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Evidence of Recent Jungle Yellow-Fever Activity in Eastern Panama*

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Outbreaks of jungle yellow fever in man have been recorded twice from eastern Panama of recent years, first in 1948 and again in 1956. Since then, a close surveillance has been maintained on virus activity in eastern Panama. Recent field observations and serological tests on 402 monkey sera indicate that there was an outbreak of yellow fever among monkeys of southern Darién Province some time between 1963 and 1965. It does not appear that the outbreak has spread as yet to other areas. Virus transmission may have been permanently disrupted during the drought which affected the region in 1965. However, if the virus had managed to survive this unfavourable period, an epizootic wave might have evolved, invading forested areas immediately east of the Panama Canal, now inhabited by a dense non-immune human population.

During the eighteenth and nineteenth centuries, yellow fever was a well-known endemic disease in the isthmus of Panama. Gorgas initiated his intensive measures against the mosquito *Aedes aegypti* soon after the start of the twentieth century. His campaign was so successful that by the fall of 1905 urban yellow fever had been completely eradicated from Panama. From that year until 1948, no autochthonous cases of the disease were seen again in the isthmus (Trapido & Galindo, 1956).

During the months of November and December 1948, several cases of a rapidly fatal infectious disease appeared among wood-cutters and hunters in the area of Cerro Azul, some 20 miles east of Panama City. On 14 January 1949, these cases were officially reported as yellow fever, on the basis of post-mortem histopathological examination of liver specimens (Herrera et al., 1949).

From 1948 to 1956, outbreaks of the disease were noted among humans and monkeys in central and western Panama, Costa Rica, Nicaragua, eastern Honduras and eastern and central Guatemala (Trapido & Galindo, 1956; Rodaniche & Galindo, 1957). Virus activity in these outbreaks took the form of a wave which moved slowly westward and northward from the Panama Canal through the tropical forests of Central America, leaving in its wake several hundred human fatalities and a greatly reduced population of howler monkeys (*Alouatta* sp.). The last known outbreak caused by this epizootic wave was detected in 1956, when howler monkeys were reported dying near the Guatemalan-Mexican border. Yellow fever was diagnosed after histopathological examination of a liver specimen obtained from one of these dead monkeys (C. M. Johnson, personal communication). The last report of yellow-fever activity in humans in Panama caused by the 1948 wave was of a human death in February 1952, in the vicinity of Burica Point, Chiriqui Province, near the southern end of the frontier between Panama and Costa Rica (Elton, 1952) (see the accompanying figure).

Early in September 1956, signs of a second wave of jungle yellow fever were detected when virus was isolated from a pool of *Haemagogus lucifer* mosquitos collected in the Mandinga forests of Panama, some 10 miles north of the Cerro Azul area where the 1948 outbreak occurred (see figure). This isolation was followed a few weeks later by a fatal human case and by further isolations from mosquitos captured in the Cerro Azul area (Rodaniche et al., 1957). During the summer and fall of 1957, outbreaks of yellow fever similar to those of 1949 were noted near Buena Vista on the eastern shore of the Panama

