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ON THE TRANSMISSION OF SIMIAN MALARIA IN BRAZIL

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SUMMARY

We have recently proved that *Anopheles (Kerteszia) cruzi* is the vector of simian malaria in the State of São Paulo, and our data also indicate that it is the probable vector in other areas of Eastern and Southern Brazil. In the Amazon Region the carrier is still unknown; near Manaus, *Anopheles (Kerteszia) neivai* is suspected, but no guess can be made in relation to the enzootic foci of the Amapá Territory, where there are several species of anophelines of other subgenera with acrodendrophilic habits.

In our opinion, in some areas of Brazil *A. cruzi* transmitts both the simian and human malarias, in others only the monkey parasites. The reasons for such situations are discussed on the basis of the biology of the vector and the habits of the human populations.

INTRODUCTION

In Brazil, simian malaria has been found up to the present in two regions: the Amazon, where only the quartan-like *Plasmodium brasilianum* has been detected, and the Eastern and Southern coastal forests, where both *P. brasilianum* and the benign-tertian-like *P. simium* are present. *P. brasilianum* has been recorded in fourteen species or varieties of Brazilian monkeys, while *P. simium* apparently occurs in only two. The infection rates vary according to area and species of primate, in some places malaria being practically holoenzootic in some species of monkeys.

During the recent years a search for the vectors of simian malaria is being carried out in several parts of the country (Map 1). These investigations led to the discovery that in one highly enzootic forest of the State of São Paulo monkey malaria is transmitted by *Anopheles (Kerteszia) cruzi*, which our data suggest to be also the vector in other areas of Eastern and Southern Brazil. In the Amazon Region, however, although near Manaus *Anopheles (Kerteszia) neivai* might be a carrier, in other foci this mosquito was not found or was too scanty, several species of other subgenera were much more numerous in the canopy and the transmission problem is far from settled.

MATERIAL AND METHODS

The transmission studies started and have been more thoroughly carried out in the Horto Florestal da Cantareira, State of São Paulo. However, they were also performed in other localities of the Eastern, Southern and Northern Regions of Brazil: Sooretama and Santa Leopoldina, in the State of Espirito Santo; the surroundings of Joinvile and Campo Alegre, in the State of Santa Catari-

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na; the vicinity of Guaiba, in the State of Rio Grande do Sul; Pôrto Mauá and the Manaus-Itacoatiara Road, in the State of Amazonas; and Serra do Navio and Tracajatuba, in the Amapá Territory (Map 1).

The work consists of mosquito captures on baits placed on the ground and on platforms or hammocks near the forest canopy, and identification of the insects in order to screen the acrodendrophilic species, i.e., those that prefer to feed in the canopy and which are, therefore, more likely to transmit the monkey parasites. Dissections for the search of sporozoites in the salivary glands is also performed. Although all mosquitoes are collected and identified, in the accompanying Tables we include only the anophelines, because the non-anophelines (culicines and sabethines) are not supposed to transmit mammalian plasmodia.

