

STUDIES ON MONKEY MALARIA IN THE VICINITY OF MANAUS, STATE OF AMAZONAS, BRAZIL

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SUMMARY

In the vicinity of Manaus we found five species of monkeys infected with *Plasmodium brasilianum*: *Cebus apella apella* (capuchin-monkey), *Alouatta seniculus straminea* (red howler-monkey), *Ateles paniscus paniscus* (red-faced spider-monkey), *Chiropotes chiropotes* (red-backed saki) and *Saimiri sciureus* (squirrel-monkey). The infection of this last species in Brazil is here recorded for the first time. No marmosets were found parasitized.

Comparative studies performed in a low forest at Pôrto Mauá and a high, thick forest along the Manaus-Itacoatiara Road, showed that primates are abundant in both, but while marmosets and the small squirrel-monkeys prevail in the former area, the larger monkeys are predominant in the latter. The simian malaria infection rate verified through blood examination (Table I) was much lower in the low forest than in the high forest — 1.8%, against 15.8%, respectively. In the former area only one squirrel-monkey was found infected (2.5%), while in the latter, malaria parasites were detected in capuchins (19.2%), spider-monkeys (19.2%), howler-monkeys (16.7%) and sakis (14.3%).

In attempts to clarify the transmission of monkey malaria, comparative mosquito captures were performed in both areas, on baits placed at ground level and near the canopy of the trees (Table II). Of the acrodendrophilic anophelines, one species, *Anopheles neivai*, was present and numerous only in the high forest, and was the only one found infected with sporozoites; another species, *Chagasia bonnea*, was strikingly predominant there. The bromeliad-breeding *A. neivai* belongs to the same subgenus, *Kerteszia*, as *A. cruzi*, the incriminated vector of simian malaria in Southern Brazil.

INTRODUCTION

Since June 1965 we have been searching for plasmodia in monkeys and marmosets from the forests in the vicinity of Manaus, capital of the State of Amazonas, Brazil.

The finding, in March 1966, of *Plasmodium brasilianum* in a spider-monkey, *Ateles paniscus paniscus*, shot in the jungle along the road linking Manaus to the village

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of Itacoatiara, lead us not only to intensify the search for plasmodia in the local primates, but also to perform entomological studies aiming at the finding of a natural vector of simian malaria in the area. For comparison, we carried out similar studies in a neighbouring area of quite different landscape, Pôrto Mauá.

In this note, we present the results of the work performed in the two areas until May, 1968. The data obtained prior to December, 1966 appeared in a previous article⁴, but the original informations gathered since then, justify the publication of the present paper.

MATERIAL AND METHODS

Both areas chosen for the observations are located in the municipality of Manaus, in a stretch of territory situated at about 3° 8' South and between 59.° and 60.° West. In this equatorial forest the temperature and humidity are high throughout the year, and heavy rains pour during the long wet season. Meteorological data obtained at the field station of the Instituto Nacional de Pesquisas da Amazônia in the one-year period comprised between October 1966 and September 1967, revealed average monthly temperatures ranging from 25.4°C to 28.7°C, the lowest temperature recorded being 19.6°C and the highest 38.0°C; relative humidity ranged from 71% to 91% and the rainfall totalled 2,305.5 millimeters for the twelve months, varying from 3.4 millimeters in August to 532.7 millimeters in March.

One of the two areas studied, Pôrto Mauá, is a swamp forest, with small or moderately tall trees with slender trunks and abundant undergrowth, at the left margin of the Negro River, about 10 kilometers southeast of Manaus.

The other area includes several sites in the thick forest formed by trees of great height, along the road linking Manaus to Itacoatiara, 286 kilometers eastward. This rather sinuous road crosses three main rivers, one of them twice. Between Rivers Preto da Eva (kilometer 80) and Urubu (kilometer 200), at every five kilometers, narrow paths emerge at right angles, alternatively, from the left and right sides of the main-

road. These paths vary in length and are usually crossed by brooks in their lower portions. Bromeliads are frequent on the trees located on those depressions of the terrain. It was in the jungle along these narrow paths — and not in the main road — that nearly all monkeys were obtained.

