THE SUSCEPTIBILITY OF HOWLER MONKEYS TO YELLOW FEVER VIRUS[1]

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Andrew Balfour (1914) is responsible for one of the earliest references in the literature to the possible role of howler monkeys as a reservoir for the virus of yellow fever. Thirty-six years ago, on a visit to the island of Trinidad, he had investigated a small outbreak of that disease that had involved nine Americans and one Trinidadian, who were working in the forest near the pitch lake. These men were drilling for oil at the end of a road which extended far into the heart of the high woods. It was said that red howler monkeys were present in those woods.

Balfour was impressed by an old saying among the negroes of Trinidad to the effect that they could always tell when there was going to be an epidemic of yellow fever on the island, because prior to its appearance red howler monkeys were found dying and dead in the high woods.

In June 1935 Abel Tavares Lacerda of the Yellow Fever Service of Brazil was investigating an outbreak of that disease near Patos in the State of Minas Gerais. He learnt from the local farmers that four or five dead howler monkeys had been found in the forest near Rio Preto in the district of Unai. After visiting the area in question he ascertained that the information was quite correct and that this was not the first occasion when howler monkeys had died in the forests of that region. Previous outbreaks of a fatal disease among howlers had generally occurred in years of excessive rainfall.

Howler monkeys immune to yellow fever have been captured in eight of the 20 Brazilian states including Pará in the north and Rio Grande do Sul in the far south. The importance of the genus *Alouatta* therefore has become appreciated more and more as surveys of immunity among primates have been extended throughout this country.

Almost no laboratory investigations, however, have previously been carried out with howler monkeys, largely because they were considered very difficult animals to keep in captivity. Davis (1931) infected a single specimen of the red howler monkey, *Alouatta seniculus*, by mosquito bite, and he recovered virus both by blood transfer to a rhesus and by feeding a fresh batch of *Aedes aegypti*. This is the only successful transmission experiment on record. It seemed well worth-while therefore to make further studies on the susceptibility of howler monkeys to yellow fever. This report will summarize the results of those investigations.

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Waddell and Taylor (1945) have pointed out that the mere fact that an animal circulates yellow fever virus following artificial introduction of the virus does not necessarily imply that the animal may be a potential natural host. The epidemiologist would like to know whether a given vertebrate and a suitable insect vector can serve for repeated cyclic passages of that virus.

In earlier studies on the susceptibility of various Brazilian animals to yellow fever Laemmert (1943, 1944, 1946 and 1948) found that reptiles and amphibians were resistant while certain rodents and marsupials were slightly susceptible. Only among marmosets, however, did he encounter a group of animals that consistently circulated virus in a high titer and became specifically immune. Later Waddell and Taylor (1945) confirmed Laemmert's observation that *Callithrix* marmosets could serve for cyclic passages of the disease in combination with *A. aegypti* as the insect fector.

MATERIALS AND METHODS

Vertebrate hosts. Two species of howler monkeys have been tested.[3] Specimens of *Alouatta caraya* Humboldt were obtained near Malhada in the southwestern corner of the State of Bahia. They were shipped by boat from Malhada to Bom Jesus da Lapa and by plane from Lapa to Rio de Janeiro. Three young females of *Alouatta guariba clamitans* Cabrera were captured near Ibirama in the State of Santa Catarina. The latter were brought to Rio by automobile and endured the ardous journey surprisingly well. Howler monkeys, which arrived at the yellow fever laboratory in good condition, have survived for many months on a diet of bananas, eggs, milk and *Cecropia* leaves. A male and a female of *Alouatta caraya* are shown in the accompanying photograph. Males of this species are black while immatures and females are light buff in color.

Arthropod vectors. The small shipment of three *Alouatta guariba clamitans* arrived during the rainy season when *Aedes leucocelaenus* mosquitoes were abundant and so it was possible to use that species in cyclic transmission studies with them. A group of eight *Alouatta caraya*, which were caught at Malhada, reached the yellow fever laboratory at the beginning of the following dry season, when *Haemagogus capricornii*, *H. spegazzinii* and *A. leucocelaenus* are almost unobtainable in the environs of Rio de Janeiro. After giving careful thought to that situation we resolved to attempt a series of cyclic transmissions with them using *Aedes scapularis* as the insect vector.

