a single specimen of the subspecies *clarki* from the collections made at Yaruca where monkeys had died earlier, but none was taken in the year-long collections made at Lancetilla, and it was not recovered at La Masica.

The sabethine mosquitoes have never been adequately studied for their ability to transmit virus due to the difficulties encountered in keeping them alive and inducing them to feed in captivity (Whitman, 1951), but on one occasion virus was recovered from a mixed lot of these mosquitoes (Shannon, Whitman, and Franca, 1938). In our studies in Panama we considered *Sabethes chloropterus* suspect on epidemiological grounds (Galindo, Trapido, and Carpenter, 1950). This species was present at the time of the La Masica epizootic, but was far outnumbered by *Trichoprosopon magnus*. Nothing is known of the vector status of the latter, although another *Trichoprosopon*, the species *frontosus*, has been shown to be capable of transmitting the virus by bite in the laboratory (Whitman, Tullock and Waddell as quoted in Waddell, 1949). Despite the fact that *T. magnus* was the most abundant truly arboreal mosquito taken, we would tend to discount its importance on ecological grounds. It breeds in a species of *Calathea* which occurs in low swampy forest. Thus it has not in our experience, been associated in significant numbers with the occurrence of yellow fever, which up to this point in Middle America has been on elevated slopes. It might, of course, be a secondary vector in this special circumstance, though not involved elsewhere. While the numbers of *Sabethes chloropterus* were smaller, this species has invariably been present at all yellow fever episodes investigated by us and we still consider it as suspect. Experiments on this and other sabethines to determine their ability to transmit yellow fever virus are much needed.

The superintendents of the United Fruit Company Hospital at Tela and the Salvatore D'Antoni Hospital at La Ceiba were informed of the positive diagnosis of yellow fever in monkeys, asked to be on the lookout for clinical cases of yellow fever, and to save sera from fever patients in whom a diagnosis of malaria could not be established. No yellow fever in humans was detected and no sera obtained, but mild and inapparent infections such as those described by Kerr (1951) could readily have been missed. We have no first hand information on the fatal case reported from San Pedro Sula in the "Tropical Medicine and Hygiene News" of December 1954. Militating against the appearance of human infections would be the fact that some vaccination in the area had been carried on prior to the epizootic, although we encountered numbers of persons who had not been vaccinated. Urban human cases were not expected, as *Aedes aegypti* is thought to have been eradicated from the area by residual DDT spraying carried on by the Honduran government.

The results of attempts to recover yellow fever virus from the mosquitoes frozen on CO₂ ice will be reported separately with Dr. Enid de Rodaniche.

SUMMARY AND CONCLUSIONS

1. Howling monkeys (*Alouatta*) died of histopathologically proven yellow fever in July and early August, 1954, on the coastal plain near La Masica, Honduras, in an ecological situation unlike that of epizootics experienced during the preceding five years in Panama and lower Central America. No naturally proven vector mosquitoes could be found on the coastal plain where the monkeys died.
2. Small numbers of *Haemagogus spegazzinii falco*, a proven natural vector

in South America, were taken on mountain slopes in the tropical rain forest some 10 to 20 kilometers or more from the place where the monkeys died, but no evidence could be obtained of monkey mortality there. The authors believe the north coast of Honduras to be at or near the northern limit of the range of *H. spegazzinii falco*.

3. *Haemagogus equinus*, which has been shown to be capable of transmitting yellow fever in the laboratory, but from which virus has never been certainly recovered in the field, was the only *Haemagogus* species recovered at the place where the monkeys died near La Masica. This species which is widespread in Middle America has been taken by us in the Atlantic drainage as far north as the southwestern corner of the Mexican state of Tamaulipas which borders Texas on the Gulf Coast.

4. Aside from the predominantly ground-level biting *Psorophora* and *Mansonia*, which are not considered to be involved in the yellow fever transmission, the commonest arboreal mosquito was *Trichoprosopon magnus*. The ability of this mosquito to transmit virus is unknown. Another sabethine mosquito whose status as a vector is also not known, *Sabethes chloropterus*, was present in moderate numbers. This species has been present at other recent yellow fever episodes in Panama and lower Central America.

5. No human cases of yellow fever were recognized in the immediate area of the epizootic during, or in the months following the monkey fatalities.

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