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SIMIAN MALARIA IN BRAZIL

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In Brazil simian malaria is widely spread, being frequent in the Amazon region (10% of primates infected) and even more in the forested coastal mountains of the Southeastern and Southern regions (35% and 18% infected, respectively), but absent in the semi-arid Northeast.

Only two species of plasmodia have been found: the quartan-like Plasmodium brasilianum *and the tertian-like* P. simium, *but the possible presence of other species is not excluded.* P. brasilianum *is found in all enzootic foci, but P. simium was detected only on the coast of the Southeastern and Southern regions, between parallels 20°S and 30°S.*

Nearly all hosts are monkeys (family Cebidae, 28 species harbouring plasmodia out of 46 examined), and very rarely marmosets or tamarins (family Callitrichidae, 1 especies out of 16). P. brasilianum *was present in all infected species,* P. simium *in only two.*

The natural vector in the Southeastern and Southern regions was found to be Anopheles cruzi, but has not been conclusively identified in the Amazon.

One natural, accidental human infection due to P. simium *was observed.*

There is no evidence of the relation of simian to human malaria in the Southeastern and Southern regions, where human malaria was eradicated in spite of the high rates of monkeys infected, but in the Amazon recent serological studies by other workers, revealing high positivity for P. brasilianum/P. malariae *antibodies in local indians, would suggest that among them malaria might possibly be regarded as a zoonosis.*

Key words: Simian malaria – Brazil

When Don Eyles, in 1960, was accidentally infected by mosquito bite while working with the simian *Plasmodium cynomolgi* in the United States, this fact stimulated extensive studies on simian malaria in various parts of the world. Three years later, while I was in London as a student at Prof. P.C.C. Garnham's laboratory, he suggested Dr J.L. Bruce-Chwatt, then at the World Health Organization, to put me in charge of a programme sponsored by the organization, for a nation-wide simian malaria survey in Brazil. I first hesitated in taking that responsibility because at that time in Brazil, simian plasmodia had been detected in only three monkeys: the original uakari from the Amazon and two howlers from the Southeastern region. In August of the same year (1963) a guard of the Cantareira forest rservation in São Paulo, came to my laboratory at the Medical School asking if we were interested in a howler-monkey he had captured. In the very first microscopic field of a stained smear of the animal's blood I found a plasmodium and, fortunately, marked it, because no other parasite could be detected in that and other smears. In September Bruce-Chwatt came to Brasil for the VII International Congress on Tropical Medicine and Malaria held in Rio de Janeiro and visited

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my laboratory in São Paulo asking what I had decided conceming my participation in the simian Malaria survey. Showing him the howler's parasite I accepted his proposal, because, I had learned that monkey malaria was present in Cantareira, only 25 km from the Medical School. The ensuing survey lasted for ten years, 1964 to 1973, and its principal results have been summarized by us elsewhere (Deane, 1967a; 1969; 1972; 1976; 1988 and Deane et al., 1969, 1971). More recently, other studies on the subject were reported (Arruda, 1985; Lourenço-de-Oliveira, 1988, 1990; Deane et al., 1989).

However, the story of simian malaria in Brasil started many years before.

Although in 1905 Laveran stated that he had seen malaria parasites in the blood of orangutans, it was only in 1907 that the plasmodia of non-human primates were described, all from the Orient (Halberstaedter & Prowazeck, 1907; Mayer, 1907, 1908). On the following year it was discovered that simian malaria was also present in the American Continent. By examining the blood of a bald-uakari, *Cacajao calvus*, received from the state of Amazonas, Brazil (where it is sometimes called "macaco-inglês" or English-monkey, because of its red face and white body) and which was being exhibited at Hagenbeck's Circus in Hamburg, Gonder & von Berenberg-Gossler (1908) found a parasite that they named *Plasmodium brasilianum*. The examination of the animal's blood for a fortnight led to a detailed description of the parasite's morphology, to the verification of its similarity with the human *P. malariae* and the quartan periodicity of its erythrocytic schizogony (von Berenberg-Gossler, 1909).

This parasite was later found in numerous monkeys of several species from other American countries but it had to wait for 56 years to be seen in Brazil. It remained the only species of simian malaria parasite known in the Americas until 1951.

In 1939 Prof. Flávio da Fonseca, of the Butantã Institute, while studying a strain of yellow fever virus in Brazilian monkeys, was impressed by the unusual temperature curve presented by a young howler-monkey, *Alouatta fusca*, that had been captured at the Itapecirica forest near São Paulo. Examining the animal's blood he found a malaria parasite that he recorded as *P. brasilianum* in a paper on yellow fever. In 1950 I asked Prof. Fonseca who was visiting my laboratory at the Malariology Institute, for more details on his finding of the first and only report of *P. brasilianum* in Southern Brazil. I was then much interested in a study of *P. brasilianum* because, during an extensive survey of human malaria and its vectors along the Amazon, my wife, Maria Deane, and I had found that *P. malariae* was more prevalent in jungles than in towns and, due to similarities between this species and. *P. brasilianum*, we had thought of the possibility of man and monkey sharing the same parasite. Stimulated by my enquiry, on his return to São Paulo Prof. Fonseca went through the monkey's blood smears he had examined ten years before and discovered the parasite to he a very different species, a tertian-like plasmodium which he named *P. simium* in 1951. In 1955 Fonseca and Garnham found and studied the blood forms from a second howler also caught in Itapecirica (Garnham, 1966).

As you know, *P. brasilianum* is very similar to the human *P. malariae.* Its blood forms are identical or almost so, although at least in some strains the schizonts tend to be larger and present in somewhat enlarged red cells. The blood forms are also indistinguishable in ultrastructure (Coatney et al., 1972) and no differences have been found in the sporogonic (Collins et al., 1969; Coatney et al., 1972) or in the exoerythrocytic (Garnham et al., 1963; Sodeman et al., 1969) cycles. Monoclonal antibodies against the surface of *P. malariae* sporozoites cross-react with *P. brasilianum* sporozoites and vice-versa (Cochrane et al., 1984, 1985) and, further, it was demonstrated that the sequence of the genes for the immunodominant epitopes in the circumsporozoite protein is the same for the two species (Lal et al., 1988). If all these data be accepted as sufficient proofs of identity with the human quartan parasite, *P. brasilianum* will become a synonym, because *P. malariae* was named twenty-seven years before.

Plasmodium simium, of tertian periodicity, resembles *P. vivax* and *P. ovale* in its blood stages, being less amoeboid and with coarser and more precocious Schuffner's dots than the former and not tending to the oval forms of the latter. Its sporogony resembles that o *P. vivax* and the exoerythrocytic forms are said to be only slightly smaller (Collins et al., 1969a).