Several considerations were influential in our reaching that decision. In the first place Causey and Santos (1949) have shown that *A. scapularis* are present throughout the dry and cold months at Passos in southern Brazil; second, that species can live under laboratory conditions for periods of from one to three months. Finally, we were anxious to obtain an adequate explanation of how the virus of sylvan yellow fever actually does survive the winter months in the southern part of this country. Kumm (in press) has pointed out that in south Brazil from July to October inclusive rainfall is at a minimum, *Haemagogus*

[3] We are grateful to Dr. Remington Kellogg of the United States National Museum for verifying our identifications of these monkeys.

mosquitoes are rarely encountered and human cases of jungle yellow fever are conspicuous by their absence.

Three lots of *A. aegypti* were used, one of them to initiate the series of cyclic transmissions with *A. scapularis*, and the other two lots were utilized to infect two more specimens of *Alouatta caraya*.

Virus strain. The Olímpio Cristo strain of jungle yellow fever virus was employed throughout. Fox and Manso had isolated that strain at Santa Leopoldina in the State of Espírito Santo in January 1940 (Laemmert 1944).

Virus determinations. To determine the virus concentration in howler monkeys, they were bled daily from the femoral vein and after the blood had coagulated,

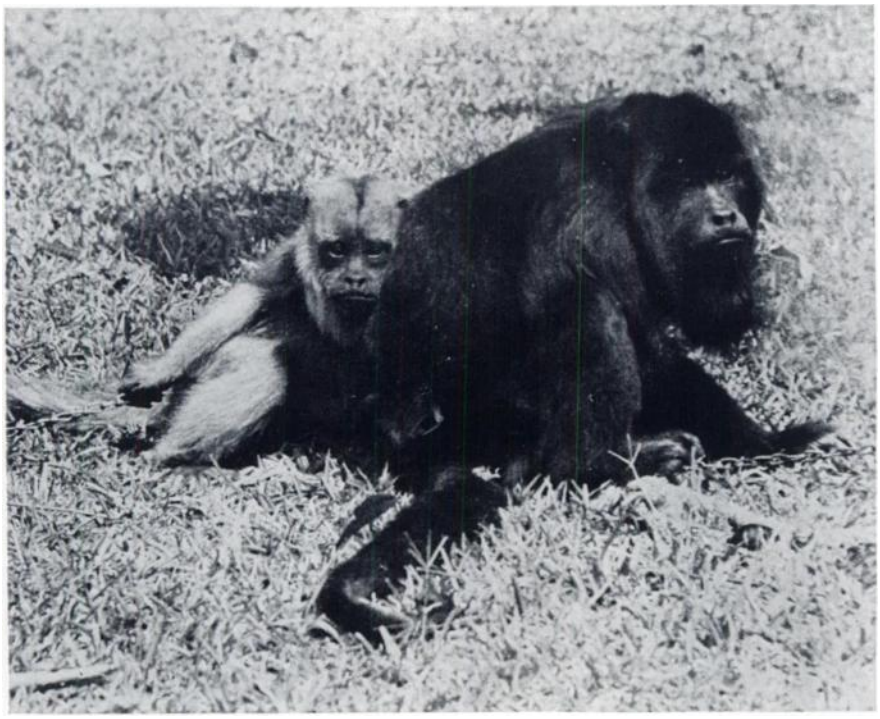

FIG. 1

the serum was separated by centrifugation and titrated in decimal dilutions. The diluent employed was 10 per cent normal human serum saline. White mice of the Swiss strain in groups of six each were inoculated with successive decimal dilutions of serum. Each mouse received 0.03 cc. of the mixture intracerebrally. All mice were observed for 21 days and survivors were reinoculated intracerebrally with approximately 100 MLD of French neurotropic virus. Survivors of the challenge inoculation were considered to have been immunized by the first intracerebral injection. The titer prevailing each day was determined by the method of Reed and Muench (1938), which gives an estimate of the 50 per cent end point. All monkeys and marmosets were bled before use and their sera examined for immunity by the intracerebral neutralization test of Theiler (1933).

