

SURVEY OF PRIMATES CAPTURED IN PANAMÁ, R. P. DURING THE YEARS 1952-1956 FOR PROTECTIVE ANTIBODIES AGAINST YELLOW FEVER

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Surveys of the rural population of Panamá for protective antibodies against yellow fever, conducted with serums collected in 1937 by Johnson (1938) and in 1941-1942 by Kumm and Crawford (1943) indicated that jungle yellow fever was endemic in San Blas on the Atlantic side of the Continental Divide and in Darien and the lower half of the Province of Panamá (adjacent to Darien) on the Pacific side. In recent years, however, extensive vaccination has limited the epidemiological value of such studies of the human population, and forest animals, especially monkeys, have been substituted. In the wake of the 1948 outbreak of jungle yellow fever in Panamá, Clark (1950) undertook a general survey of shot forest animals finding a high proportion of all bloods positive for neutralizing antibodies. The author simultaneously began a study of captured forest animals, housed, bled and tested at the Gorgas Memorial Laboratory. The use of animals captured alive has the advantage that it eliminates the frequent troublesome nonspecific and unsatisfactory reactions obtained with serum from shot animals. This survey begun in 1949 (Rodaniche, 1952) has been continued on a limited scale up to the present time. In the present publication results of mouse protection tests with serums of primates captured during the five year period, 1951-1956, are presented.

MATERIALS AND METHODS

The monkeys were usually acquired by purchase, occasionally as gifts. The majority were received from neighboring districts within easy transportation distance of the laboratory and relatively few from the distant provinces. Most of the animals were captured in the Bayano River valley and the environs of Chepo, Pacora and Cerro Azul directly west of the Bayano where human yellow fever cases were identified in 1948 and again in 1956. Since the 1948 outbreak immunized a large proportion of the simian population of the affected areas, an attempt was made to obtain animals born since that time. Of the larger longer-lived species, nonsuckling juveniles more than 6 months of age were preferred. Of the smaller shorter lived species both adults and juveniles were considered useful. All but one of Panamá's six native primate species were represented in this survey, including squirrel marmosets (*Marikina geoffroyi*), red and black spider monkeys (*Ateles geoffroyi panamensis* and *robustus*), night monkeys (*Aotus zonalis*), whiteface monkeys (*Cebus capucinus*) and howling monkeys (*Alouatta palliata*).

The animals were bled from the femoral vein, occasionally from the heart, with aseptic technique. Effort was made to maintain them alive for further study

but many died spontaneously within a few days or weeks of acquisition. Serums were stored at approximately -20°C . until a sufficient number had accumulated for study. The intracerebral mouse protection test devised by Theiler (1933), with certain modifications described by Bugher (1948), was employed in all tests. For initial testing French neuroadapted virus desiccated in vacuo was so diluted as to provide an inoculum of between 50 and 100 LD/50 per mouse, six mice being used for each serum test group. The few serums which gave unsatisfactory results on initial investigation were passed through a Seitz filter and retested. In most instances this procedure eliminated the factor responsible and a satisfactory result was obtained. A survival ratio of 4/4, 4/5, 5/5, 5/6, or 6/6 was considered a positive test.

Serums giving a positive result with 50-100 LD/50 of virus were stored and later titrated against higher serial concentrations of virus, the same technique being employed as for the preliminary test. Depending on the quantity of serum available, one or more of the following virus concentrations were employed, approximately 200, 500, 1,000, 10,000 and 100,000 LD/50. In some instances, especially among the marmosets, insufficient serum was available to reach an endpoint. This titration of antibodies was carried out in order to avoid misinterpretation of results due to nonspecific factors or to cross-reactions produced by previous infection with antigenically related viruses. A high titer of antibody against yellow fever was considered the strongest evidence of the specificity of the reaction. As a further check serums were also tested for mouse-protecting antibodies against a Central American strain of Ilhéus virus isolated by the author (Rodaniche, 1956).

RESULTS AND INTERPRETATION

A total of 395 primates was examined during the five year period of this survey, including 197 squirrel marmosets, 100 spider monkeys, 47 night monkeys, 24 whiteface monkeys and 27 howling monkeys. The two former species were the only ones yielding positive bloods. Failure to find immune animals among the night, whiteface and howling monkeys may depend on a number of factors, including total numbers received, geographic distribution, habits and innate resistance. In Table 1 results are classified according to species of primate and year of capture and in Table 2 according to general geographic distribution.

The recent demonstration of cross-reactions between the immunologically related arthropod-borne viruses of Group B has forced us to reexamine criteria for the interpretation of positive serological reactions in yellow fever. Casals and Brown (1954) find the greatest serological crossing in the hemagglutination inhibition reaction, less in the complement-fixation, and least in the neutralization test. However, such overlap may occur in variable degree in the neutralization test, especially where low virus titers are used, and must be taken into consideration. Interpretation is rendered still more difficult by recent studies showing distribution of certain members of Group B, especially dengue, St. Louis and Ilhéus viruses in areas of Central and South America where yellow fever is known to exist. In Panamá no evidence has been presented in recent years for

TABLE 1

Results of the intracerebral protection test using 50-100 LD/50 of yellow fever virus, according to species of monkeys and year of capture