Plasmodium braslianum and *P. simium* remain up to now, the only species of simian malaria parasites known to occur in the Neotropics and, therefore, in Brazil. However, it is possible that other species exist in Brazilian monkeys, because most natural infections have been detected by the examination of few smears and only once. Morphological differences have been seen in some strains of *P. brasilianum*, but they could not be regarded as specific distinctions because the same strain may show different morphological details when passaged to monkeys of different species, as it has been pointed out before by other authors (Taliaferro & Taliaferro, 1934).

THE SIMIAN MALARIA SURVEYS

In this report we include the results of the examination of Brazilian non-human primates for the search of plasmodia by various workers: Deane (1976), Arruda (1985), Lourenço-de-Oliveira (1988, 1990) and Deane et al. (1989).

I started to examine the blood of primates more than fifty years ago, in 1937, when engaged in the team led by Evandro Chagas that performed, in the Brazilian Amazon, the pioneer studies on Neotropical Kala-azar, carried out in the forests of Abaetetuba, Pará state. As Evandro thought that Kala-azar is originally autochtonous in this continent and should have a wild reservoir host, he put me in charge of the search of the parasite in wild mammals. We examined Giemsa stained smears of the animals' spleen in search of leishmaniae, but also looked for blood parasites – plasmodia trypanosomes and microfilariae (Deane, 1961). Years later I looked for plasmodia during surveys on wild reservoirs of *Trypanosoma cruzi* and other trypanosomes in Pará state, or, with my wife (Deane, 1964, 1967), on reservoirs of Kala-azar in Ceará state (Deane, 1956), and with the specific aim of searching for plasmodia in one area in Pará state (Deane & Damasceno, 1961). Although trypanosomes were frequently found, no plasmodia were detected in those surveys. However, different results were obtained when we later performed the afore mentioned country-wide simian malaria survey. In such study some monkeys were brought by local hunters, others were bought from local dealers, a few were caugth in traps, but, as anesthetizing bullets did not prove efficient in the jungle, most of the animals were shot and necropsied in the forest; blood was obtained through cardiac puncture of the moribunds and a piece of spleen was removed for the search of malarial pigment. A considerable proportion of the animals were used by colleagues for other parasites or for pathology or genetic studies.

During the building of dams for hydroelectric plants in Brazil the animals of the areas to be flooded were captured to be transported to dry portions of the surrounding forest. This procedure permits that numerous animals be examined in a relatively short time. Arruda (1985) took advantage of one of such situations, so that in a single locality, Tucurui, Pará state, she examined the blood of more than two thousand primates in three months. In 1988 Lourenço-de-Oliveira did the same in the hydroelectric plant of Balbina, Amazonas state and two years later examined some golden lion tamarins in Rio de Janeiro state, while we did a simian malaria survey when a hydroelectric plant was being built in Cachoeira Samuel, Rondônia state (Deane et al., 1989). In the nation-wide survey under my responsibility (Deane, 1976) I had many collaborators, the most constant of which were Joaquim Ferreira Neto and my wife. The other surveys were performed each in a single locality.

THE GEOGRAPHICAL DISTRIBUTION OF SIMIAN MALARIA AND ITS PARASITES

Brazil holds 26 states in five major climatic regions (Fig. 1)*. In the forested warm and humid Amazon region the climate is equatorial, in the Northeastern region it is semiarid, in the Southeastern it is tropical, in the Southern it is sub-tropical and in the West-Central it is also tropical but with sparse vegetation.

The states included in each region are seen in the map.

Simian malaria has been surveyed in 118 localities from nearly all Brazilian states, except Rio Grande do Norte and Paraíba, in the Northeast.

*The localities examined in the states of Maranhão, Tocantins and Mato Grosso are Amazonian in climate. Therefore, we included those states in the Amazon region, although they officially belong to the Northeastern and West-Central regions, respectively.