The routine followed in the capture and examination of the primates has been described in previous publications^{1, 4}: the monkeys, shot in the jungle, were bled by cardiac puncture on the spot, while moribund; from each monkey, two thick blood smears and five thin blood smears were made for the search of plasmodia, while spleen prints were also prepared, to detect malarial pigment. Skull and skin of each animal were preserved for the specific identification of the host.

All primates from the Manaus-Itacoatiara road were obtained between kilometers 104 and 200.

In the search for the vectors of simian malaria, entomological studies were also performed in Pôrto Mauá and in the Manaus-Itacoatiara Road during a whole year — October 1966 through September 1967 — in order to cover all seasons. Mosquitoes were caught while feeding on baits simultaneously placed at ground level and near the forest canopy — 10 meters above the ground at Pôrto Mauá, and 15 to 25 meters at the Manaus-Itacoatiara Road, near kilometers 135, 160, 175 and 190. The captures were performed either during the day or in the first hours of the night.

The insects were brought to the laboratory in glass jars covered inside with a thin layer of humidified plaster of Paris, and kept at low temperature in a portable refrigerator. On the next day, they were identified to species and some were dissected for the search of sporozoites in the salivary glands.

RESULTS

1. Malarial infection in monkeys

A total of 169 primates were examined, including 157 monkeys and 12 marmosets. Table I presents the number of specimens

of each species examined and positive, in Pôrto Mauá and in the Manaus-Itacoatiara Road.

The primate fauna differs in the two areas: in the low forest of Pôrto Mauá, squirrel-monkeys (*Saimiri sciureus*) are common, and pied tamarins (*Marikina bicolor*) rather frequent, while capuchin-monkeys (*Cebus apella apella*), and golden-headed sakis (*Pithecia chrysocephala*) are scarce, and the other species were not found. In the high forests of the Manaus-Itacoatiara Road, no squirrel-monkey was seen, golden-headed sakis and pied tamarins were scanty, but the remaining species were present in much larger numbers: capuchin-monkeys, red howler-monkeys (*Alouatta seniculus straminea*), red-faced spider-monkeys (*Ateles paniscus paniscus*), and red-backed sakis (*Chiropotes chiropotes*).

In Pôrto Mauá only 1 out of 55 primates — a squirrel-monkey — showed malaria parasites in the blood, or 1.8 per cent. None revealed malarial pigment in the spleen smears.

In the Manaus-Itacoatiara Road, the blood of 18 out of 114 specimens was positive for plasmodia, or 15.8 per cent. The parasites were found in four species of monkeys: capuchin-monkeys — 5 positives out of 26 examined, or 19.2 per cent; red howler-monkeys — 4 in 24, or 16.7 per cent; red-faced spider-monkeys — 5 of 26, or 19.2 per cent; and red-backed sakis — 4 of 28, or 14.3 per cent.

Besides, malarial pigment was detected in the spleen smears of the following monkeys with negative blood, in the Manaus-Itacoatiara Road: 3 capuchins, 1 howler, 1 spider-

TABLE I

Primates examined for plasmodia in the low forest of Pôrto Mauá and in the high forest of the Manaus-Itacoatiara Road, State of Amazonas, Brazil, from June 1965 to May 1968.

All infections were due to *Plasmodium brasilianum*

Species of monkey	Pôrto Mauá			Manaus-Itacoatiara Road		
	Examined	Positive		Examined	Positive	
		no.	%		no.	%
<i>Cebus apella apella</i> (capuchin-monkey)	4	—	—	26	5	19.2
<i>Alouatta seniculus straminea</i> (red howler-monkey)	—	—	—	24	4	16.7
<i>Ateles paniscus paniscus</i> (red-faced spider-monkey)	—	—	—	26	5	19.2
<i>Chiropotes chiropotes</i> (red-backed saki)	—	—	—	28	4	14.3
<i>Pithecia chrysocephala</i> (golden-headed saki)	3	—	—	6	—	—
<i>Saimiri sciureus</i> (squirrel-monkey)	40	1	2.5	—	—	—
<i>Marikina bicolor</i> (pied tamarin) *	8	—	—	4	—	—
Total	55	1	1.8	114	18	15.8