RESULTS

Anopheles cruzi, *a proved vector of simian malaria in the State of São Paulo*

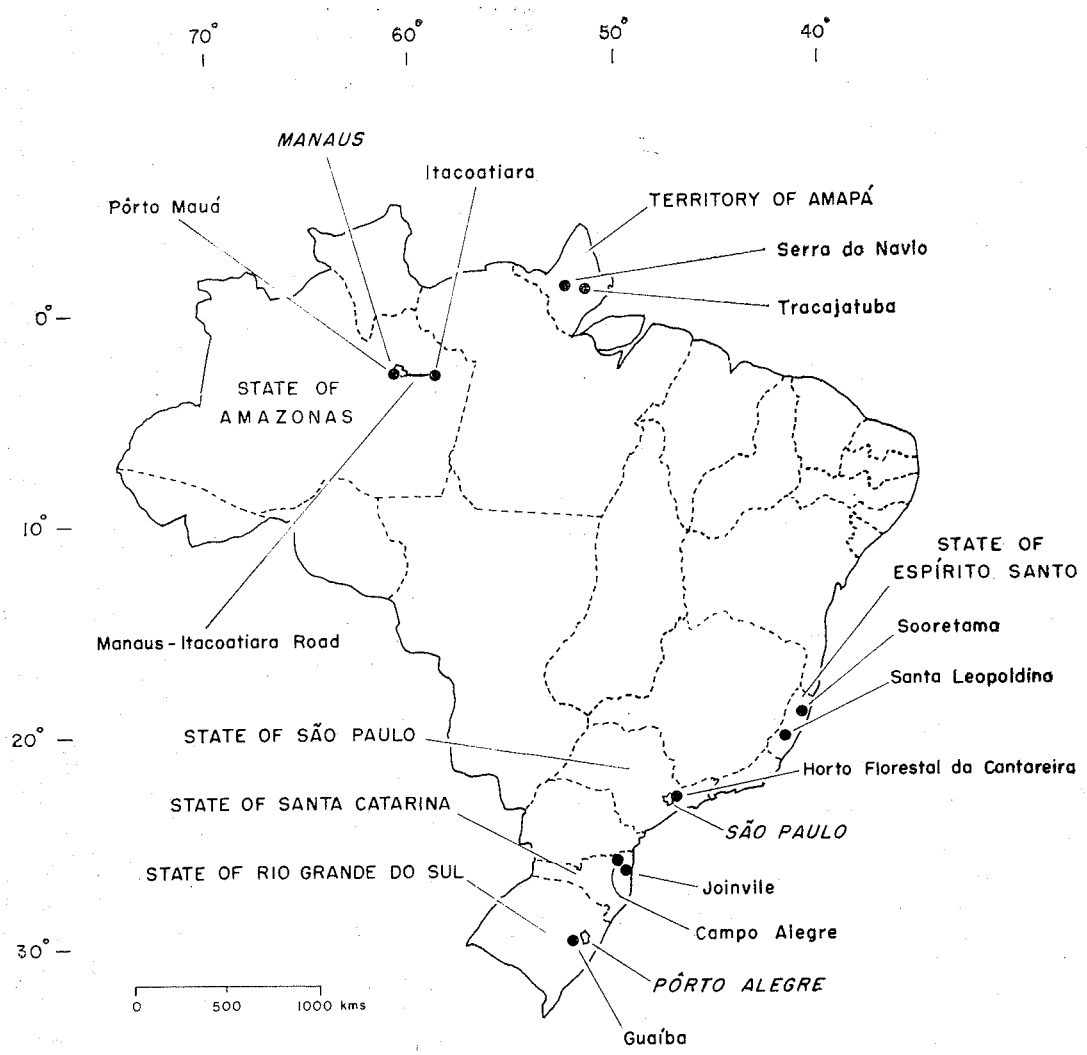
The Horto Florestal da Cantareira is an extensive forest reservation on hilly terrain, in the outskirts of the city of São Paulo, barely 25 kilometers distant from the senior author's laboratory. The only human dwellers are thirty forest guards and their families, but wild animals are plentiful. Among the primates there are very numerous howler-monkeys *(Alouatta fusca)*, some black-horned capuchins *(Cebus apella nigritus)* and marmosets *(Callithrix aurita)*, and rare titis *(Callicebus personatus)*. Malaria is extremely frequent among the howlers, more than 60% of them showing plasmodia in the blood and almost 80% either having a patent parasitemia or revealing malarial pigment in the spleen semears.

The mosquito captures early showed that the only anopheline to be caught in large numbers near the canopy was *Anopheles (Kerteszia) cruzi*, an observation confirmed during the subsequent captures carried out for more than six years (Table I). Besides, this mosquito — which breeds only in bromeliad plants, mostly epiphytes —, was the only species to be found naturally infected with sporozoites, with a global infection rate of 2% (Table VI). The sporozoites found resemble those of primate plasmodia and probably belonged to the howlers' parasites, because human malaria does not occur in the area and many other local mammals were examined with negative results. Trying to

TABLE I

Anopheline mosquitoes captured in the Horto Florestal da Cantareira, State of São Paulo, Brazil, on baits placed on the ground and on platforms in the forest canopy (January 1964 to March 1970). In this area 62% of the howler-monkeys harboured plasmodia

Species of anopheline	Ground	Canopy
Anopheles (Kerteszia) cruzi	18	2587
Anopheles (Myzorhynchella) lutzi	93	21
Anopheles (Myzorhynchella) antunesi	16	3
Anopheles (Myzorhynchella) parvus	2	—
Anopheles (Nyssorhynchus) strodei	15	1
Anopheles (Nyssorhynchus) noroestensis	—	1
Anopheles (Arribalzagia) intermedius	1	—
Chagasia fajardoi	2	4
Total	147	2617
Hours spent	507	488