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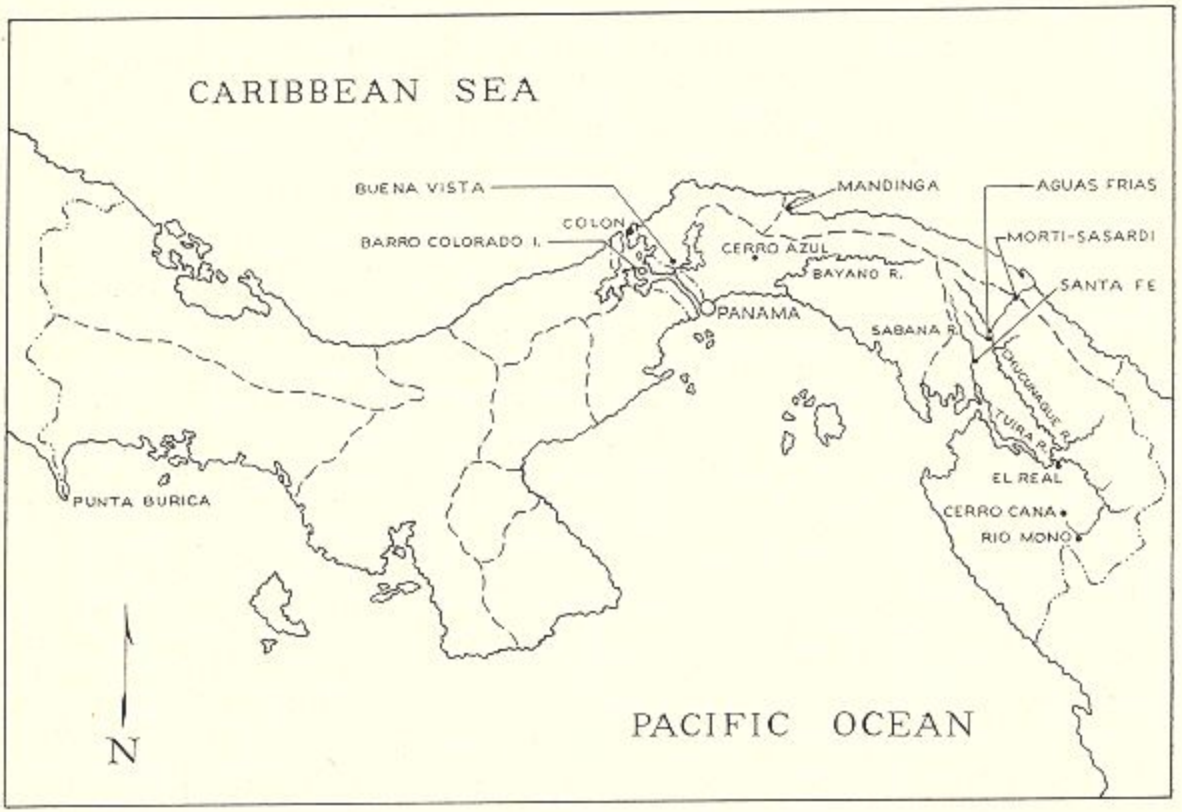
JUNGLE YELLOW FEVER INVESTIGATION SITES IN PANAMA



Canal, with isolation of virus from the blood of a man who recovered and from forest mosquitos (Galindo & Rodaniche, 1964). However, in contrast to what happened in the 1948 wave, the virus failed to cross the canal into western Panama and with the advent of the dry season yellow-fever activity ceased east of the Canal as well (Galindo & Rodaniche, 1964).

From 1957 to date, a close surveillance for signs of yellow-fever activity in eastern Panama has been maintained. This surveillance has included attempts to isolate virus from day-flying forest mosquitos collected in strategic areas and serological tests to detect acquired immunity in monkeys inhabiting these areas. Information gathered in these surveillance activities points to the absence of yellow fever in eastern Panama from 1957 to at least 1963 (Galindo & Rodaniche, 1964; M. A. Grayson, unpublished data).

The present report includes serological data obtained from tests on monkey blood collected in eastern Panama during 1965 and 1966, complemented by ecological observations which give information as to possible recent yellow-fever activity in the area.

METHODS

Monkeys as indicators of jungle yellow-fever activity

It is a known fact that all Panamanian monkeys are capable of becoming infected with yellow-fever virus. However, the level of viraemia and the resistance to infection vary in the different groups of primates.

There are five genera of monkeys in eastern Panama—namely, *Ateles* (spider monkey), *Alouatta* (howler monkey), *Cebus* (capuchin monkey), *Saguinus* (marmoset) and *Aotes* (night monkey). Of these five groups, the spider and capuchin monkeys show a much greater resistance to yellow-fever infections than the others. Spider monkeys, when infected, usually show some symptoms and high virus titres in the blood, but seldom die as a consequence of the infection. Capuchin monkeys resist

infection even better than spider monkeys. They seldom show any symptoms and rarely die from yellow fever. Moreover, in the field they do not seem to become as easily infected as spider monkeys, nor do they have such high virus titres as the latter. The three other groups (howler monkeys, marmosets and night monkeys) are very susceptible to infection. All of them usually sicken when infected and have high virus titres for several days, while a large percentage die. Thus, theoretically, when yellow-fever virus gets to an area inhabited by all these types of monkeys, large numbers of howlers and marmosets will die. Night monkeys, though highly susceptible, are hidden in tree holes at the time of day when the mosquito vectors are active, so not many of them are liable to become infected. On the other hand, many spider and capuchin monkeys become infected, but few die. The result is that howlers and marmosets are greatly reduced in numbers, leaving only the few specimens which survive infection and become immune and those which were not infected. Populations of spider and capuchin monkeys are hardly affected, as these monkeys seldom die as a result of yellow-fever infections.

It is evident from the above discussion that periodic observations of population densities of the different species of monkeys inhabiting a particular forest should offer substantial evidence as to the presence in the immediate past of yellow fever in the area. The validity of this theory was demonstrated in Barro Colorado Island, Panama Canal Zone, after a yellow-fever wave passed through the area in 1949 (Collias & Southwick, 1952; Trapido & Galindo, 1956).