* This species is a marmoset. All others listed in this Table are monkeys

TABLE II

Number of mosquitoes captured on baits placed at ground level and on platforms built near the canopy of trees, in Pôrto Mauá and in the Manaus — Itacoatiara Road, State of Amazonas, Brazil, from October 1966 to September 1967

Species of mosquito	Pôrto Mauá		Manaus-Itacoatiara Road	
	Ground	Platform*	Ground	Platform**
Tribe ANOPHELINI				
<i>Anopheles (Kerteszia) neivai</i>	—	—	8	77
<i>Anopheles (Arribalzagia) mediopunctatus</i>	28	706	2	22
<i>Anopheles (Arribalzagia) shannoni</i>	4	48	—	9
<i>Anopheles (Arribalzagia) intermedius</i>	3	19	—	—
<i>Anopheles (Anopheles) mattogrossensis</i>	1	1	—	—
<i>Anopheles (Nyssorhynchus) darlingi</i>	—	1	—	—
<i>Anopheles (Nyssorhynchus) albitarsis</i>	—	1	—	—
<i>Anopheles (Nyssorhynchus) oswaldoi</i>	83	114	3	2
<i>Anopheles (Nyssorhynchus) nuñeztovari</i>	129	88	1	1
<i>Anopheles (Nyssorhynchus) triannulatus</i>	29	36	—	4
<i>Anopheles (Nyssorhynchus) spp.</i>	35	37	1	11
<i>Chagasia bonneae</i>	1	3	2	118
Tribe CULICINI				
<i>Culex (Culex) coronator</i>	60	—	—	1
<i>Culex (Culex) spp.</i>	127	3	10	15
<i>Culex (Melanoconion) chrysonotum</i>	1	1	13	4
<i>Culex (Melanoconion) spp.</i>	19	11	84	93
<i>Haemagogus spp.</i>	—	—	1	5
<i>Aedes (Ochlerotatus) fulvus</i>	51	12	—	2
<i>Aedes (Ochlerotatus) serratus</i>	3	—	1	—
<i>Aedes spp.</i>	3	—	1	—
<i>Psorophora (Grabhamia) cingulata</i>	76	9	7	—
<i>Psorophora (Janthinosoma) ferox</i>	4	—	—	—
<i>Psorophora (Janthinosoma) lutzi</i>	1	5	—	—
<i>Psorophora (Janthinosoma) albipes</i>	1	—	—	—
<i>Psorophora (Janthinosoma) circumflava</i>	133	158	27	60
<i>Psorophora (Janthinosoma) lanei</i>	—	1	—	—
<i>Psorophora (Janthinosoma) spp.</i>	—	—	2	6
<i>Mansonia (Mansonia) titillans</i>	140	353	8	17
<i>Mansonia (Mansonia) indubitans</i>	176	835	18	61
<i>Mansonia (Mansonia) humeralis</i>	112	66	—	—
<i>Mansonia (Mansonia) amazonensis</i>	1	1	—	—
<i>Mansonia (Rhynchoaenia) albicosta</i>	2	—	—	—
<i>Mansonia (Rhynchoaenia) fasciolata</i>	8	8	—	—
<i>Mansonia (Rhynchoaenia) venezuelensis</i>	28	46	—	—
<i>Mansonia (Rhynchoaenia) sp.</i>	24	9	12	69
<i>Aedomyia squamipenna</i>	2	—	—	1
Tribe SABETHINI				
<i>Trichoprosopon (Trichoprosopon) digitatum</i>	—	—	1	—
<i>Wyeomyia (Dendromyia) confusa</i>	—	—	2	—
<i>Sabethes (Sabethes) spixii</i>	—	—	—	1
<i>Sabethes (Sabethes) cyaneus</i>	—	—	—	1
<i>Sabethes (Sabethes) amazonicus</i>	—	—	—	7
<i>Sabethes (Sabethoides) tridentatus</i>	—	—	—	4
<i>Sabethes (Sabethoides) glaucodaemon</i>	—	—	1	2
<i>Sabethes spp.</i>	—	—	1	16
Total	1285	2572	206	609
Hours spent	33	33	141	141