MAP 1 — Map of Brazil, showing the localities where studies on transmission of simian malaria have been performed.

demonstrate the simian origin of the sporozoites we inoculated positive salivary glands of wild-caught *A. cruzi* into squirrel-monkeys *(Saimiri sciureus)* which we had previously demonstrated to be susceptible to *P. simium*. Such inoculations were apparently unsuccessful. Believing these failures could be due to some detail in the manipulation of the parasites for inoculation, as explained in a precedent article, we recently tried another experiment, the result of which was quite rewarding. A very young howler-monkey *(Alouatta belzebul belzebul)* caught in the Amazon Region and brought to São Paulo by airplane was kept in our laboratory where its blood was examined for malaria parasites nineteen times during two months. After this "quarentine" it was taken to the Horto Florestal da Cantareira in a large-mesh wire cage inside a bobbin-net enclosure, every afternoon, from 2 to 11 March 1970. The cage was hoisted on to the canopy of a tree, where, on a platform, a boy was used as bait for the mosquitoes. The anophelines alighting on him were caught before biting and transferred to the bobbin-cage with the monkey. The cage remained in the canopy for the night, but, on the fol-

lowing mornings it was brought to the laboratory, where the mosquitoes were identified and dissected for sporozoites; all mosquitoes were seen to belong to a single species, *Anopheles cruzi*, and two of the 80 specimens that had fed on the monkey showed sporozoites in the salivary glands. They had bitten the howler on March 5 and 6. Daily blood examination was performed thereafter, and on April 13 the first plasmodia were seen in the thick smears and on the next day they were numerous enough to be detected in the thin smears, being typical *Plasmodium simium;* later, *P. brasilianum* also appeared. As the howler came from the Amazon, where *P. simium* has never been recorded, and since its blood had been adequately examined with negative results before the experiment, the present infection was undoubtedly transmitted by *A. cruzi*. And so this mosquito is a confirmed vector in the Horto Florestal da Cantareira. It became the first proved vector of simian malaria not only in Brazil, but also in the Neotropical Region.

Anopheles cruzi, *the probable vector of simian malaria in other areas of Eastern and Southern Brazil*

In several other localities of Eastern and Southern Brazil our data indicate that *A. cruzi* must also be the vector.

In the Eastern State of Espirito Santo, for instance, simian malaria studies were undertaken in the two already mentioned areas, the forest plains of Sooretama and the forested mountains of Santa Leopoldina. In Sooretama howler-monkeys *(Alouatta fusca)* are scanty, but capuchin-monkeys *(Cebus apella)* and titis *(Callicebus personatus)* are numerous. In Santa Leopoldina howlers are often seen, but woolly spider-monkeys *(Brachyteles arachnoides)* are the most numerous monkeys. In Sooretama all monkeys examined were negative, while in Santa Leopoldina 31.2% showed malaria parasites in the blood smears. *Anopheles cruzi* was not found in Sooretama, while in Santa Leopoldina it was not only the most numerous mosquito but also the only one definitely acrodendrophilic (Table II). It correspond-

TABLE II

Anopheline mosquitoes captured in the State of Espirito Santo, Brazil, on baits placed on the ground and on platforms in the forest canopy (November 1967 to June 1968) in two areas: Sooretama, where monkey malaria was not found, and Santa Leopoldina, where 31.2% of the monkeys harboured plasmodia *

Species of anopheline	Sooretama		Santa Leopoldina	
	Ground	Canopy	Ground	Canopy
Anopheles (Kerteszia) cruzi	—	—	1	189
Anopheles (Myzorhynchella) lutzi	—	—	8	3
Anopheles (Myzorhynchella) parvus	—	—	4	1
Anopheles (Nyssorhynchus) strodei	—	—	—	1
Anopheles (Nyssorhynchus) oswaldoi	1	—	—	—
Anopheles (Arribalzagia) mediopunctatus	9	60	—	—
Anopheles (Arribalzagia) intermedius	1	—	—	—
Anopheles (Arribalzagia) fluminensis	1	2	—	—
Anopheles (Stethomyia) kompi	3	—	—	—
Chagasia fajardoi	—	—	1	2
Total	15	62	14	196
Hours spent	26	26	54	54

* Adapted from DEANE et al. (1968) [9]