The serological responses to a yellow-fever antigen also show distinct immunological patterns for the various species of wild primates inhabiting the forests of eastern Panama. Soon after the passage of yellow-fever virus through a forest, howler monkeys and marmosets will become scarce while a small number, particularly juveniles, will show antibodies against the yellow-fever virus. Spider and capuchin monkeys will be almost as common as before the arrival of the virus, but a larger percentage of the spider monkeys than of the capuchin monkeys will show antibodies in the blood, as the former seem to become infected more readily than the latter.

A combination of these two methods has been used in the work reported here, to arrive at conclusions regarding recent yellow-fever activity in forested areas of eastern Panama.

Collection of monkey sera

The majority of serum samples were obtained by shooting monkeys in selected forested areas and bleeding them by heart puncture immediately after retrieval. The rest were secured in the laboratory from monkeys from the Cerro Azul and Bayano River areas (see figure) purchased from hunters and Indians. The great majority of monkeys shot were still alive at the time of bleeding. No attempt was made to bleed monkeys that had been badly shot through the heart or abdomen. The heads of all monkeys yielding a blood sample after shooting were removed and labelled with the same number as the corresponding blood specimen. All blood samples were placed inside a portable kerosene refrigerator soon after being taken, and were kept at approximately 4°C for periods ranging from 4 to 10 days before being sent to the central laboratory. Monkey heads were skinned and the skulls were thoroughly cleaned and dried before being shipped to Panama City for age determination.

At the laboratory, all blood samples showing bacterial contamination or a high degree of haemolysis were discarded. Sera were separated from the rest of the specimens and stored at −20°C until ready to be tested.

Serological tests

All stored sera were subjected to a preliminary haemagglutination-inhibition (HI) test against a number of group-B antigens, including indigenous and sometimes non-indigenous viruses. Sera that were positive only to the yellow-fever antigen or that gave a higher titre against this antigen than against all other group-B arboviruses used were later subjected to mouse neutralization (NT) tests against yellow-fever virus and sometimes also against Bussuquara virus. Samples of the HI-negative sera selected at random were also included in the neutralization tests as controls. When a large number of sera from one area gave a positive reaction in the HI and NT tests, the positive sera were further tested by the complement-fixation (CF) technique.

The methods used in the HI, CF and NT tests will now be briefly described.

Haemagglutination-inhibition test. The technique described by Clarke & Casals (1958) was used. Primary incubations were done at 4°C overnight and the tests were carried out at room temperature (22°C to 24°C) after the addition of goose erythrocytes. All sera were treated with kaolin for removal of non-specific inhibitors.

Complement-fixation test. A modification of the Fulton & Dumbell (1949) microtechnique was used in CF testing. Primary incubations were done at 4°C overnight and the tests were carried out at 37°C in an incubator for 30 minutes after the addition of the haemolytic system. At least two units of antigen were used in the tests.

Neutralization test. Weanling white mice inoculated intracerebrally were used in the neutralization test. Titration end-points were calculated by the method of Reed & Muench (1938). Untreated sera were mixed with virus suspensions prepared from infected suckling mouse brain and were incubated at 37°C for one hour prior to injection in mice. The reaction was considered positive when 4 or 5 out of 5 inoculated mice survived during the 2-week examination period.

Use of skulls for determination of age of monkeys

In 1962, more than 50 skulls of monkeys from the Rio Mono area were sent to Dr Charles O. Handley of the US National Museum, Washington, D.C., in an attempt to establish criteria for determination of the approximate age of the specimens. After a study of these skulls and of material deposited at the US National Museum, Dr Handley was able to separate specimens into 5 age-groups which are quite adequate for evaluation of the age of eastern-Panama monkeys in the light of past episodes of jungle yellow fever recorded from that area.

FIELD ACTIVITIES

As in previous surveillance years, an area in southern Darién Province was chosen for the collection of samples of monkey blood in 1965. The area selected for this activity was that of Cerro Cana in the Pirre range of mountains, not far north of the Panamanian-Colombian border (see figure).

Once examination of the Cerro Cana serum samples revealed the possibility that jungle yellow fever had been recently active in the region, a number of other areas in eastern Panama were chosen to determine how far virus activity had spread toward the Panama Canal. The hunting areas will now be described briefly, with mention of the dates on which hunting operations took place and the number of monkeys that were bled in each area.