RESULTS

Nine howler monkeys of the species *Alouatta caraya* were tested, the first seven of them in a series of cyclic transmissions with *A. scapularis* as the insect vector. *Alouatta caraya* Nos. 8 and 9 were merely bitten by infected lots of *A. aegypti* and then tested daily for the titer of virus in their blood stream.

Two of the *Alouatta caraya* used in the cyclic transmission experiment did not become infected. No. 2 was bitten by a lot of *A. scapularis*, which had incubated virus for 23 days, during which time unfortunately the thermostatic control of the warm room was not entirely satisfactory and the temperature of that room fell from 28°C. to 24°C. for about a week. Howler monkey No. 2 died on the sixth day after infection but it did not die of yellow fever. Six survivors of the same lot of *A. scapularis* that fed originally on howler monkey No. 1 and later bit monkey No. 2 did, however, transmit the disease by bite to *Alouatta caraya* No. 3 after they had incubated virus for a total of 44 days.

Similarly, howler monkey No. 7, which was bitten by a single *A. scapularis* after the latter had incubated virus for 28 days at 28°C., died on the fourth day but not from yellow fever. The cause of its death was never determined.

The other five *Alouatta caraya*, which were used in the cyclic transmission experiment with *A. scapularis*, all died of yellow fever. Figure 2 shows that the virus was passed in series from an infected marmoset through four howler monkeys in succession and then back to two more normal marmosets. *Alouatta caraya* No. 4 died on the third day with a fulminating attack of the disease. Because its death was so rapid, no mosquitoes imbibed blood on that day and for that reason another howler monkey, No. 5, was bitten by another lot of *A. scapularis*, which had fed 41 days previously on *Alouatta caraya* No. 3.

Table 1 indicates the titers of circulating yellow fever virus that were found in the experimental monkeys. The lot of *A. scapularis*, which fed on *Alouatta caraya* No. 1 on the fifth day, when its titer was $10^{-7.6}$, was the lot which was later used to infect howler monkey No. 3. Similarly other lots of *A. scapularis*, which had been infected at peak titers, were selected to transmit the virus to monkeys Nos. 5 and 6 in succession.

It should be emphasized at this point that the Olímpio Cristo strain of yellow fever virus was the one that was used in these studies. That strain usually produced a fatal outcome in from three to 10 days. Other strains of yellow fever virus, such as the Almada strain from Ilhéus, Bahia, might have brought about different results. Because immune howler monkeys have been captured in many parts of Brazil it is obvious that not all strains of jungle yellow fever are equally lethal to the two species of *Alouatta* which were tested in these experiments.

The gross pathology in fatal cases among howler monkeys was not always characteristic. Usually the coagulation time of the blood was prolonged and the liver hyperemic. Sometimes the liver had the boxwood color, which is typical of fatal cases of that disease among rhesus monkeys. Haemorrhages were also present at times in the mucous membrane of the stomach wall and one autopsy showed that the stomach contained black vomitus.

A number of lots of *A. scapularis* which had fed upon infected howler monkeys

and which were not needed to maintain the cyclic passages, were tested after appropriate incubation periods by allowing the surviving insects to bite non-immune *Callithrix* marmosets. The results obtained in that study have been summarized in Table 2.

Two lots of *A. scapularis*, which fed on *Alouatta caraya* No. 1 on the third day when the latter was circulating virus in a titer of $10^{-2.3}$, never became infected. Lots 4, 5 and 6 fed on that same howler monkey on the fourth day