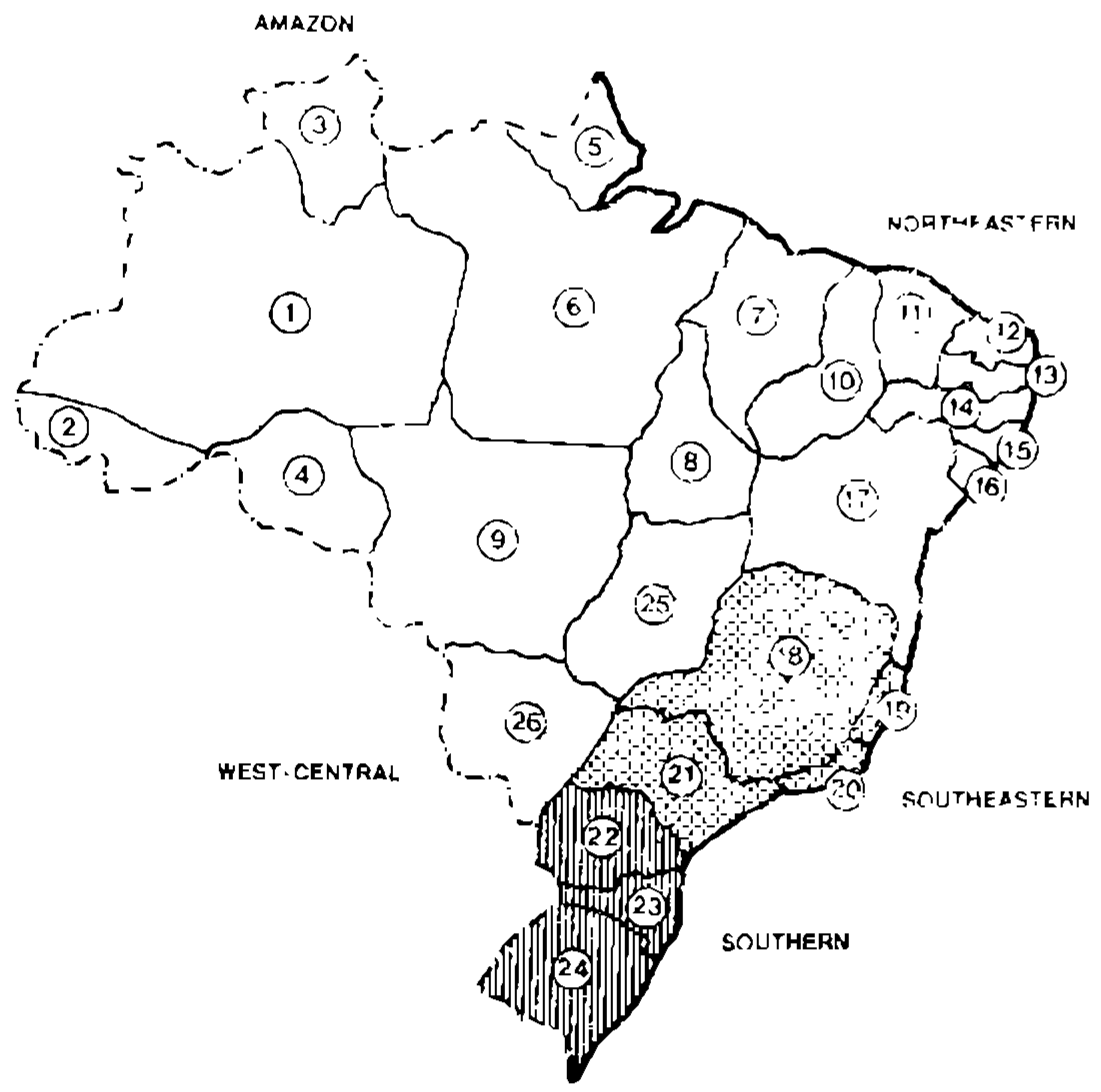

Fig. 1: Brazil regions and states. 1 - Amazonas. 2 - Acre. 3 - Roraima. 4 - Rondônia. 5 - Amapá. 6 - Pará. 7 -Maranhão. 8 - Tocantins. 9 - Mato Grosso. 10 - Piauí. 11 - Ceará. 12 - Rio Grande do Norte. 13 - Paraíba. 14 - Pernambuco. 15 - Alagoas. - 16 Sergipe. 17 - Bahia. 18 - Minas Gerais. 19 - Espírito Santo. 20 - Rio de Janeiro. 21 - São Paulo. 22 - Paraná. 23 - Santa Catarina. 24 - Rio Grande do Sul. 25 - Goiás. 26 - Mato Grosso do Sul.

Of the 4585 primates examined 655 showed malaria parasites in the blood, or 14.3% (Table I). The infection rates were not uniformely distributed but varied with the different regions. In the Amazon, the infection rate was 10.1%. In the Northeast, where forests and primates are scarce, none of the animals were found infected. The same was observed in the small primate sample examined from the West-Central region. The highest infection rate was recorded in the Southeastern region: 35.6%; next came the Southern region with 17.9%.

As to the species of malaria parasites (Table I, Fig. 2), the number of primates infected with *P. brasilianum* was much higher than those with *P. simium*. The proportions for Brazil as a whole were 73.9% for *brasilianum* alone, 17.7% for *simium*, 4.9% for mixed infections with both, 0.5% for *P. brasilianum* together with a non identifiable species and 3% with plasmodia which could not be determined. While in Amazônia *P. brasilianum* was the only species identified, in the Southeast it prevailed, accounting for 46.3% of the positive blood samples against 37.5% for *P. simium* as single species, 9.6% mixed and 6.6% non identifiable. In the Southern region *P. simium* was predominant - 42.4% against 33.3% for *P. brasilianum*, but mixed infections reached 18.2%, and 6.1% could not be identified.

Plasmodium brasilianum was detected in all enzootic localities and accounted for all identifiable parasites in the Amazon. *P. simium* was found in a much more restricted area, only in the coastal forests of the Southeastern and Southern regions, between parallels 20°S and 30°S, the latter being also the southern limit of *P. brasilianum*.

It should be emphasized, however, that we are not sure *P. simium* is absent from the rest of Brazil, the Amazon region included. We have seen in a few Amazonian monkeys some parasites definitely larger than the usual for *P. brasilianum* and in somewhat enlarged red cells, and if it was not for the lack of Schuffner's dots, they would be identified as *P. simium*.

It should be reminded that the real prevalence of simian malaria in Brazil and also of mixed infections must be much higher than shown here, because the great majority of the primates were examined only once and only a few were splenectomized, and it is expected that sub-patent infections were missed.

Within each region (Table II), in the Amazon, Pará state accounted for almost 72% of the animals (because of Arruda's survey in Tucuruí) and in the Southeast 60% of the primates were from São Paulo state (because of the Cantareira forest reservation where we had a permanent station for seven years).

In the Amazon the infection rates were high in al states, but in the Southeastern region, where more than one half of the infected primates were from São Paulo state, none were found in Minas Gerais state.

THE SIMIAN HOSTS

Brazil holds one of the most varied simian fauna for a single country in the world. Both families of Ceboidea are present and well represented here: the Cebidae, or true monkeys, and the Callitrichidae, or marmosets and tamarins. All eleven genera of Cebidae and five of Callitrichidae are found.

The former include *Alouatta* (howler-monkeys), *Aotus* (night-monkeys or owl-monkeys),