Cerro Cana

Located south of El Real in southern Darién Province (see figure). On 28 July 1965, one scientist and three technicians were transported into the area by a US Air Force helicopter. The base jungle camp was established near the top of the mountain in a cloud forest at 4200 ft (1300 m) above sea level. In view of the fact that only a few groups of howler and capuchin monkeys were seen near the camp location, most of the monkey hunting activity was moved down to heights of 2000 ft-3000 ft (600 m-900 m), between the base camp and the Valley of Cana. A total of 67 monkeys were bled from 30 July to 11 August. Of these, 32 were howler monkeys (*Alouatta palliata*), 27 spider monkeys (*Ateles grisescens*) and 8 capuchin monkeys (*Cebus capucinus*). Although many howler monkeys were observed, particularly above the 3000-ft (900-m) level, it was noted that the groups were made up of at most 4 monkeys, many of them being solitary specimens.

Rio Mono

This collecting station was established at the junction of the Mono and upper Tuira rivers, south-east of Cerro Cana, at an elevation of 500 ft (150 m) above sea level (see figure). The base camp was located above the Mono river in a thick, continuous stand of tropical rain forest. The same site had served as base camp for a similar expedition in 1962. A total of 115 monkey sera was obtained between 22 March and 17 April 1966. Of these, 88 were from spider monkeys and 23 from capuchin monkeys. No howlers or marmosets (*Saguinus geoffroyi*) were seen or heard despite the fact that in 1962 these two monkeys constituted 29.2% and 35.4% respectively of all those bled. Mr Charles Myers (personal communication), a keenly observant herpetologist, who collected extensively in this area in July 1965, was also impressed by the lack of audible and visual evidence of howler monkeys.

Aguas Frías

This is a small stream which empties into the upper Chucunaque river, just below the Mortí tributary, in northern Darién Province (see figure). The Aguas Frías area was visited between 13 May and 19 June 1966. Since the base camp was located in an extensive cativo (*Prioria copaifera*) swamp, most of the hunting was done about 4 to 5 miles (6 to 8 km) north-west of camp, on a ridge covered with transitional tropical rain-forest which separates the Chucunaque, Bayano and Sabana rivers. A total of 91 monkeys were bled, 47 being capuchin, 36 spider and 8 howler monkeys. Groups of 10 or more howlers were common, but few members of

this species were obtained because efforts were concentrated on the spider monkeys, which constitute a much better serological indicator of yellow-fever activity.

Cerro Azul

In view of the fact that the spot where yellow fever was detected in 1956 has been largely cleared for agricultural purposes, the collecting station was established about 5 miles (8 km) to the north, on the headwaters of the Rio Piedras, at 2400 ft (730 m) above sea level. The station is located between the Cerro Azul and Mandinga areas, both of which yielded yellow-fever virus in 1956 (see figure). Forest cover is of the tropical rain-forest type, and continues unbroken eastward to the Panamanian-Colombian border in Darién Province. Thus, any yellow-fever activity occurring in northern Darién could spread into this area without any physical or ecological barriers to its progress. A total of 54 monkey sera was obtained at the Rio Piedras station between 7 and 31 January 1966, from 21 howler monkeys, 16 capuchin, 15 red spider monkeys (*Ateles geoffroyi*) and 2 night monkeys (*Aotes trivirgatus*). All groups of howler monkeys noted were made up of 10 or more specimens and were normal in appearance.

PURCHASED MONKEYS

The Gorgas Memorial Laboratory has always been interested in the study of diseases of wild primates. Thus, monkeys brought to the laboratory by hunters and jungle inhabitants are routinely purchased. Recently, these purchases have been greatly increased in consequence of a very active research project on monkey malaria carried out by Dr Martin D. Young and Dr James Porter.

A total of 138 sera of infant, juvenile and very young adult monkeys were obtained from Dr Porter for testing. Of these, 34 were from spider monkeys, 33 from night monkeys, 15 from capuchin monkeys, 42 from marmosets and 14 from howler monkeys. All these monkeys were obtained in 1965 or 1966 along the Pacific slopes of the continental divide, between the Cerro Azul area and the upper Bayano River basin (see figure).

RESULTS OF SEROLOGICAL TESTS

Cerro Cana area

Of the 67 sera collected, 60 were subjected to a preliminary HI test; 12 specimens (20%) gave a positive reaction for yellow fever. The break-down by type of monkey and age-group is found in Table 1. As may be noted, almost all (11 out of 12) of the positive specimens were from spider monkeys.