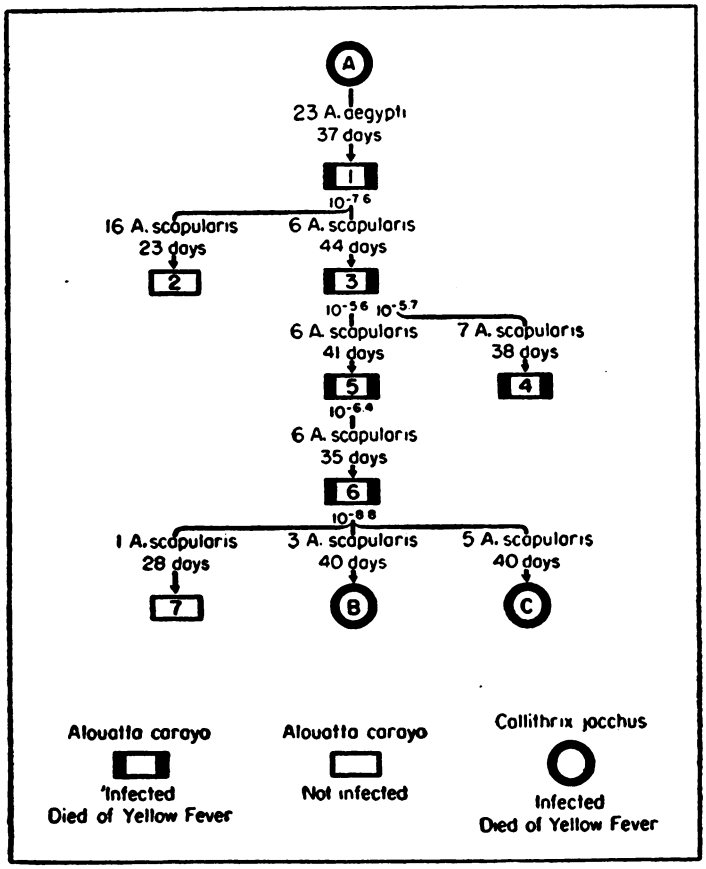

FIG. 2. CYCLIC TRANSMISSION OF YELLOW FEVER VIRUS IN ALOUATTA CARAYA WITH AEDES SCAPULARIS

of its illness. Lot 4, which had incubated virus for 24 days, did not transmit by bite, but lots 5 and 6 both produced positive results. Sixteen *A. scapularis* of lot 3, which had been infected on *Alouatta caraya* No. 3 on the third day, also transmitted yellow fever virus by bite. The results shown in Table 2 are similar to the findings from a study of individual mosquitoes, which are being reported elsewhere by Kumm and Laemmert (to be published). The lowest titer at which transmission was obtained by bite was $10^{-3.0}$.

TABLE 1

Titrations of yellow fever virus, Olimpio Cristo strain, circulating in howler monkeys

MONKEY USED	INFECTED BY	LOG OF TITER OF CIRCULATING VIRUS EACH DAY									
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Alouatta caraya No. 1	23—*Aedes aegypti*	—	—	$10^{-2.3}$	$10^{-5.3}$	$10^{-7.6}$	*				
A. caraya No. 3	6—*Aedes scapularis*	—	—	$10^{-3.0}$	$10^{-5.4}$	$10^{-5.7}$	$10^{-5.6}$	$10^{-5.3}$	$10^{-4.0}$	$10^{-3.6}$ *	
A. caraya No. 4.	7—*Aedes scapularis*	—	$10^{-3.2}$	$10^{-5.6}$ *							
A. caraya No. 5	6—*Aedes scapularis*	—	Trace	$10^{-3.4}$	$10^{-5.1}$	$10^{-6.4}$	$10^{-5.5}$	$10^{-5.3}$	$10^{-3.4}$	$10^{-3.4}$	$10^{-1.3}$
A. caraya No. 6	6—*Aedes scapularis*	0	0	$10^{-3.6}$	$10^{-5.3}$	$10^{-6.3}$	$10^{-3.6}$	*			
A. caraya No. 8	40—*Aedes aegypti*	Trace	$10^{-4.0}$	$10^{-7.3}$	$10^{-7.5}$	$10^{-7.0}$ *					
A. caraya No. 9	39—*Aedes aegypti*	Trace	$10^{-3.0}$	$10^{-3.9}$	$10^{-6.0}$ *						
Alouatta guariba clamitans No. 10	7—*Aedes leucocelaenus*	Trace	$10^{-3.4}$	$10^{-5.1}$	$10^{-3.0}$	*					
A. guariba clamitans No. 11	10—*Aedes leucocelaenus*	$10^{-1.5}$	$10^{-4.5}$	$10^{-7.0}$	$10^{-4.3}$ *						
A. guariba clamitans No. 12a	2—*Aedes leucocelaenus*	—	0	$10^{-2.0}$	$10^{-5.4}$	$10^{-6.9}$	—	—	—	—	*

* = Died of yellow fever.
— = No information.