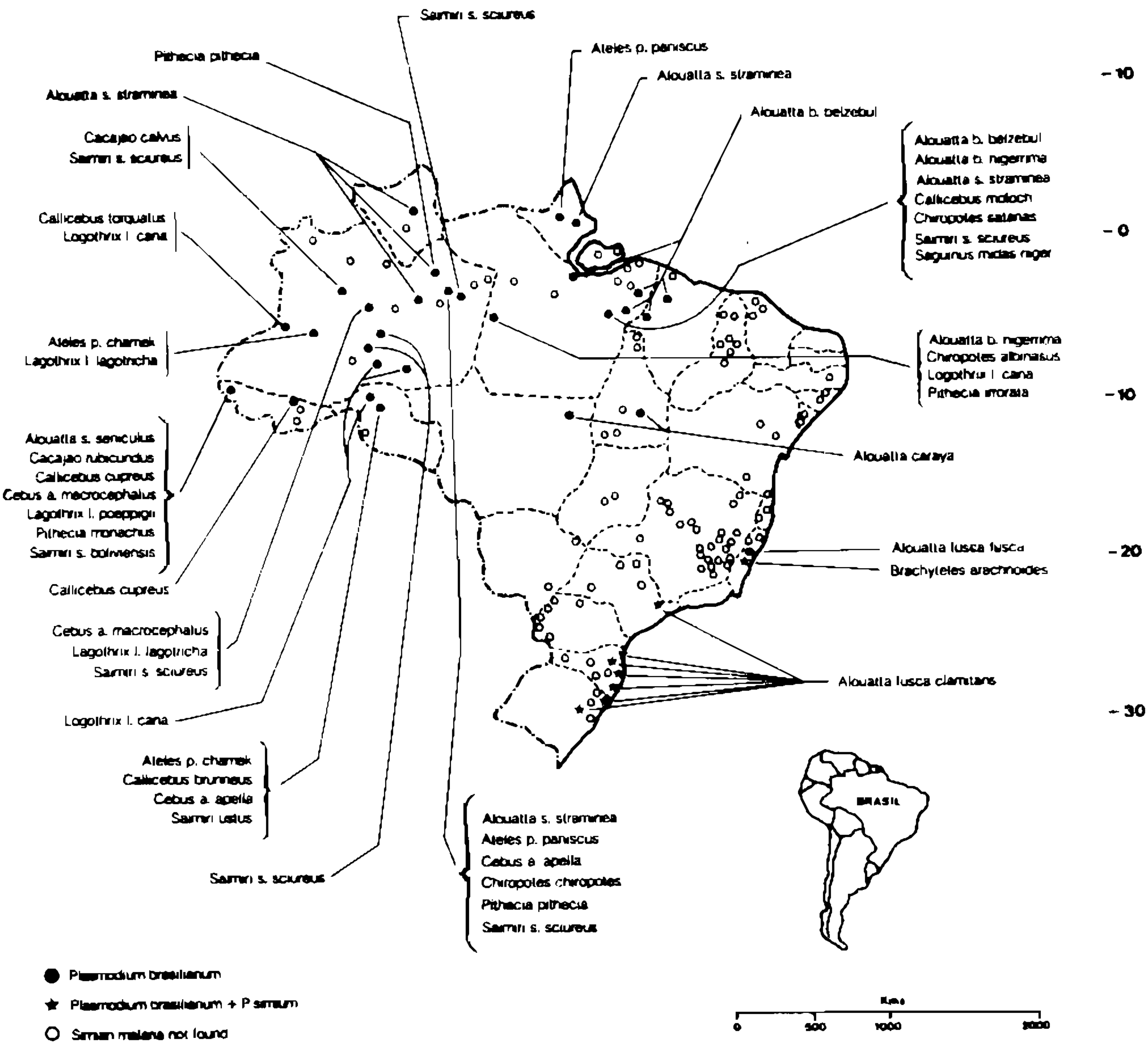

Fig. 2: simian malaria in Brazil distribution of parasites and hosts.

Ateles (spider-monkeys), *Brachyteles* (wooly-spider-monkeys), *Cacajao* (uakaris), *Callicebus* (titis), Cebus (capuchin-monkeys), *Chiropotes* (bearded sakis), *Lagothrix* (wooly-monkeys), *Pithecia* (sakis) and *Saimiri* (squirrel-monkeys). Among the Callitrichidae the genera were *Callimico* (Goeldi's marmosets), *Callithrix* (marmosets), *Cebuella* (pygmy-marmoset), *Leontopithecus* (golden lion tamarins) and *Saguinus* (tamarins)*.

The regional distribution of the genera are found in Fig. 3. All genera, except *Brachyteles* and *Leontopithecus*, are present in the Amazon. In the semi-arid Northeast only *Alouatta*, *Cebus* and *Callithrix* are to be encountered, while in the upper Southeast these groups are frequent, but *Callicebus*, *Brachyteles* and *Leontopithecus* also occur, the two latter being restricted to this region. In the lower Southeast one finds *Alouatta*, *Cebus*, *Callicebus* and *Callithrix*, while in the south of the Southern region only *Alouatta* and *Cebus* are usually to be found (in many areas only *Alouatta*). All these genera are easy to distinguish, but the identification of many species and sub-species often requires specialists. In this report, simians of all genera present in Brazil were examined, except *Callimico*.

Of the primates examined (Table III) 4076 were Cebidae of eleven genera and 48 species or sub-species, the corresponding figures for the Callitrichidae being 509, 4 and 16.

Nearly one-half of the animals were *Alouatta*, but other genera were well represented, such as *Saimiri* and *Cebus*, followed by *Callicebus*, *Aotus* and *Chiropotes* among the Cebidae and *Callithrix* and *Saguinus* among the Callitrichidae.

*We are very grateful to Drs Fernando de Avila Pires, Cory Carvalho and Horacio Schneider for the identification of many of the primates or the help on the systematics of the hosts.

TABLE I

Simian plasmodia in Brazil: frequency according to regions, as detected in blood smears, 1937-1990[a]. Percent in brackets

		Frequency of species among total with *Plasmodium*					
Regions	Primates examined	Total with *Plasmodium*	*P. brasilianum*	*P. simium*	*P. brasilianum* + *P. simium*	*P. brasilianum* + *P.* sp.	*P.* sp
Amazon	3472	350 (10.1)	347 (99.1)	–	–	3 (0.9)	–
Northeastern	146	–	–	–	–	–	–
Southeastern	764	272 (35.6)	126 (46.3)	102 (37.5)	26 (9.6)	–	18 (6.6)
Southern	184	33 (17.9)	11 (33.3)	14 (48.4)	6 (18.2)	–	2 (6.1)
West-Central	19	–	–	–	–	–	–
Total	4585	655 (14.3)	484 (73.9)	116 (17.7)	32 (4.9)	3 (0.5)	20 (3.0)

a: from Deane (1976), Arruda (1985), Lourenço-de-Oliveira (1988, 1990) and Deane et al. (1989).

The global malaria infection rate was 14.3%, but there was a striking difference as to infection between the two families of hosts: nearly all infections were in the Cebidae, the rates being 16% for this family and only 0.8% for the Callitrichidae. Of the former, only one genus *Aotus*, was consistently negative, in spite of being one of the principal experimental hosts for human malaria and also shown to be experimentally susceptible to *P. brasilianum*, as mentioned by Coatney et al. (1972). On the other hand, among the Callitrichidae parasites were detected in only one genus, *Saguinus*.

Among the Cebidae high infection rates were obtained in *Alouatta, Ateles* and *Lagothrix*, while only few *Cebus* had malaria; as for *Saimiri*, a genus so common that more than 89,000 specimens were imported by the United States within six years (Cooper, 1968), the majority of individuals are naturally infected in some areas, none in others, a fact probably related to the absence of an appropriate vector.

Plasmodium brasilianum was found in all genera (except *Aotus*), *P. simium* only in *Alouatta* and *Brachyteles*.

Concerning the simian species and sub-species (Table IV), among the *Alouatta* one species showed the highest infection rate of all primates: 53.2% for *Alouatta fusca clamitans;* in Cantareira, over 62% of specimens of this monkey showed plasmodia in the blood and additional 20% without detectable parasitemia had malaria pigment in the spleen smears, suggesting a holoenzootic condition.

On the other hand, among the Callitrichidae malaria parasites were detected (by Arruda, 1985) in only 4 *Saguinus midas niger* out of 178 examined, all other marmosets and tamarins being negative, even in areas where monkeys were found infected, sometimes heavily so. I used to think that in Brazil callitrichids were never naturally infected, before Prof. Mauro Barretto told me he had found plasmodia in one *Callithrix geoffroyi* examined for *T. cruzi* (unpublished).

Regarding the distribution of hosts of simian malaria (Fig. 2), in the Southeast only three species of hosts were found with parasites: *A. fusca clamitans, A. fusca fusca* and *Brachyteles arachnoides*, the first of which was also seen infected in the South. All other primates with malaria were in the Amazon.

In all of the 64 simian species or subspecies examined, plasmodia were detected in 29, of which 21 had never been found infected before.