* Ten meters above the ground

** Fifteen to 25 meters above the ground

monkey and 4 sakis. This raises to 27 the number of monkeys with evidence of present or past malarial infection, or 23.7 per cent.

All infections, in both areas — Pôrto Mauá and the Manaus-Itacoatiara Road — were due to a single species of malaria parasite, *Plasmodium brasilianum* GONDER & BERENBERG-GOSSLER, 1908⁶.

In the blood of the great majority of specimens of all species of monkeys and marmosets, microfilariae and trypanosomes of several species were also detected. The trypanosomes will be discussed in a separate publication.

2. The search for the vectors of simian malaria

The comparison between the mosquito fauna of Pôrto Mauá and the Manaus-Itacoatiara Road studied during a whole year is shown in Table II.

In the former area, the density of mosquitoes was much higher than in the latter, the average numbers of specimens per hour of capture being, respectively, 58.3 and 2.9.

Counting only the anophelines, which are the usual vectors of mammalian malaria, we will see that in Pôrto Mauá a total of 1367 specimens were caught in 66 hours, or 20.7 per hour, against 261 specimens in 282 hours, or 0.9 per hour in the Manaus-Itacoatiara Road. Not only the density of anophelines was greater in Pôrto Mauá, but the number of species was also higher. However, one species of anopheline, *Anopheles (Kerteszia) neivai*, quite numerous in the Manaus-Itacoatiara Road, was not found in Pôrto Mauá; another species, *Chagasia bonnea*, was much more abundant in the former area; all other species of anophelines were more numerous, or much more so, in Pôrto Mauá.

When comparing mosquitoes caught on baits placed at ground level and on the platforms, one should remember that in Pôrto Mauá the canopy is much lower than in the Manaus-Itacoatiara Road (see footnote, Table II).

In both areas nearly all species of anophelines showed a preference for feeding

near the canopy. This preference was very definite for *Chagasia bonnea*, *Anopheles neivai*, *A. mediopunctatus*, *A. shannoni* and *A. intermedius*.

The following 471 mosquitoes were dissected for the search of sporozoites in the salivary glands: 72 *Anopheles neivai*, 17 *A. mediopunctatus*, 7 *A. shannoni*, 5 *A. oswaldoi*, 1 *A. nuñeztovari*, 100 *Chagasia bonnea*, 3 *Culex chrysonotum*, 112 *C. (Melanocnion)* sp., 3 *Haemagogus* sp., 1 *Aedes julvus*, 1 *Aedes* sp., 2 *Psorophora cingulata*, 47 *P. circumflava*, 1 *P. albipes* e 4 *Psorophora* sp., 17 *Mansonia titillans*, 24 *M. indubitans*, 34 *Mansonia* spp, 1 *Wyeomyia confusa*, 3 *Sabethes amazonicus*, 2 *S. tridentatus*, 1 *S. cyaneus* and 14 *Sabethes* spp. Only 1 *Anopheles neivai* was positive; it had been caught on a human bait, 25 meters above the ground, near km 135 of the Manaus-Itacoatiara Road.

COMMENTS

By studying primate malaria, comparatively, in the two neighbouring but ecologically different areas, we verified that in the high forest the infection was very frequent, reaching nearly one-fourth of the simian population, while in the low forest only one among 55 primates was found infected.

The infection of *Saimiri sciureus* by *Plasmodium brasilianum* is here reported for the first time in Brazil, but this species of monkey had been previously found parasitized in Colombia^{7, 8} and Peru⁵. The parasitism of all the other four species of monkeys had been previously recorded by us from the Manaus-Itacoatiara Road^{2, 4}.