The two additional specimens of *Alouatta caraya* that were bitten by infected *A. aegypti*, died promptly on the fourth and fifth days of their illnesses, virus in circulation attaining maximum titers of $10^{-6.0}$ and $10^{-7.5}$ respectively.

TABLE 2

Susceptibility of groups of Aedes scapularis *to yellow fever virus circulating in howler monkeys at various titers*

MOSQUITO LOT NUMBER	TITER OF VIRUS IN SOURCE MONKEY	INCUBATION PERIOD IN DAYS	NUMBER OF MOSQUITOES FED ON TEST MARMOSET	RESULT IN MARMOSET
1	$10^{-2.3}$	25	12	Negative
2	$10^{-2.3}$	46	4	Negative
3	$10^{-3.0}$	28	16	Positive
4	$10^{-5.3}$	24	38	Negative
5	$10^{-5.3}$	28	15	Positive
6	$10^{-5.3}$	45	15	Positive
7	$10^{-5.4}$	28	28	Positive
8	$10^{-5.6}$	28	17	Positive
9	$10^{-7.5}$	34	5	Positive
10	$10^{-7.5}$	53	3	Positive
11	$10^{-7.5}$	62	2	Positive
12	$10^{-7.6}$	45	3	Positive
13	$10^{-8.8}$	40	3	Positive
14	$10^{-8.8}$	40	5	Positive