Concerning the species of plasmodia in the various hosts, P. *simium* was found in nature only in *A. fusca clamitans* and *B. arachnoides,*

TABLE II

Simian plasmodia in Brazil: frequency according to Regions and States, as detected in blood smears, 1937-1990[a]

Regions and States	Primates examined	Total with *Plasmodium* No.	Total with *Plasmodium* %	Species of *Plasmodium*: *P. brasilianum*	*P. simium*	*P. brasilianum* + *P. simum*	*P. brasilianum* + *P.* sp.	*P.* sp.
Amazon								
Amazonas	552	89	16.1	87	–	–	2	–
Acre	93	12	13	11	–	–	1	–
Roraima	1	1		1	–	–	–	–
Rondônia	251	19	7.6	19	–	–	–	–
Amapá	29	3	10.3	3	–	–	–	–
Pará	2492	215	8.6	215	–	–	–	–
Tocantins	22	3	13.6	3	–	–	–	–
Mato Grosso	4	1	25	1	–	–	–	–
Maranhão	28	7	25	7	–	–	–	–
Northeastern								
Piaui	34	–	–	–	–	–	–	–
Ceará	11	–	–	–	–	–	–	–
Pernambuco	3	–	–	–	–	–	–	–
Alagoas	10	–	–	–	–	–	–	–
Sergipe	11	–	–	–	–	–	–	–
Bahia	77	–	–	–	–	–	–	–
Southeastern								
Minas Gerais	180	–	–	–	–	–	–	–
Espírito Santo	92	10	10.9	7	–	3	–	–
Rio de Janeiro	28	–	–	–	–	–	–	–
São Paulo	464	262	56.5	119	102	23	–	18
Southern								
Paraná	29	–	–	–	–	–	–	–
Santa Catarina	100	25	25	10	11	3	–	1
Rio Grande do Sul	55	8	14.5	1	3	3	–	1
West Central								
Mato Grosso do Sul	4	–	–	–	–	–	–	–
Goiás	15	–	–	–	–	–	–	–
Total	4585	655	14.3	484	116	32	3	20

a: from Deane (1976), Arruda (1985), Lourenço-de-Oliveira (1988, 1990) and Deane et al. (1989).

in all other primates only *P. brasilianum* was observed.

In the nation-wide survey (Deane, 1976) non-human primates other than the original hosts were found to be susceptible to the simian Neotropical plasmodia in Brazil: *P. brasilianum* experimentally infected the marmoset *C. jacchus* (Deane et al., 1966c), *P. simium* infected the same marmoset plus *Ateles paniscus, Lagothrix lagotricha* and *Saimiri sciureus*, species absent from the territory of this plasmodium (Deane et al., 1966d, 1965; Deane and Okumura, 1965, 1965a).

The first experimental infection of *S. sciureus* with the human *P. vivax* was also obtained in this survey (Deane et al., 1966b, 1967).

COURSE OF NATURAL INFECTIONS

These were followed in specimens of *S. sciureus, A. paniscus, L. lagotricha* and *A. fusca clamitans* with *P. brasilianum* and the last monkey species with *P. simium*.

Parasitemia was nearly always low, but more persistent in *P. brasilianum* infections.

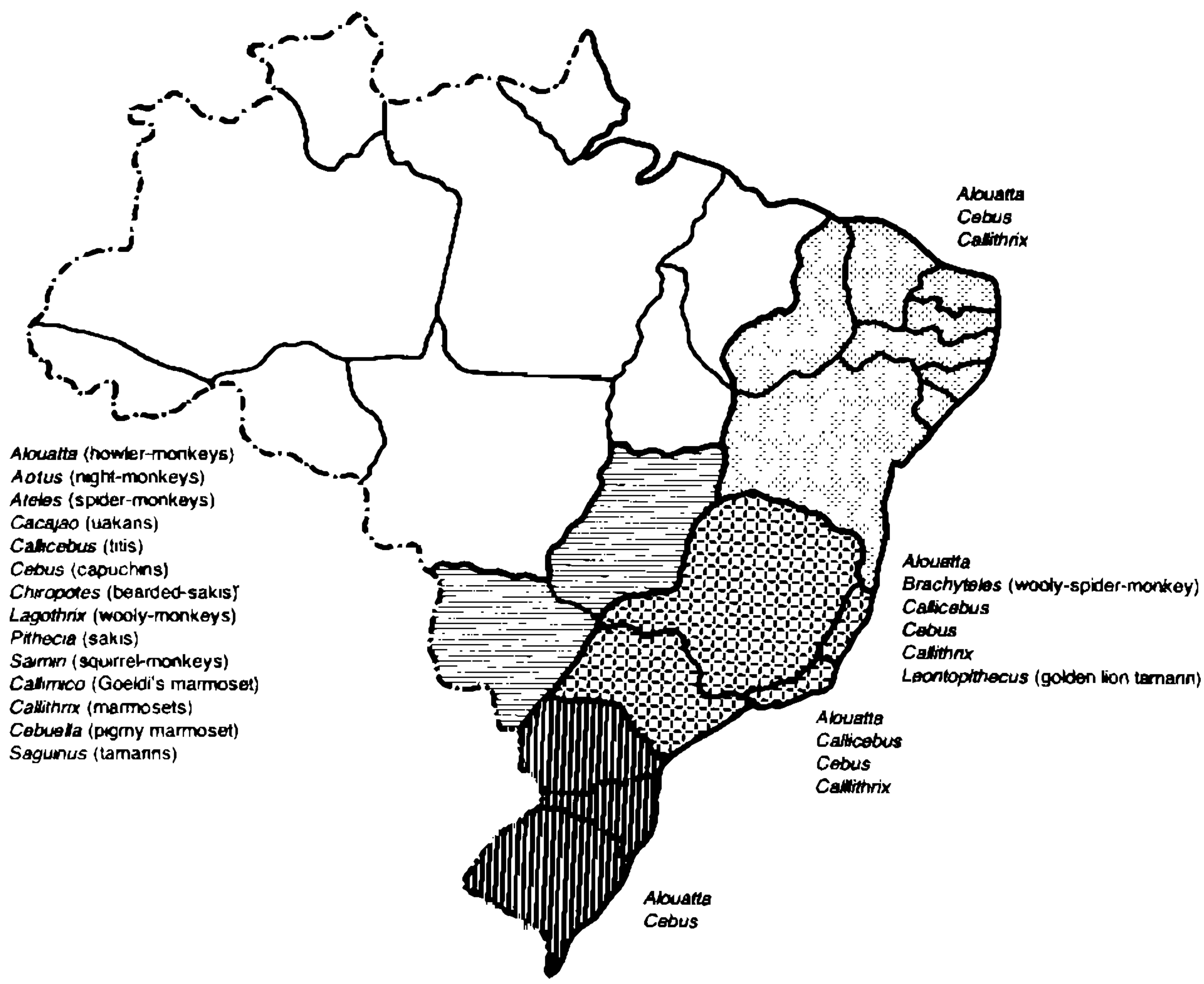

Fig. 3: Brazil – distribution of genera of primates.