Concerning the natural transmission of simian malaria, the figures presented in Table II suggest that *Anopheles neivai* might be an important vector. Most species of anophelines, both in Pôrto Mauá and in the Manaus-Itacoatiara Road, showed a definite acrodendrophily. However, by comparing the anopheline fauna of the two areas, it will be seen that in the Manaus-Itacoatiara Road, where the malarial infection rate of monkeys was much higher, two species of anophelines were abundant near the canopy: *Chagasia bonnea* and *Anopheles neivai*. The latter was the only species of anophe-

line present in the Manaus-Itacoatiara Road and apparently absent in Pôrto Mauá, where the former species, although present, was very scarce.

The number of mosquitoes dissected for sporozoites was small, but the only natural infection recorded was detected in a specimen of *A. neivai*. The sporozoites found could be of non simian origin, since the possibility that other local vertebrates might harbour plasmodia has to be considered; however, it is worth reminding that the infected *A. neivai* was caught while feeding near the forest canopy.

One can not rule out the possibility of other anophelines being vectors (*Anopheles mediopunctatus*, for example), but it is worth pointing out that the bromeliad-breeding *Anopheles neivai* belongs to the same subgenus, *Kerteszia*, as *A. cruzi*, which has been incriminated as a vector of simian malaria in Southern Brazil, State of São Paulo³.

RESUMO

Estudos sôbre malária de macacos nas vizinhanças de Manaus, Estado do Amazonas, Brasil

Nos arredores de Manaus achamos cinco espécies de macacos infetados com o *Plasmodium brasilianum*: *Cebus apella apella* (macaco-prego), *Alouatta seniculus straminea* (guariba vermelho), *Ateles paniscus paniscus* (coatá de cara vermelha), *Chiropotes chiropotes* (cuxiu) e *Saimiri sciureus* (macaco-de-cheiro). A infecção desta última espécie no Brasil é aqui assinalada pela primeira vez. Examinamos com resultado negativo alguns exemplares de outras espécies: o macaco *Pithecia chrysocephala* (parauacu) e o saguí *Marikina bicolor* (mico de coleira).

Estudos comparativos efetuados numa floresta baixa, de várzea, em Pôrto Mauá e numa floresta alta e densa ao longo da Estrada de Rodagem Manaus-Itacoatiara, mostraram que os primatas abundam em ambas, mas enquanto os saguís e os pequenos macacos-de-cheiro predominam na primeira área, os macacos maiores prevalecem na segunda. A taxa de infecção malárica cons-

tatada através o exame de sangue (Tabela I) mostrou-se bem menor entre os macacos da mata baixa do que entre os da mata alta — 1,8% contra 15,8%, respectivamente. Na primeira achamos um único macaco-de-cheiro parasitado (2,5%), enquanto na última encontramos plasmódios em macacos-prego (19,2%), coatás (19,2%), guaribas (16,7%) e cuxius (14,3%). Incluindo-se os macacos com sangue negativo mas com pigmento malárico no baço, a proporção de exemplares com evidência de infecção malárica presente ou passada permanece a mesma na mata baixa, mas eleva-se a 23,7% na floresta alta.

Tentando esclarecer a transmissão da malária simiana, efetuamos capturas comparativas de mosquitos nas duas áreas, simultaneamente em iscas colocadas no solo e perto da copa das árvores (Tabela II). Dos anofelinos acrodendrôfilos, uma espécie, *Anopheles neivai*, foi somente encontrada, (e em número elevado), na mata alta, sendo ainda a única em que constatamos infecção por esporozoitos; outra espécie, *Chagasia bonneae*, predominou nitidamente lá. O *A. neivai*, cujas larvas criam em bromélias, pertence ao mesmo subgênero, *Kerteszia*, que o *A. cruzi*, por nós anteriormente incriminado de transmitir a malária de macacos no Sul do Brasil.

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