TABLE 1
RESULTS OF HI TESTS ON CERRO CANA MONKEY SERA WITH YELLOW-FEVER VIRUS

Source of serum	No. tested	No. positive	Age of positive specimens
Spider monkey	24	11	2 juveniles, 3 young adults and 6 adults
Howler monkey	29	1	1 early juvenile
Capuchin monkey	7	0	—
Total	60	12	

Table 2 shows the HI titres of the various positive specimens against 3 group-B arboviruses known to occur in the area—namely, yellow fever, St Louis encephalitis and Bussuquara. Table 3 presents the results of CF tests against a number of group-B antigens, performed on all HI-positive sera and on 3 negative ones selected at random. Of the 3 HI-negative samples, 2 were also negative by CF, the third being anti-complementary. Of the 12 HI-positive specimens tested by CF, 2 were anti-complementary; 9 of the remaining 10 gave positive reactions with the yellow-fever antigen. As expected in arboviruses of group B, there were many HI and CF cross-reactions against the various antigens. However, with one exception, all sera gave as high or higher HI and CF titres with yellow-fever antigen as with any of the other group-B antigens used. The exception, specimen T-00182, obtained from a senile female spider monkey, gave a strong positive HI reaction with the 3 group-B antigens used, but was stronger against St Louis encephalitis and Bussuquara viruses than against yellow fever. In the CF test, this specimen reacted against all group-B antigens used, but reactions were strongest for yellow fever and Bussuquara.

Mouse neutralization tests performed on all HI-positive and a sample of HI-negative sera showed that the 3 sera which were negative in the HI test were also negative in the NT test. Of the 12 sera positive in the HI test, 11 were also positive in the NT test against yellow-fever virus. On the other

TABLE 2

HI TITRES OF CERRO CANA MONKEY SERA AGAINST THREE GROUP-B ARBOVIRUSES

Source of serum	Specimen No.	Titre against 4 units of antigen [a]		
		Yellow fever	St Louis encephalitis	Bussuquara
Spider monkey	T 00111	20	0	0
Spider monkey	T 00119	40	0	20
Spider monkey	T 00182	160	640	320
Spider monkey	T 00183	160	0	80
Spider monkey	T 00186	20	0	0
Spider monkey	T 00188	80	0	0
Spider monkey	T 00189	80	0	40
Spider monkey	T 00190	20	0	0
Spider monkey	T 00228	40	0	0
Spider monkey	T 00230	80	0	40
Spider monkey	T 00231	80	0	40
Howler monkey	T 00127	40	0	0

[a] 0 = less than 1:20.

TABLE 3

CF TESTS ON CERRO CANA MONKEY SERA AGAINST GROUP-B ARBOVIRUSES [a]

Results of yellow-fever HI test	Specimen No.	St Louis virus	Dengue 2	Yellow fever	Bussu-quara	Ilheus	Normal mouse brain	Remarks [b]
+	T 00111	4	4	8	0	0	0	
+	T 00119	4	0	4	0	0	0	
+	T 00127	–	–	–	–	–	–	AC
+	T 00182	16	8	32	32	16	0	
+	T 00183	4	4	32	4	4	0	
+	T 00186	0	0	0	0	0	0	
+	T 00188	0	0	8	0	0	0	
+	T 00189	–	–	–	–	–	–	AC
+	T 00190	4	0	4	0	0	0	
+	T 00228	0	0	4	0	0	0	
+	T 00230	4	0	16	4	0	0	
+	T 00231	0	0	8	0	0	0	
–	T 00110	0	0	0	0	0	0	
–	T 00164	0	0	0	0	0	0	
–	T 00168	–	–	–	–	–	–	AC

[a] Test done with 2 units of antigen. Titres given as 0 are less than 1:4.
[b] AC = anti-complementary.

hand, with the exception again of specimen T-00182, all these sera were negative in the NT tests with Bussuquara virus. Another significant finding was that, of the 12 monkeys which were positive for yellow-fever antibodies (HI, CF or NT), 3 were early juveniles probably less than 2 years old, and 3 were very young adults probably no more than 4 years old.

Rio Mono area

A total of 77 sera from Rio Mono was tested by HI against 6 group-B antigens, namely, those of yellow fever, St Louis encephalitis, Ilheus encephalitis, dengue 2, Bussuquara and Powassan. Of these, 15 specimens gave the highest titre with yellow fever, or gave a titre higher than 1:40 only with the yellow-fever antigen. These were considered positive for yellow fever (see Table 4); 8 additional sera gave higher titres with other group-B antigens than for yellow fever, and are not considered here as yellow-fever-positive sera. Of the 15 positive sera, 14 came from spider monkeys and 1 from a capuchin monkey; 2 of the positive specimens were obtained from juvenile monkeys probably less than 3 years of age, 1 from a spider monkey and 1 from a capuchin monkey.