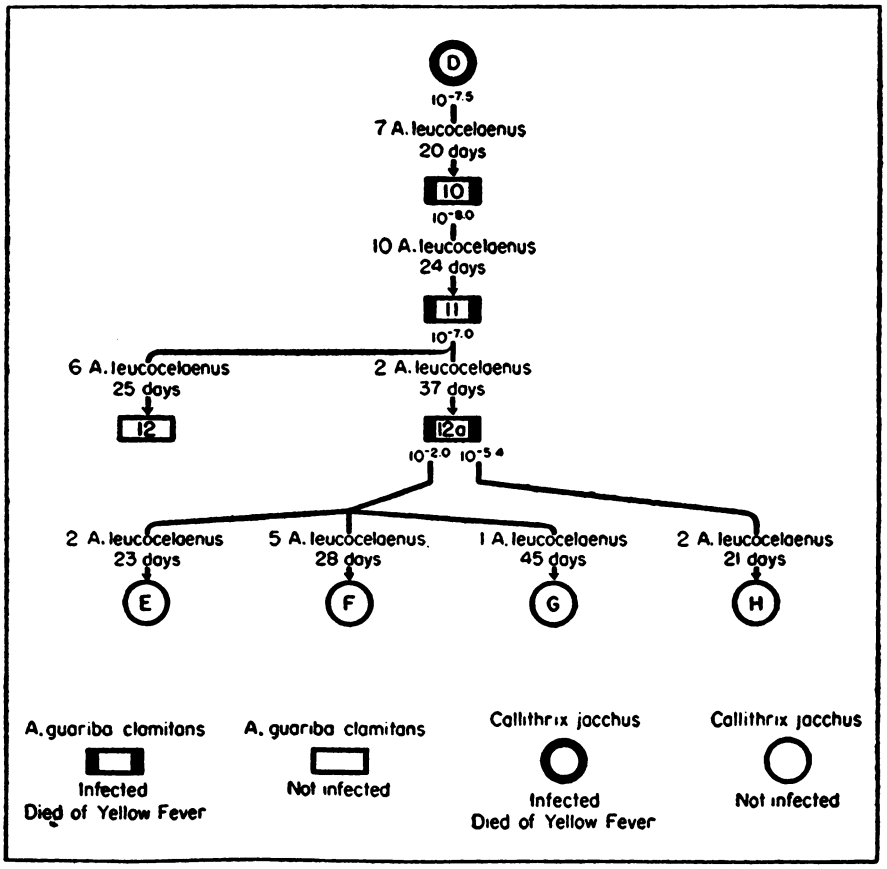

FIG. 3. CYCLIC TRANSMISSION OF YELLOW FEVER VIRUS IN ALOUATTA GUARIBA CLIMITANS WITH AEDES LEUCOCELAENUS

Table 1 also indicates that the three *Alouatta guariba clamitans*, which were used in an attempt at cyclic transmission with *A. leucocelaenus*, circulated virus in high titers before they died. It was thought for several days that *Alouatta guariba clamitans* No. 12 might survive and so no blood specimens were withdrawn from the sixth to the ninth days after infection, but that monkey finally succumbed on the tenth day. The results of the cyclic transmission experiment with *Alouatta guariba clamitans* are shown graphically in Figure 3.

In this experiment the virus was passed successfully from an infected marmoset through three howler monkeys in succession. The virus strain was lost in an attempt to infect marmosets from the third howler monkey. The probable reason for failure with marmosets E, F and G was that the mosquitoes which bit them had fed on howler monkey No. 12a on the third day when the latter was circulating virus in a titer of only $10^{-2.0}$. It has been found in other studies that such a titer of Olímpio Cristo virus circulating in a howler monkey is usually insufficient to infect mosquitoes. The two *A. leucocelaenus*, which bit marmoset H, may have received sufficient virus originally, but the incubation period in them was probably too short.

It is also interesting to observe that howler monkey No. 12 was not infected by a group of six *A. leucocelaenus*, which had incubated virus for 25 days. That same animal, which was then redesignated as No. 12a, did however develop the disease when bitten by two *A. leucocelaenus* of that same lot of mosquitoes after they had undergone 37 days of extrinsic incubation.

DISCUSSION

It was pointed out in the introduction that *A. scapularis* was selected as a potential arthropod vector for a series of cyclic transmission experiments with *Alouatta caraya*, because those monkeys arrived in Rio de Janeiro at a time of the year when *H. capricornii*, *H. spegazzinii* and *A. leucocelaenus* were unobtainable. The fact that there is such a period during the winter months in south Brazil during which the usual sylvan vectors disappear and human cases of the disease do not occur is important. A satisfactory explanation, however, of how the virus survives the four unfavorable months of each year has not yet been found. As the period during which virus circulates in the vertebrate host rarely exceeds 10 days, it has been concluded that the true reservoir must be the arthropod vector.

In south Brazil certain species of sabethine mosquitoes as well as *Psorophora ferox* and *A. scapularis* remain fairly abundant in the forests throughout the entire year. None of the commoner species of sabethines have proved to be good laboratory vectors of yellow fever. While *P. ferox* can be infected with yellow fever virus and the virus will remain active in the bodies of those mosquitoes for as long as they live, this species rarely, if ever, transmits the virus by bite. The same inability to transmit by bite characterizes *Aedes serratus*, another common sylvan species.

A. scapularis on the other hand, is different. It is a hardy species which lives a long time, has a long radius of flight and has been found in certain areas

throughout the winter months. It transmits the disease readily by bite under laboratory conditions and has proved to be quite adequate for maintaining cyclic transmissions among howler monkeys. However, *A. scapularis* has not yet been found infected in nature and until specimens have been taken in the dry season naturally infected with yellow fever virus, we will not be certain that this species does provide the true explanation of the survival of the disease during the winter months in south Brazil.

SUMMARY

1. Two species of Brazilian howler monkeys were found to be highly susceptible to the Olímpio Cristo strain of yellow fever virus when transmitted by mosquito bite.

2. Four cyclic transmissions were obtained with *A. scapularis* and three with *A. leucocelaenus*.

3. The facility with which laboratory transmissions were obtained with *A. scapularis* suggests a possible explanation for the survival of virus in the winter months in south Brazil.

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