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SURVEY OF LIVE FOREST ANIMALS FOR PROTECTIVE ANTIBODIES AGAINST YELLOW FEVER IN PANAMA, R. P.

ENID C. DE RODANICHE

Gorgas Memorial Laboratory, Panama, R. P.

In November and December of 1948 a small outbreak of jungle yellow fever occurred near the town of Pacora in the Province of Panama, R. P. (Herrera *et al.* 1949; Courtney 1950). These cases represented the first to be identified here since 1907. In order to provide information concerning the vertebrate hosts of the disease in this area, a survey of live forest mammals for protective antibodies was initiated. Surveys of this kind have been conducted in Africa by Haddow *et al.* (1947), in Colombia by Bugher and coworkers (1944) and in Brazil by Laemmert *et al.* (1946) and Kumm and Laemmert (1950), but had not previously been undertaken in Panama. Later Clark (1950 and 1952) with the cooperation of the Yellow Fever Service, began a study of the immune status of shot arboreal forest animals in representative sections of the Republic. Although the survey of Clark was much more extensive and complete than the one here reported, it was considered of value to continue the study of live-caught animals as these present certain advantages over shot animals in the conduct of serological tests: *i.e.* the avoidance of nonspecific reactions due to contamination and hemolysis and the possibility of repeated bleedings to clarify unsatisfactory or inconclusive results.

MATERIALS AND METHODS

Animals were obtained principally by purchase. In most instances the animals were brought directly to the laboratory by local trappers. A few animals were bought in the market and their place of origin was unknown. This method of obtaining animals had the disadvantage that the majority were received from neighboring districts within easy transportation distance of the laboratory and relatively few from the more distant parts of the country. Blood was obtained either from the femoral vein or directly from the heart and the serum removed within 24 hours. Sera were stored in a deep-freeze compartment at approximately −20°F. until a sufficient number had been accumulated to test, a period never exceeding two months. Although Hughes and Perlowagora (1950) found that prolonged storage may alter the specificity of some nonprimate bloods, it does not affect sterile primate bloods. An effort was made to keep the animals alive for a period of at least two weeks after the first bleeding when they were retested for possible alterations in the neutralizing capacity of their sera. It was not possible to retest all the marmosets and night monkeys since many of these small and delicate animals died as a result of the initial bleeding or other causes.

The virus strain employed was the French neurotropic, received through the courtesy of Dr. Max Theiler of the Rockefeller Foundation. The standard intraperitoneal protection test in adult Swiss albino mice as devised by Sawyer and

Lloyd (1931) was employed as we considered it the most practical for a laboratory of limited facilities. Ten per cent virus suspensions were used and intraperitoneal inoculations made one hour after intracerebral injection of 2 per cent corn starch solution. The more recent tests of Bugher (1940) and Whitman (1943) are more sensitive in detecting minimal amounts of antibody. However, great sensitivity is at times disadvantageous in general survey work. As Smithburn (1951) says, "in the conduct of immunity surveys the purpose of which is to define the limit of the disease, it may be more important to exclude with certainty all nonimmunes than to determine the exact per cent of immunity. . . . In such cases it is unnecessary to employ a sensitively balanced test and it may be unwise to do so."

During the three year period 1949–1951, a total of 209 monkeys and 58 other animals were tested. For greater facility in presentation, data concerning primates has been considered separately from that concerning nonprimates. Animal species were identified in some instances by the Smithsonian Institution, Washington, D. C. and in others in accordance with the descriptions of Goldman (1920).

TABLE 1

Results of the intraperitoneal protection test in Swiss mice for yellow fever with primate sera

SPECIES OF MONKEY	RESULTS OBTAINED				
	Positive	Negative	Inconclusive	Unsatisfactory	Total
Squirrel marmosets (*Marikina geoffroyi*)	5	74	3	3	85
Howler monkeys (*Alouatta palliata palliata* and *A. palliata aequatorialis*)	0	19	0	0	19
Whiteface monkeys (*Cebus capucinus capucinus* and *Cebus capucinus imitator*)	0	21	2	2	25
Red spider monkeys (*Ateles geoffroyi panamensis*)	4	20	0	0	24
Black spider monkeys (*Ateles fusciceps robustus*)	2	19	0	0	21
Night monkeys (*Aotus zonalis*)	1	28	5	1	35
Total	12	181	10	6	209

RESULTS OF TESTS ON PRIMATE BLOODS

In the protection tests employing primate bloods, a total of 12 positives was encountered among the 209 monkeys examined. Five of 85 squirrel marmosets, 4 of 24 red spider monkeys, 2 of 20 black spider monkeys and one of 35 night monkeys gave positive tests. Spider and howler monkeys proved most satisfactory for testing both because their large size facilitated bleeding and because their sera yielded clear-cut positive or negative results that could be reproduced with utmost fidelity both with serum stored for varying periods of time and with serum freshly obtained by rebleeding at appropriate intervals. Squirrel marmosets, night monkeys and whiteface monkeys gave a number of inconclusive and unsatisfac-

tory results. These animals also showed the greatest number of spontaneous parasitic and bacterial infections. Inconclusive results on the first bleeding were