TABLE III

Anopheline mosquitoes captured in the State of Santa Catarina, Brazil, on baits placed on the ground and in the forest canopy, in two areas: Joinvile, with 46.7% of the howler-monkeys harbouring plasmodia and with endemic human malaria, and Campo Alegre, with 43% of simian malaria but no human malaria (July 1965 to May 1970)

Species of anopheline	Joinvile		Campo Alegre	
	Ground	Canopy	Ground	Canopy
Anopheles (Kerteszia) cruzi	1468	2034	136	1135
Anopheles (Myzorhynchella) lutzi	2	—	—	—
Anopheles (Myzorhynchella) antunesi	4	3	6	—
Anopheles (Anopheles) tibiamaculatus	1	—	—	—
Chagasia fajardoi	—	—	90	565
Total	1475	2037	232	1700
Hours spent	50	50	28	28

ed to 90.5% of the anophelines obtained in all captures and to 96.4% of those caught in the canopy.

In the Southern State of Santa Catarina the forested coastal mountains harbour numerous howler-monkeys *(Alouatta fusca)* and fewer capuchins *(Cebus apella)*. In localities in the neighbourhood of Joinvile, where 46% of the howlers showed malaria parasites, *A. cruzi* was the only abundant anopheline, both near the ground and at the canopy (99.5% and 99.9%, respectively), the other species being so scarce that could hardly be responsible for the high prevalence of simian malaria. In another area, near Campo Alegre, where 43% of the howlers harboured plasmodia, *A. cruzi* was also the most abundant acrodendrophilic species, although another anopheline, *Chagasia fajardoi*, was also numerous in the canopy (Table III). However, here again *A. cruzi* was the only mosquito to be found naturally infected with sporozoites (Table VI). In other parts of Santa Catarina (like Campos Novos), where howler-monkeys are abundant but *Kertesziae* are absent or rare, simian malaria has not been found.

In the other Southern State studied, Rio Grande do Sul, the landscape is very different from that of the previously mentioned areas. The terrain is an extensive plain, interspersed with small remnants of forests along rivers or creeks. These forests are also inhabited by numerous howlers *(Alouatta fusca)*, but no other primates. In one of such forests, in Guaiba, we found 60% of the monkeys infected with malaria parasites. A few hours spent in mosquito captures revealed that *A. cruzi* was again the only anopheline to be present in numbers at the canopy.

The simian malaria transmission problem in North Brazil

However, in Northern Brazil *A. cruzi* does not exist. Therefore we started attempts to find the vectors of simian malaria in the Amazonian enzootic foci. This was done in two areas quite far apart: the vicinity of Manaus, in the State of Amazonas, and two localities in the Amapá Territory. In the former, the search was carried out in two ecologically distinct areas: Pôrto Mauá, a swamp forest where the primates were chiefly marmosets *(Saguinus bicolor)* and monkeys of small size *(Saimiri sciureus)* and where only 1.8% of the monkeys harboured plasmodia, and the high forest along the Manaus-Itacoatiara Road, where numerous species of the larger monkeys, such as capuchins *(Cebus apella)*, howlers *(Alouatta seniculus straminea)*, spider-monkeys *(Ateles paniscus paniscus)* and sakis *(Pithecia pithecia* and *Chiropotes satanas chiropotes)*

TABLE IV

Anopheline mosquitoes captured in the State of Amazonas, Brazil, on baits placed on the ground and in the forest canopy (October, 1966 to September, 1967) in two localities: Pôrto Mauá, where only 1.8% of the monkeys showed plasmodia, and the Manaus-Itacoatiara Road, where the simian malaria infection rate was 15.8%*

Species of anopheline	Pôrto Mauá		Manaus-Itacoatiara Road	
	Ground	Canopy	Ground	Canopy
Anopheles (Kerteszia) neivai	—	—	8	77
Anopheles (Nyssorhynchus) darlingi	—	1	—	—
Anopheles (Nyssorhynchus) albitarsis	—	1	—	—
Anopheles (Nyssorhynchus) oswaldoi	83	114	3	2
Anopheles (Nyssorhynchus) nuneztovari	129	88	1	1
Anopheles (Nyssorhynchus) triannulatus	29	36	—	4
Anopheles (Nyssorhynchus) spp.	35	37	1	11
Anopheles (Anopheles) mattogrossensis	1	1	—	—
Anopheles (Arribalzagia) mediopunctatus	28	706	2	22
Anopheles (Arribalzagia) shannoni	4	48	—	9
Anopheles (Arribalzagia) intermedius	3	19	—	—
Chagasia bonneae	1	3	2	118
Total	313	1054	17	244
Hours spent	33	33	141	141