The animals had fever (but rarely chills), apathy and loss of apetite during the days of schizogony, and developed loss of weight and anemia, but usually recovered spontaneously.

In a few specimens examined at different hours rupture of schizonts occurred at about 12 A.M. in both parasite species.

After splenectomy parasitemia rose sharply, in some cases to more than 1,000-fold with both plasmodia and about 40,000-fold in a howler with *P. simium*, some animals dying in cachexy.

TRANSMISSION IN NATURE

The search for the vectors of simian malaria in Brazil started in the forest reservation of Cantareira, in the outskirts of the city of São Paulo where the abundant howler-monkey population was revealing a very high rate of infection with both plasmodial species. It was later extended to forests of other states, as Santa Catarina, Rio Grande do Sul, Espirito Santo, Amazonas and Amapá (Fig. 4). Recently, areas in the hydroelectric plants in Balbina and Samuel, in the states of Amazonas and Rondônia respectively, are being surveyed by our group with the use of IRMA and monoclonal antibodies for the search and species identification of sporozoites, but results have not yet been published.

In our own survey, work consisted of anopheline captures simultaneosly performed on baits placed on the ground and on platforms or small hammocks near the forest canopy and the identification of mosquito species in order to screen the acrodendrophilic ones, i.e., those that preferred to feed at the canopy and which, therefore, would more likely transmit the simian parasites. In Cantareira, within a few days it was verified that the only species of anopheline to be caught in large numbers at the canopy was *Anopheles* (*Kerteszia*) *cruzi*, which breeds only in water collected on bromeliad plants, chiefly the epiphytic ones. During ten years study in this area, that first impression was supported not only in Cantareira (Table V), but also in the areas of simian malaria in the Southeastern and Southern regions. In Cantareira *A. cruzi* accounted for 94% of the anophelines and showed to be more than 99% acrodendrophilic. That supposition of its role as the vector, was

TABLE III

Simian plasmodia in Brazil: frequency according to families and genera of primates, as detected in blood smears, 1937-1990[a]

Families and genera of primates	Number of species	Primates examined	Total with *Plasmodium* No.	Total with *Plasmodium* %	Species of *Plasmodium*: *P. brasilianum*	*P. simium*	*P. brasilianum* + *P. simium*	*P. brasilianum* + *P.* sp.	*P.* sp.
Family Cebidae									
Alouatta (howler-monkeys)	8	2193	524	23.9	355	116	30	–	20
Aotus (night-monkeys)	3	154	–	–	–	–	–	–	–
Ateles (spider-monkeys)	3	57	14	24.6	14	–	–	1	–
Brachyteles (wooly spider-monkeys)	1	22	3	13.6	1	–	2	–	–
Cacajao (uakaries)	3	12	4	33.3	4	–	–	–	–
Callicebus (titis)	9	181	14	7.7	14	–	–	1	–
Cebus (capuchins)	9	599	9	1.5	9	–	–	–	–
Chiropotes (bearded-sakis)	3	152	18	11.8	18	–	–	–	–
Lagothrix (wooly-monkeys)	3	73	25	34.2	25	–	–	1	–
Pithecia (sakis)	3	85	2	2.4	2	–	–	–	–
Saimiri (squirrel-monkeys)	3	548	38	6.9	38	–	–	–	–
Total	48	4076	651	16.0	480	116	32	3	20
Family Callitrichidae									
Callithrix (marmosets)	6	250	–	–	–	–	–	–	–
Cebuella (pigmy mamoset)	1	1	–	–	–	–	–	–	–
Leontopithecus (golden lion tamarin)	1	28	–	–	–	–	–	–	–
Saguinus (tamarins)	8	230	4	1.7	4	–	–	–	–
Total	16	509	4	0.8	4	–	–	–	–
Total, both families	64	4585	655	14.3	484	116	32	3	20

a: from Deane (1976), Arruda (1985), Lourenço-de-Oliveira (1988, 1990) and Deane et al. (1989).

reinforced when *A. cruzi* was seen to be the only species of anopheline found naturally infected with sporozoites in the enzootic areas (Table XI). The simian origin of the sporozoites was not identified for at the time the IRMA with monoclonal antibodies had not yet been developed, but the vectorial role of *A. cruzi* was finally confirmed in one experiment undertaken in Cantareira, in which it transmitted both simian parasites (Deane et al., 1970a, 1971a). One young howler-monkey, *A. 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 Cantareira in a large-mesh wire cage inside a bobbin-net enclosure. The cage was hoistoned on to the canopy of a tree where, on a platform, a boy was used as bait. 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 and, on the following morning it was brought to the laboratory, where the mosquitoes were identified and dissected for sporozoites: all were seen to belong to a single species, *A. cruzi*, and two out of the 89 specimens that had fed on the monkey showed sporozoites in the salivary glands. Daily blood examination was performed thereafter, and typical *P. simium* appeared, to be folloowed by *P. brasilianum*. As the howler had come from Maranhão, in the Amazon, where *P. simium* has never been detected, the infection was undoubtedly transmitted by *A. cruzi*, a species that so became the first proven vector of simian malaria not only in Brazil but also in the