TABLE 2

Places of origin of primates tested and year of capture

PLACE OF ORIGIN	NO. RECEIVED PER YEAR			
	1949	1950	1951	Total
Panama, west of Canal				
Province of Chiriqui				
Squirrel marmosets	0	1	0	1
Whiteface monkeys	0	1	5	6
Howler monkeys	0	2	2	4
Province of Bocas del Toro				
Red spider monkey	1	0	0	1
Provinces of Veraguas, Cocle, Herrera and Los Santos				
Red spider monkeys	2	0	1	3
Whiteface monkeys	1	0	2	3
Howler monkeys	0	0	1	1
Province of Panama, west of Canal				
Squirrel marmosets	7	5	4	16
Red spider monkeys	1	0	2	3
Night monkeys	0	1	1	2
Canal Zone				
Squirrel marmosets	0	0	2	2
Howler monkeys	1	0	1	2
Panama, east of Canal				
Province of Colon, east of Canal				
Squirrel marmosets	0	1	0	1
Red spider monkeys	1	1 (1)*	2	4 (1)
Whiteface monkeys	0	0	1	1
Province of Panama, east of Canal				
Squirrel marmosets	21 (2)	10	33 (3)	64 (5)
Red spider monkeys	5 (3)	3	5	13 (3)
Black spider monkeys	1	6 (1)	6	13 (1)
Whiteface monkeys	0	2	5	7
Howler monkeys	3	4	5	12
Night monkeys	8	6	19 (1)	33 (1)
Province of Darien				
Black spider monkeys	2 (1)	2	2	6 (1)
Whiteface monkeys	1	1	1	3
Origin Unknown				
Squirrel marmosets	0	1	0	1
Black spider monkeys	0	1	1	2
Whiteface monkeys	2	0	3	5
Sum total	57 (6)	48 (2)	104 (4)	209 (12)

* Numbers in parenthesis represent numbers of positive reactors obtained.

obtained in a total of 18 monkeys, including 7 squirrel marmosets, 4 whiteface monkeys and 7 night monkeys. Unsatisfactory results were obtained on first bleeding in 10 of the animals, including 4 squirrel marmosets, 5 whiteface mon-

keys and one night monkey. We were able to repeat the test after rebleeding in 19 of these 28 animals giving an initial inconclusive or unsatisfactory result. Of 13 originally classified as inconclusive, 8 became negative, 4 continued inconclusive and one gave an unsatisfactory result. Of 6 classified originally as unsatisfactory, 4 became negative, one gave an inconclusive test and one was again unsatisfactory. These results are tabulated in Table 1.

In Table 2 are presented data concerning the places where the various monkeys were trapped and the year of their acquisition by the laboratory. A total of 40 monkeys was received from the section extending west of the Canal Zone toward the Costa Rican border. Thirty-nine of these were from the Pacific side of the Continental Divide and only one from the Atlantic side. There were no positives in this group nor among 4 monkeys obtained from the western part of the Canal Zone. A total of 157 monkeys was received from the section extending east of the

TABLE 3

Age of monkeys tested

SPECIES OF MONKEY	ADULTS		JUVENILES	
	No. tested	No. positive	No. tested	No. positive
Squirrel marmosets	72	5	13	0
Howler monkeys	2	0	17	0
Red spider monkeys	8	0	16	4
Black spider monkeys	4	1	17	1
Whiteface monkeys	12	0	13	0
Night monkeys	29	1	6	0
Total	127	7	82	5

Canal Zone toward the Colombian border. Of these, 12 gave a positive test. The exact place of origin of 8 was unknown. The great majority of all monkeys (142) were received from the Province of Panama, east of the Canal. Fifty-seven primates were acquired in 1949, of which 6 gave a positive test: 48 in 1950 with 2 positives and 104 in 1951 with 4 positives.

In Table 3 is presented a classification of monkeys in accordance with their ages. The large majority of marmosets and night monkeys were adults when received. Of the larger monkey species the majority were juveniles. This preponderance of juveniles among the spider, howler and whiteface monkeys resulted of course in a lower percentage of positives than would be found in a balanced group. However, positive results among them have greater epidemiological significance as they indicate that yellow fever had been active recently in the areas in question. Of 6 positive spider monkeys 5 were classified as juveniles, probably 8 to 18 months of age when purchased. Three of these were received in 1949 and 2 in 1950. Four were from the vicinity of the Bayano River and one from the vicinity of the Gatuncillo River. As the studies of Soper and coworkers (1938) indicate that antibodies acquired passively from the mother are lost in 6 months, it may be concluded that yellow fever was active in these areas in 1948 and 1949.