* Adapted from DEANE et al. (1968) [6]

are present and where the plasmodial infection rate among the monkeys was much higher, 15.8%. In Pôrto Mauá (Table IV) *Kertesziae* were not found, but several species of anophelines were numerous near the canopy; one of them, *Anopheles (Arribalzagia) mediopunctatus,* was particularly abundant. In the Manaus-Itacoatiara Road, however, two species of acrodendrophilic anophelines were usually present: *Chagasia bonneae* and *Anopheles (Kerteszia) neivai,* the latter belonging to the same subgenus as *A. cruzi* and also breeding in bromeliad plants. The examination of salivary glands of local mosquitoes revealed sporozoites in only 1 *A. neivai* of 72 examined, and none in *C. bonneae* (Table VI). We suspect, therefore, that *A. neivai* might be a vector in the area.

In the Amapá Territory, however, in spite of mosquito captures being performed for more than one year in two foci, we reached at no conclusion as to the probable vectors. In two localities surveyed we found simian malaria: in one, Serra do Navio, the infection rate was 12.5%, in the other, Tracajatuba, 16.6%. In Serra do Navio the mosquito density was rather low (Table V), *Anopheles (Kerteszia) neivai* was present but very scarce, other species of anophelines being more numerous in the canopy; in Tracajatuba anophelines were quite abundant but no *Kertesziae* were seen and, of the ten species of anophelines captured, the majority was found in large numbers near the canopy. Some of them, like *A. mediopunctatus* and *A. shannoni,* are well known as canopy feeders, but a surprising result was the presence or abundance, in the canopy, of species of the subgenus *Nyssorhynchus,* like *A. triannulatus, A. nuneztovari, A. oswaldoi, A. brasiliensis* and *A. darlingi,* which are not supposed to be acrodendrophilic. Therefore, in Tracajatuba the transmission problem deserves further attention.

TABLE V

Anopheline mosquitoes captured in the Territory of Amapá, Brazil, on baits placed on the ground and on platforms near the forest canopy (October 1968 to November 1969), in two areas, Serra do Navio and Tracajatuba, where, respectively, 17% and 12.5% of the monkeys harboured plasmodia

Species of anopheline	Serra do Navio		Tracajatuba	
	Ground	Canopy	Ground	Canopy
Anopheles (Kerteszia) neivai	—	2	—	—
Anopheles (Nyssorhynchus) darlingi	—	—	186	161
Anopheles (Nyssorhynchus) albitarsis	—	—	4	1
Anopheles (Nyssorhynchus) braziliensis	—	—	1	23
Anopheles (Nyssorhynchus) oswaldoi	450	44	76	135
Anopheles (Nyssorhynchus) nuneztovari	23	1	206	101
Anopheles (Nyssorhynchus) triannulatus	25	46	975	3216
Anopheles (Anopheles) peryassui	—	—	17	7
Anopheles (Arribalzagia) mediopunctatus	11	47	51	678
Anopheles (Arribalzagia) shannoni	—	—	62	603
Anopheles (Arribalzagia) intermedius	4	—	63	13
Anopheles (Arribalzagia) minor	—	—	2	—
Total	513	140	1643	4938
Hours spent	101	101	121	121

TABLE VI

Anopheline mosquitoes examined for sporozoites in three enzootic areas of simian malaria in Brazil, 1964 to 1970. In brackets, number of mosquitoes positive

Species of anopheline	State of São Paulo (Horto Florestal da Cantareira)	State of Santa Catarina	State of Amazonas (Manaus-Itacoatiara Road)
Anopheles (Kerteszia) cruzi	1230 (24)	1134 (8)	—
Anopheles (Kerteszia) neivai	—	—	72 (1)
Anopheles (Myzorhynchella) antunesi	13	4	—
Anopheles (Myzorhynchella) lutzi	46	—	—
Anopheles (Nyssorhynchus) oswaldoi	—	—	5
Anopheles (Nyssorhynchus) strodei	14	—	—
Anopheles (Nyssorhynchus) nuneztovari	—	—	1
Anopheles (Arribalzagia) mediopunctatus	—	—	17
Anopheles (Arribalzagia) shannoni	—	—	7
Chagasia fajardoi	4	539	—
Chagasia bonneae	—	—	100
Total	1307 (24)	1677 (8)	202 (1)

Enzootic areas of simian malaria, without or with human malaria also transmitted by Anopheles cruzi

An interesting fact revealed by these studies was that the vector of simian malaria, *Anopheles cruzi,* is a well known vector of human malaria. We observed, however, that in some areas it transmitts only monkey malaria, while in others it conveys both the simian and human malaria parasites. We believe that these situations are related to differences in behaviour of *A. cruzi* in the various areas or to the habits of the human populations.