Species of monkey	Year of capture and result					
	1952 T (P, I, U)	1953 T (P, I, U)	1954 T (P, I, U)	1955 T (P, I, U)	1956 T (P, I, U)	Total T (P, I, U)
Marmoset.....	5 (0, 1, 0)	33 (3, 0, 0)	24 (2, 0, 0)	70 (3, 2, 0)	65 (4, 0, 0)	197 (12, 3, 0)
Spider monkey.....	3 (0, 0, 0)	4 (0, 0, 0)	37 (1, 0, 1)	7 (0, 0, 0)	49 (8, 1, 0)	100 (9, 1, 1)
Night monkey.....	2 (0, 0, 0)	10 (0, 0, 0)	8 (0, 0, 0)	7 (0, 0, 0)	20 (0, 0, 0)	47 (0, 0, 0)
Whiteface monkey.....	2 (0, 0, 0)	5 (0, 0, 0)	7 (0, 0, 1)	10 (0, 0, 0)	0	24 (0, 0, 1)
Howling monkey.....	7 (0, 1, 0)	7 (0, 1, 0)	2 (0, 1, 0)	3 (0, 0, 0)	8 (0, 0, 0)	27 (0, 3, 0)
Total.....	19 (0, 2, 0)	59 (3, 1, 0)	78 (3, 1, 2)	97 (3, 2, 0)	142 (12, 1, 0)	395 (21, 7, 2)

First figure represents total; figures in parentheses positive, inconclusive and unsatisfactory results respectively.

TABLE 2

Places of origin of primates tested

Province	Species of monkey					
	Spider	Marmoset	Night	Whiteface	Howling	Total
Darien.....	17 (1)	12 (1)	3	1	3	36 (2)
Panama east of Canal						
Area #1.....	67 (8)	151 (9)	39	6	17	280 (17)
Area #2.....	5	6	1	0	0	12
Panama west of Canal...	0	23 (1)	2	1	0	26 (1)
Colon west of Canal.....	1	1 (1)	0	0	1	3 (1)
Los Santos.....	0	0	0	3	0	3
Chiriqui.....	5	2	1	10	4	22
Bocas del Toro.....	1	0	0	0	0	1
Unknown.....	4	2	1	3	2	12
Total.....	100 (9)	197 (12)	47	24	27	395 (21)

Area 1—Including Bayano River Valley and section extending westward to Canal Zone embracing environs of Pacora, Chepo and Cerro Azul.

Area 2—Including area bordering Transisthmian Highway, Chagres River and Madden Lake.

Numbers in parentheses indicate monkeys giving a positive test.

the activity of any Group B virus except yellow fever. However, further study undoubtedly will demonstrate the presence of other members of the group.

The protection tests conducted in mice against Ithús virus with monkey bloods positive for yellow fever virus gave essentially negative results, i.e., no neutralization of less than 10 LD/50. On the contrary, neutralization indices

against yellow fever were usually significantly high. Serums of 16 of the 21 animals possessing neutralizing antibodies in their sera gave positive reactions against more than 500 LD/50 of virus and at least 11 against more than 1,000. Twelve of 151 adult squirrel marmosets gave positive findings. However, as little is known concerning the longevity of the marmoset in nature, it can not be stated conclusively that the immune adults of this species acquired their infection since the 1948 outbreak, although this seems probable. The highly positive tests obtained with blood of a juvenile marmoset and a juvenile black spider monkey, both captured in the Bayano area in 1954, and a juvenile marmoset captured near Pacora in 1955 give strong indication of continued minimal activity of virus in this area.

In 1956 there was a sharp increase in the percentage of positive animals received from the Bayano River Valley and the contiguous area. Seven of 44 juvenile spider monkeys (16%) gave positive protection tests, with 5 showing power to protect against at least 1,000 LD/50 of virus. In the fall of the same year, one fatal human case of yellow fever and two serologically positive recovered cases were identified from the Cerro Azul-Tocumen area. Yellow fever virus also was repeatedly recovered from mosquitoes captured in the same general zone. Kumm and Crawford (1943) previously showed that the endemic area for yellow fever in the Province of Panamá extended as far west as El Llano on the Bayano River. Recent years apparently have brought a further westward extension of this zone to embrace the Chepo-Pacora sector within a few miles of the outer limits of the District of Panamá.

One of three monkeys acquired from the Province of Colon, west of the Canal Zone, gave a positive test. This was a juvenile marmoset captured near Chagres on the Atlantic coast in July 1956, whose serum neutralized more than 500 LD/50 of virus. It is known that yellow fever was active in this area in 1950 (Elton, 1952). The presence of antibodies in the blood of this juvenile animal 6 years later strongly suggests persistent activity of jungle yellow fever there and indicates the need for more intensive epidemiological studies. The previous surveys conducted by Johnson in 1938 and Kumm and Crawford in 1943 gave no evidence of endemicity in any area west of the Canal Zone.

One adult marmoset captured near Arraijan in 1955 gave a weakly positive test. This was the only one of 51 animals acquired from the Pacific side of Panamá, west of the Canal, to give a positive reaction.

CONCLUSIONS

Results of the intracerebral mouse protection test against yellow fever with blood of 395 monkeys captured during the five year period 1951-1956 are reported. Repeated finding of strongly positive reactions in juvenile monkeys captured in the Bayano-Chepo-Pacora sector of the Province of Panamá suggests that this is an important enzoötic center. Epizoötic activity was observed in 1956 when 16 per cent of 44 juvenile monkeys from this general area gave positive results.

The finding of one positive among 3 juvenile monkeys captured in the Province of Colon, west of the Canal, suggests continued virus activity in this area, since the 1948-1951 outbreaks.

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