TABLE 4

YELLOW-FEVER ANTIBODIES IN MONKEYS OF 3 AREAS OF EASTERN PANAMA

Locality	No. tested	No. positive		No. of juveniles positive
		HI test	NT test	
Rio Mono	77	15	14	2
Aguas Frías	73	2	2	0
Cerro Azul	54	3	2	0

All HI-positive sera, and 3 of the HI-negative sera selected at random, were subjected to mouse neutralization tests. As may be noted in Table 4, of the 15 HI-positive sera tested, only 1 appeared to be negative in the NT tests.

Aguas Frías area

Of the sera collected, 73 were subjected to HI tests and only 2 gave a positive reaction with the yellow-fever antigen, 1 at a titre of 40 and 1 at 80. Both sera were obtained from adult spider monkeys estimated to be more than 6 years of age. Both HI-positive sera and 4 HI-negative sera selected at random were subjected to NT tests. All of the HI-negatives sera were also negative in the NT test. Both HI-positive sera were positive in the NT tests (see Table 4).

Cerro Azul area

All of the 54 sera were subjected to HI tests against group-B arboviruses. Of these, 3 were considered positive for yellow fever, reacting at titres of 160, 80 and 80. All these specimens were obtained from adult monkeys (2 spider and 1 capuchin) that were considered to be over 6 years old. The 3 HI-positive sera and 3 HI-negative sera were subjected to NT tests. Of the positive sera 2 were also NT positives. Of the HI-negative sera, none was positive in the NT test (see Table 4).

Purchased monkeys

A total of 138 sera from purchased juvenile and young adult monkeys from the Cerro Azul and Bayano River areas were submitted to HI rests against a yellow-fever antigen with completely negative results (see Table 5).

TABLE 5

YELLOW-FEVER HI TESTS ON SERA OF PURCHASED YOUNG MONKEYS FROM THE CERRO AZUL AND BAYANO AREAS

Type of monkey	No. tested	No. positive
Spider monkey	34	0
Night monkey	33	0
Capuchin monkey	15	0
Marmoset	42	0
Howler monkey	14	0
Total	138	0

DISCUSSION

The field observations and results of serological tests will now be discussed by localities, in terms of possible recent jungle yellow-fever activity in these areas.

Cerro Cana

An examination of the field and serological data gathered from Cerro Cana, in southern Darién Province, leads to the conclusion that there was

jungle yellow-fever activity in this area some time between 1963 and 1965.

This conclusion is based on the following considerations:

(*a*) Although in July 1965 howler monkeys were not uncommon in the area, almost all the specimens were seen or heard along the upper fringes of the yellow-fever belt, between 3500 ft and 4000 ft (1000 m and 1200 m) above sea level. All howler-monkey groups observed were made up of few individuals with many solitary specimens. On the other hand, groups of spider and capuchin monkeys were composed of many specimens, as is normal. This reduction in the howler-monkey population with little or no effect on the spider and capuchin monkeys is a common occurrence after a yellow-fever outbreak.

(*b*) At least 18% of all the monkeys and 40% of the spider monkeys showed neutralizing antibodies against yellow-fever virus and were negative against Bussuquara, also a group-B virus, confirming the specificity of the reactions. Previous experiences in Panama have shown that these high rates of positive sera are only obtained during the first two or three years after an outbreak of yellow fever.

(*c*) Positive CF antibodies probably indicates that yellow-fever infection occurred in the area during the last two or three years.

(*d*) It was found that 2 of the positive spider monkeys and the only positive howler were juveniles with milk dentition. Since they were probably less than 2 years old, they must have acquired their infections after 1963.

Rio Mono

Data from this southern Darién locality also show that it must have been visited by jungle yellow fever at about the same time as the Cerro Cana outbreak. The reasons for this conclusion follow:

(*a*) As early as July 1965, it was noted by Mr Charles Myers that something had happened to the howler monkeys, as he neither heard nor saw any specimens during the several weeks he was collecting amphibians and reptiles in the area. Later, in March and April 1966, our party failed to find or hear howlers and marmosets, but populations of spider and capuchin monkeys were noted to be normal. This is in contrast to findings during a similar expedition in 1962, when howlers and marmosets constituted a high percentage of the total sample of monkeys obtained. This phenomenon in an uninhabited virgin forest in Panama is usually indicative of recent yellow-fever activity.

(*b*) Over 19% of all the monkeys and at least 24% of the spider monkeys showed antibodies to yellow-fever virus. As explained above, these high rates are only observed soon after the virus has passed through an area.

(*c*) It was found that 1 juvenile spider and 1 juvenile capuchin monkey, probably between 1 and 3 years of age, had neutralizing antibodies against yellow-fever virus, showing that the disease must have been active in the area some time between 1963 and 1965.