In some places, like the Horto Florestal da Cantareira, State of São Paulo, and Santa Leopoldina, State of Espirito Santo, *A. cruzi* is decidedly acrodendrophilic, very few specimens coming to feed near the ground. In our comparative captures performed on baits placed on the ground and in the canopy, in the Horto and Santa Leopoldina respectively, 99.3% and 99.4% of the specimens were caught in the canopy. In such forests, although monkey malaria is very frequent or almost holoenzootic, human malaria does not occur.

In Santa Catarina conditions differ: in Campo Alegre, where *A. cruzi* is still decidedly acrodendrophilic, 89.3% of the specimens being caught in the canopy, 43% of the howler-monkeys showed malaria parasites, but human malaria is also absent; but in the Joinvile area, although still preferring the canopy, *A. cruzi* is more eclectic; in our series of captures 58% were obtained in the canopy against 42% near the ground; monkey malaria is highly prevalent (infection rate, 64%), but human malaria is also endemic.

Finally, a curious fact was observed in Guaiba, State of Rio Grande do Sul. In the already mentioned forest where 60% of the howlers had malaria parasites, we caught *Anopheles cruzi* biting both near the ground (56%) and near the canopy (44%). But in Guaiba there is no human malaria at all. How this could be explained in a place where *A. cruzi,* a proved vector of human malaria in other areas, feeds at different levels of the forest? We may attempt to answer as follows: a) in this place the patches of forest inhabited by the howler-monkeys are quite distant from the human dwellings and *A. cruzi* can find in the forest enough sources of food and does not need to fly to the houses to feed; b) this being a cattle-raising area, the domestic animals live on the fields between the forest and the houses and may form a barrier that protects the human dwellers from the bites of the few mosquitoes that eventually leave the woods; and c) the inhabitants rarely get into the forest and almost never do it after sunset, the preferential feeding time for *A. cruzi.* Therefore, the absence of human malaria in this area could be due to the lack of opportunities for *A. cruzi* to feed on man.

One of the most important aspects of the studies on the vectors of monkey plasmodia is concerned with their possible role in transmitting simian malaria to humans in nature. In Brazil, the cycles of transmission of human and simian malarias are usually independent, the former disease being conveyed by anophelines that prefer to feed near the ground, while the vectors of the latter are acrodendrophilic species. The only recorded human infection with a monkey malaria parasite *(P. simium)* in Brazil was in a man acting as bait for mosquitoes in captures performed near the canopy in the highly enzootic forest of the Horto Florestal da Cantareira. But since both *P. simium* and *P. brasilianum* are infective for man, we insist that naturally acquired infections of humans with simian plasmodia be searched in areas where *Anopheles cruzi* feeds both near the ground and in the canopy, as it does in some parts of the coastal forested mountains of the State of Santa Catarina.

RESUMO

Sôbre a transmissão da malária de macacos no Brasil

Recentemente provamos que o *Anopheles (Kerteszia) cruzi* é o transmissor da malária simiana em uma floresta do Estado de São Paulo (Horto Florestal da Cantareira), sendo também o provável vector em outras áreas do Brasil oriental e meridional. Na Região Amazônica, entretanto, não se descobriu ainda o transmissor; nos arredores de Manaus suspeita-se do *Anopheles (Kerteszia) neivai,* mas no Território do Amapá

a ocorrência de várias espécies de anofelinos de outros subgêneros com hábitos acrodendrófilos, em focos enzoóticos, torna difícil apontar um provável vector.

Pensamos que em algumas áreas do Brasil o *A. cruzi* transmite tanto a malária simiana como a humana, enquanto em outras sòmente a dos macacos. As razões disso são discutidas com base em diferenças na biologia do vector e nos hábitos das populações humanas.

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