RESULTS OF TESTS ON NONPRIMATE BLOODS

A tabulation of the results obtained in protection tests with nonprimate bloods is presented in Table 4. A total of 58 animals of 15 different species was tested, 23 having been received in 1949, 15 in 1950 and 20 in 1951. A strongly positive result was obtained with the blood of one pygmy anteater, *Cyclopes didactylus*, an adult male captured in Curundu in the Canal Zone in April 1951. Three others gave a negative and one an unsatisfactory test. Two agoutis, *Dasyprocta punctata*, a male and female juvenile respectively, both received from the vicinity of the Sabalo River in Darien in June 1951, gave a positive result when first bled on June 9. They were both rebled on September 18 when an inconclusive result was

TABLE 4

Results of intraperitoneal protection test in Swiss mice with nonprimate bloods

SPECIES OF ANIMAL	POSITIVE	NEGATIVE	INCONCLUSIVE	UNSATISFACTORY	TOTAL
Three-toed sloth (*Bradypus griseus griseus*)	0	10	1	0	11
Coati (*Nasua narica panamensis*)	0	13	0	0	13
Agouti (*Dasyprocta punctata*)	2*	9	0	0	11
Pygmy anteater (*Cyclopes didactylus dorsalis*)	1	3	0	1	5
Great anteater (*Myrmecophaga tridactyla centralis*)	0	1	0	0	1
Opossum (*Didelphis marsupialis etensis*)	0	6	0	0	6
Woolly opossum (*Caluromys laniger*)	0	1	0	0	1
Water opossum (*Chironectes panamensis*)	0	1	0	0	1
Raccoon (*Procyon cancrivorus panamensis*)	0	2	0	0	2
Kinkajou (*Potos flavus isthmicus*)	0	1	1	0	2
Paca (*Cuniculus paca virgatus*)	0	1	0	0	1
Collared peccary (*Pecari angulatus bangsi*)	0	1	0	0	1
Tapir (*Tapirella bairdii*)	0	1	0	0	1
Grison (*Grison canaster*)	0	0	0	1	1
Porcupine (*Coendou rothschildi*)	0	1	0	0	1
Total	3	51	2	2	58

* First test.

obtained. One was bled a third time on February 7, 1952 when a totally negative result was given. The first test was preformed after the sera had been stored in the frozen state for 2 weeks, the second and third tests within 24 hours of bleeding. Considerable experimental work has been done with various species of agoutis. Bugher and coworkers (1944) found this animal to be susceptible to yellow fever, as also *Cyclopes didactylus*. Laemmert (1948) found evidence of formation of antibodies in the agouti after inoculation with virus but could not demonstrate circulation of the virus.

DISCUSSION

In the present survey of 209 monkeys captured in various sections of Panama, a total of 12 positives or 5.7 per cent was obtained. The specificity of the reaction

in suitably drawn, noncontaminated primate bloods is generally acknowledged. However, the proportion of positive monkeys in this series must be considered low. Haddow *et al.* (1947) found 61 per cent of positive bloods among 150 primates studied in Uganda. Kumm and Laemmert (1950) found an average of 12.4 per cent positives among a total of 5857 monkeys examined in Brazil, and Bugher (cited by Taylor 1951) in 67 per cent of 183 primates captured or shot in a postepidemic period and 70 per cent of 44 primates in an epidemic period in the Restrepo-Villavicencio region of Colombia. However, the number of human cases in these countries has been high in comparison to Panama where the disease had remained clinically unrecognized for more than 40 years prior to the recent outbreak. Clark (1950) found 51.7 per cent positive among 201 monkeys shot in various localities east of the Canal and 30.3 per cent positive among 224 monkeys shot in localities west of the Canal. The great preponderance of juvenile animals among the larger monkeys in our series may account in part for the discrepancy. Also the use of the less sensitive intraperitoneal test and the very irregular geographic distribution of the specimens received may have influenced our results. In general, the present findings, though limited in scope as noted above, correspond to what we know of the epidemiology of the disease here. No positives were found among 44 primates from the Canal Zone and the Provinces of Chiriqui, Herrera, Cocle, Veraguas and Panama west of the Canal, in which areas human cases of yellow fever have not been recorded, and in which Kumm and Crawford (1943) found only negative human sera in a survey conducted in 1943. Ten immune monkeys were obtained from the Province of Panama, east of the Canal, where jungle yellow fever has been shown to be endemic in recent years and where the first cases of the present outbreak occurred in 1948. One immune monkey was received from the Province of Darien, and one from the Province of Colon east of the Canal where 2 cases of yellow fever occurred in 1949. Unfortunately, no specimens were received from the Province of Colon west of the Canal and only one from Bocas del Toro where human cases were registered in 1951, from one of which the author isolated a strain of virus of high antigenicity but low pathogenicity in monkeys.

Since the specificity of protection tests with nonprimate bloods has been shown to be affected by a number of variables by Koprowski (1946), no attempt will be made to interpret the temporarily positive results obtained in 2 agoutis and the positive result in one pygmy anteater until further experimental work has been completed.

CONCLUSIONS

Among 209 primates from various sections of Panama, a total of 12 gave a positive reaction in the intraperitoneal protection test for yellow fever in Swiss mice.

Of 58 other animals, one pygmy anteater and two agoutis also gave a positive test, the reaction in the latter species being temporary.

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