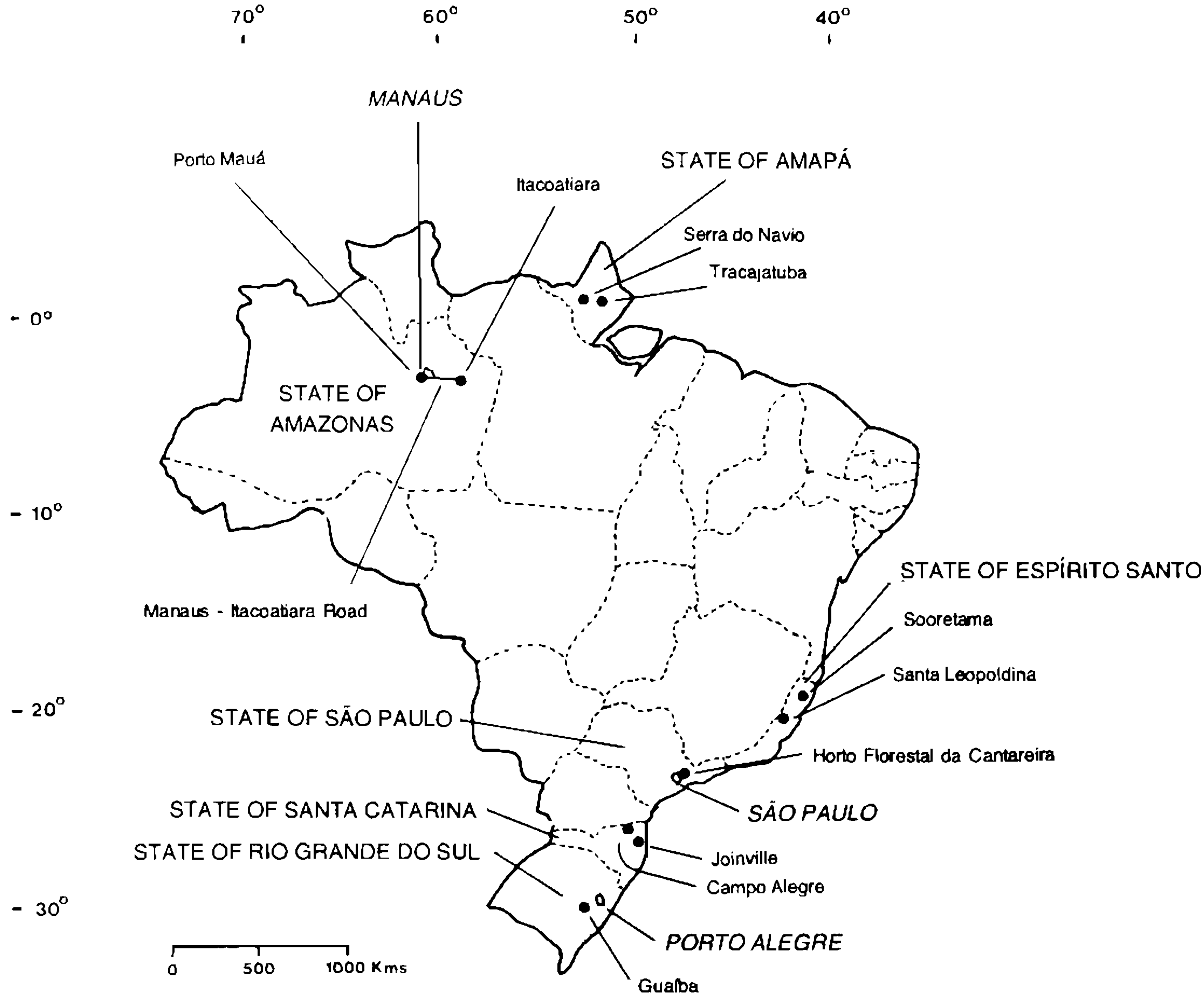

Fig. 4: localities where studies on transmission of simian malaria have been performed in Brazil.

American Continent.

In several other localities in Southeastern and Southern Brazil *A. cruzi* must also be the vector.

In the state of Santa Catarina (Table VI, Fig. 4) captures were performed in two localities: in the neighborhood of Joinvile, where 46% of the howlers harboured 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 would hardly be responsible for the high prevalence of simian malaria. In another locality, Campo Alegre, where 43% of the howlers were naturally infected, *A. cruzi* was also the most abundant acrodendrophilic species, although *Chagasia fajardoi* was also numerous in the canopy. Here again *A. cruzi* was the only mosquito to be found naturally infected with sporozoites (Table XI).

In the state of Rio Grande do Sul the landscape is very different from that of the forested mountainous areas mentioned above. It is an extensive plain, interspersed with small remnants of forests along rivers or creeks inhabited by numerous howlers but no other primates. In one of such gallery forests, in Guaiba, 60% of the monkeys showed plasmodia in the blood: a few hours spent in mosquito captures revealed that *A. cruzi* was again the only anopheline present in numbers at the canopy (Deane & Ferreira Neto, 1969).

In Espírito Santo State (Table VII) two areas were surveyed: in the forested plain of Sooretama, where no malaria was found in monkeys, *A. cruzi* was not detected; in the coastal forested mountains at Santa Leopoldina, where 31.2% of the monkeys harboured plasmodia, *A. cruzi* was the most numerous anopheline and the only one definitely acrodendrophilic, accounting for 96.4% of the anophelines caugth in the canopy (Deane et al., 1968).

These observations led us to regard *A. cruzi* as the only important vector of simian malaria in the Southeastern and Southern regions of Brazil.

TABLE IV

Simian plasmodia in Brazil: frequency according to species of primates as detected in blood smears, 1937-1990[a]

Species of primate	Primates examined	Total with *Plasmodium* No.	Total with *Plasmodium* %	*P. brasilianum*	*P. simium*	*P. brasilianum* + *P. simium*	*P. brasilianum* + *P.* sp.	*P.* sp
Family Cebidae								
Alouatta belzebul belzebul	500	83	16.6	83	–	–	–	–
Alouatta belzebul nigerrima	919	113	12.3	113	–	–	–	–
Alouatta belzebul ululata	37	–	–	–	–	–	–	–
Alouatta caraya	39	4	10.3	4	–	–	–	–
Alouatta fusca clamitans	554	295	53.2	129	116	30	–	20
Alouatta fusca fusca	41	7	17.1	7	–	–	–	–
Alouatta seniculus seniculus	10	3	30	3	–	–	–	–
Alouatta seniculus straminea	93	19	20.4	19	–	–	–	–
Aotus azarae	20	–	–	–	–	–	–	–
Aotus infulatus	108	–	–	–	–	–	–	–
Aotus nigriceps	26	–	–	–	–	–	–	–
Ateles belzebuth marginatus	4	–	–	–	–	–	–	–
Ateles paniscus chamek	16	5	31.3	5	–	–	–	–
Ateles paniscus paniscus	37	9	24.3	8	–	–	1	–
Brachyteles arachnoides	22	3	13.6	1	–	2	–	–
Cacajao calvus	4	3	75	3	–	–	–	–
Cacajao melanocephalus	4	–	–	–	–	–	–	–
Cacajao rubicundus rubicundus	4	1	25	1	–	–	–	–
Callicebus brunneus	50	8	16	8	–	–	–	–
Callicebus cupreus	16	–	–	–	–	–	–	–
Callicebus hoffmannsi	1	–	–	–	–	–	–	–
Callicebus moloch	91	5	4.7	4	–	–	1	–
Callicebus personatus melanochir	2	–	–	–	–	–	–	–
Callicebus personatus nigrifrons	2	–	–	–	–	–	–	–
Callicebus personatu personatus	14	–	–	–	–	–	–	–
Callicebus torquatus lugens	1	–	–	–	–	–	–	–
Callicebus torquatus torquatus	4	1	25	1	–	–	–	–
Cebus albifrons	18	–	–	–	–	–	–	–
Cebus apella apella	380	6	1.6	6	–	–	–	–
Cebus apella libidinosus	25	–	–	–	–	–	–	–
Cebus apella macrocephalus	30	3	10	3	–	–	–	–
Cebus apella nigritus	58	–	–	–	–	–	–	–
Cebus apella pallidus	8	–	–	–	–	–	–	–
Cebus apella robustus	61	–	–	–	–	–	–	–
Cebus apella versutus	15	–	–	–	–	–	–	–
Cebus nigrivittatus	4	–	–	–	–	–	–	–
Chiropotes albinasus	3	1	33.3	1	–	–	–	–
Chiropotes chiropotes	30	4	13.3	4	–	–	–	–
Chiropotes satanas	119	13	10.9	13	–	–	–	–
Lagothrix lagotricha cana	38	16	42.1	16	–	–	–	–
Lagothrix lagotricha lagotricha	22	5	22.7	4	–	–	1	–
Lagotrhrix lagotricha poeppigii	13	4	30.8	4	–	–	–	–
Pithecia irrorata	51	–	–	–	–	–	–	–
Pithecia monachus	18	1	5.6	1	–	–	–	–
Pithecia pithecia	16	1	6.3	1	–	–	–	–
Saimiri sciureus boliviensis	24	3	12.5	3	–	–	–	–
Saimiri sciureus sciureus	472	23	4.9	23	–	–	–	–
Saimiri ustus	52	12	23.1	12	–	–	–	–