Aguas Frías and Cerro Azul

Examination of data from these localities leads to the conclusion that there has been no recent yellow-fever activity; this conclusion is based on the following reasons:

(*a*) The low titres of positive sera in monkeys from these areas are indicative of old infections. Past experience demonstrates that as yellow-fever virus passes through these regions, it leaves in its wake relatively high rates of immunity in the monkey population which gradually decrease from year to year (Galindo & Rodaniche, 1964).

(*b*) Populations of howler monkeys were normal both at Aguas Frías and at Cerro Azul. This observation would argue against the occurrence of recent yellow-fever outbreaks in these areas, since a high mortality rate among the howlers is one of the first visible signs of a jungle yellow-fever outbreak.

(*c*) All positive sera from Cerro Azul and Aguas Frías belong to adult monkeys probably over 6 years of age, which may have been old enough to acquire their infections during the previous wave of yellow fever which swept the area in 1956 and 1957.

(*d*) A total of 138 sera of purchased juvenile and young adult monkeys from the Cerro Azul and Bayano River area showed no HI antibodies against the yellow-fever virus.

STATISTICAL ANALYSIS OF THE DATA

Examination of the totals included in Table 6, which summarizes by locality the over-all percentage of HI-positive monkey sera, will lead to the inevitable conclusion that there has been a recent outbreak of jungle yellow fever in the east of the Republic of

TABLE 6

YELLOW-FEVER-HI-POSITIVE MONKEY SERA BY LOCALITIES

Locality	Province	No. of sera tested	No. of positive sera	% positive	No. of juveniles positive
Cerro Cana	southern Darién	60	12	20.0	3
Rio Mono	southern Darién	77	15	19.5	2
Aguas Frias	northern Darién	73	2	2.7	0
Cerro Azul	eastern Panamá	54	3	5.6	0
Cerro Azul and Bayano [a]	eastern Panamá	138	0	0	0
Total		402	32	8.0	5

[a] Monkeys purchased from hunters and Indians.

Panama. However, some doubt might be cast on the contention that the data presented indicate that the recent yellow-fever activity has been restricted to southern Darién Province and has not yet spread to regions in northern Darién province and eastern Panamá Province.

In order to test the validity of this contention, the available data were subjected to statistical analysis. It must be emphasized here that the areas sampled are ecologically very similar. The large majority of monkeys bled were inhabitants of closely related types of forests in areas with qualitatively and quantitatively very similar fauna of diurnal, arboreal, forest mosquitos. The composition of the different monkey samples was also similar, with the exception of the one from Rio Mono which included no howler monkeys.

The first step taken was to determine whether it would be statistically permissible to condense the data from the four localities which yielded positive monkeys into two groups, one representing southern Darién and the other including specimens from northern Darién and eastern Panamá Provinces. To this end, the statistical significance of the difference between the positive rates of the Cerro Azul and Aguas Frías samples, as well as that between the Cerro Cana and Rio Mono specimens, was determined. The χ^2 test gave a χ^2 value of 0.12 for the samples from Aguas Frías and Cerro Azul. Since this value is much below the level of significance, there appeared to be no statistical objections to lumping data from these two localities.

A χ^2 value of 0.006 was obtained for the Cerro Cana and Rio Mono samples, which is again much below the level of significance. However, since the composition of the Cerro Cana and Rio Mono samples differed in that howlers were not found at Rio Mono, while at Cerro Cana many howlers were present in the sample, the data from the two areas were again submitted to the significance test after elimination of howler monkeys from the Cerro Cana sample. A χ^2 value of 2.28 was obtained this time. This figure is still below that corresponding to the usual significance level of 5%, which is 3.841. It thus also appears statistically sound to lump the data from Cerro Cana and Rio Mono, as representing yellow-fever immunity rates in monkeys inhabiting southern Darién Province.

As a last step, the significance test was applied to data from southern Darién (Cerro Cana and Rio Mono) as compared with those from northern Darién and eastern Panamá Provinces (Aguas Frías and Cerro Azul). A χ^2 value of 13.9 was obtained, showing that the difference between the two samples is significant, much above the 0.1% level.

From this statistical analysis it becomes evident that the difference observed in the yellow-fever immunity rates among the monkeys which inhabit the two large geographical areas mentioned above is certainly not due to chance. The contention that such a difference is probably due to the fact that jungle yellow fever was recently active in southern Darién but not in northern Darién or eastern Panamá Provinces is therefore not contradicted by statistical analysis of the data.

PRESENT STATUS OF JUNGLE YELLOW-FEVER ACTIVITY IN EASTERN PANAMA

At the date of writing, it has not been possible to determine whether yellow fever is still active in some region of Darién Province. The absence of reported human cases is not unusual, as the areas where activity of the virus has been noted are inhabited only by a few isolated groups of nomadic Choco Indians and clandestine Colombian Negroes. These areas are so remote that if a human case occurred it would probably go unnoticed.

It is quite possible that transmission of the virus was permanently disrupted during the severe drought experienced throughout the isthmus of Panama during the first half of 1965. If this was the case, several years will probably elapse before the virus is able again to break through the barrier of immune vertebrate hosts in southern Darién. On the other hand, if small centres of virus activity managed to remain through the unfavourable drought period, they will gradually flare up into larger and larger epizootic outbreaks during the present rainy season (May to December 1966).[1] Such outbreaks are apt to evolve quickly into a wave of yellow fever moving toward the Panama Canal that, if not detected in time, is likely to cause a considerable number of human cases in the areas of the Bayano River and the Cerro Azul mountains, which are now much more densely populated by non-immune persons than during the 1956-57 outbreak.

Plans are being drawn up to establish a permanent surveillance station in the critical Mortí-Sasardí area in northern Darién Province (see figure). Yellow-fever virus will have to funnel through this area in order to pass from the extensive forests of Darién Province into the narrow forested belt along both slopes of the continental divide at the level of the upper Bayano River basin. These plans include the use of sentinel monkeys, attempts to isolate yellow-fever virus from diurnal, forest-canopy mosquitos, and close surveillance of the monkey population for signs of yellow-fever activity.

[1] In fact, close surveillance of the Mortí-Sasardí area in Northern Darién Province did not reveal any signs of yellow-fever activity in 1966.

RÉSUMÉ

En raison de poussées épidémiques de fièvre jaune survenues en 1948 et en 1956-1957, une étroite surveillance est exercée au Panama oriental pour dépister tout signe de reprise d'activité de la maladie. On tente notamment d'isoler le virus amaril à partir des vecteurs potentiels et on pratique des examens sérologiques parmi la population simienne pour déceler la présence d'anticorps. D'autre part l'étude de la densité relative des diverses populations de singes fournit de précieuses indications, car certaines espèces (singes hurleurs, ouistitis) sont décimées par la maladie tandis que d'autres (singes capucins, singes araignées) sont particulièrement résistantes à l'infection.

Une première expédition de chasse au singe a été organisée en 1965 dans la province du Darien méridional. Les singes hurleurs étaient inhabituellement rares. Soixante sérums prélevés chez trois espèces ont été examinés par les épreuves d'inhibition de l'hémagglutination, de fixation du complément et de séroneutralisation. Douze d'entre eux (20%) donnaient une réaction positive avec le virus amaril; onze avaient été prélevés chez des singes araignées où le taux de positivité atteignait 45%. Trois des échantillons positifs provenaient de jeunes singes âgés de moins de 2 ans, ce qui semble démontrer qu'une épidémie de fièvre jaune de brousse a sévi dans la région entre 1963 et l'été 1965.

Au cours d'une deuxième expédition, dans une région voisine, on a noté une absence inhabituelle des singes hurleurs et des ouistitis. Dix-neuf pour cent des 77 sérums examinés et 24% des sérums prélevés chez des singes araignées renfermaient des anticorps antiamarils. Deux sérums de singes âgés de moins de 3 ans donnaient une réaction positive, ce qui suggère également l'existence récente d'une épidémie de fièvre jaune de brousse dans la région.

A l'occasion de deux enquêtes menées dans d'autres endroits du Panama, aucune mortalité anormale n'a été constatée parmi les singes hurleurs. Dans un cas, 2 sérums seulement sur 73, et tous deux recueillis sur des animaux âgés de plus de 6 ans, étaient positifs pour la fièvre jaune. Dans l'autre cas, seuls 3 singes âgés de plus de 6 ans, sur 54, étaient porteurs d'anticorps antiamarils. D'autre part, 138 singes achetés par le laboratoire et originaires des mêmes régions ont fourni des sérums dépourvus d'anticorps.

Selon les auteurs, ces résultats démontrent l'activité récente du virus amaril dans la province du Darien méridional, les provinces du Darien septentrional et du Panama oriental étant épargnées. Le fait que la région atteinte a souffert d'une extrême sécheresse en 1965 a peut-être entravé la transmission de l'infection. Si le virus avait survécu, on aurait pu assister éventuellement, durant la saison des pluies, à une extension de foyers actuellement limités et à des épizooties dans la zone avoisinant le canal de Panama. Les conséquences auraient pu en être désastreuses pour la population humaine à forte densité et non immune qui y vit actuellement.

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