Cont.

Species of primate	Primates examined	Total with *Plasmodium* No.	Total with *Plasmodium* %	Species of *Plasmodium*: *P. brasilianum*	*P. simium*	*P. brasilianum* + *P. simium*	*P. brasilianum* + *P.* sp.	*P.* sp
Family Callitrichidae								
Callithrix argentate emiliae	4	–	–	–	–	–	–	–
Callithrix aurita coelestis	6	–	–	–	–	–	–	–
Callithrix geoffroyi	30	–	–	–	–	–	–	–
Callithrix humeralifer	2	–	–	–	–	–	–	–
Callithrix jacchus	67	–	–	–	–	–	–	–
Callithrix penicillata jordani	141	–	–	–	–	–	–	–
Cebuella pygmaea	1	–	–	–	–	–	–	–
Leontopithecus rosalia	28	–	–	–	–	–	–	–
Saguinus bicolor	12	–	–	–	–	–	–	–
Saguinus fuscicollis	26	–	–	–	–	–	–	–
Saguinus imperator	2	–	–	–	–	–	–	–
Saguinus labiatus grisoevertex	5	–	–	–	–	–	–	–
Saguinus labiatus labiatus	2	–	–	–	–	–	–	–
Saguinus midas midas	2	–	–	–	–	–	–	–
Saguinus midas niger	178	4	2.2	4	–	–	–	–
Saguinus mystax	3	–	–	–	–	–	–	–
Total	4585	655	14.3	484	116	32	3	20

a: from Deane (1976), Arruda (1985), Lourenço-de-Oliveira (1988, 1990) and Deane et al. (1989).

What about the rest of this country?

What are the vectors in the Amazon, where so many species of primates are naturally infected and where *A. cruzi* is not present?

Published reports on this subject refer to studies performed in the states of Amazonas and Amapá and a few data are also available from states of Pará, Maranhão and Tocantins.

In Amazonas State the search was first implemented in two ecologically distinct areas (Table VIII, Fig. 4): Porto Mauá, a swamp forest where there were chiefly marmosets and monkeys of the smaller species and where only 1.8% harboured plasmodia; and the Manaus-Itacoatiara Road, where numerous species of the large monkeys were present and the plasmodial infection rate among them was higher – 15.8%. In Porto Mauá, anophelines were more abundant, most species being more numerous in the canopy, chiefly *A. mediopunctatus* and *A. oswaldoi*, while in the Manaus-Itacoatiara Road, of acrodencrophilic anophelines two species were numerous: *A. (Kerteszia) neivai* and *Chagasia bonneae*, the former being a bromeliad-breeding species, belonging to the same subgenus as *A. cruzi* (Table VIII). One of the 52 *A. neivai* dissected had sporozoites in the salivary glands, while all 100 *C. bonneae* were negative (Table XI). However, we can not incriminate *A. neivai* as a vector because the sporozoites were decidedly different from those seen in *A. cruzi*: they were shorter and thicker and we suspect they were not of mammalian origin.

In Amapá State (Table IX, Fig. 4), in spite of mosquito captures being performed for more than one year, no conclusion was reached as to the probable vectors. In the localities surveyed, in Serra do Navio, with 17% of the primates infected, the anopheline density was rather low, *A. neivai* was present but very scarce, *A. oswaldoi* was much more numerous near the ground, while *A. triannulatus* and *A. mediopunctatus* prevailed at the canopy. In Tracajatuba, with 12.5% of simian malaria, more species were acrodendrophilic, like *A. oswaldoi, A. mediopunctatus A. shannoni and A. triannulatus*. Even *A. darlingi* was almost as numerous in the canopy as near the ground. However, as the platform here was in a steep area, acrodendrophily could not be properly evaluated.

TABLE V

Anopheline mosquitoes captured in the Cantareira forest reservation, Brazil, on baits placed on the ground and on platforms in the forest canopy (1964-1970). In this area 62 per cent of the lowler monkeys harbored plasmodia[a]

Species of anopheline	Ground	Canopy
Anopheles (Kerteszia) cruzi	18	2,587
Anopheles (Nyssorhynchus) lutzi	93	21
Anopheles (Nyssorhynchus) autunesi	16	3
Anopheles (Nyssorhynchus) parvus	2	–
Anopheles (Nyssorhynchus)strodei	15	1
Anopheles (Nyssorhynchus) evansae	–	1
Anopheles (Anopheles) intermedius	1	–
Chagasia fajardoi	2.	4
Total	147	2,617
Hours spent	507	488

a: from Deane et al., 1971.

TABLE VI

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 per cent of the howler monkeys harboring plasmodia and with endemic human malaria, and Campo Alegre, with 43 per cent of simian malaria but no human malaria (1965-1970)[a]

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

a: from Deane et al., 1971.

Coming back to the state of Amazonas, a visit to São José near Fonte Boa, the territory of *Cacajao calvus*, the original host from which *P. brasilianum* was described, all three specimens of this uakari locally examined did harbour this plasmodium, and of the few anophelines caught at the canopy *A. trian-nulatus* was dominant (Almeida & Deane, 1970).

In areas where simian malaria was detected in the states of Maranhão and Tocantins, respectively, Alto das Guaribas and Brejo and Monte do Carmo, where 50% and 43% of howlers harboured plasmodia, several anopheline species were eclectic as to vertical distribution or tended to be acrodendrophilic, like *A. oswaldoi, A. triannulatus, A. mediopunctatus* and *Chagasia bonneae*, but we have no evidence that one of them is a simian malaria vector; they are also present and sometimes abundant in areas without monkey plasmodia (Ferreira Neto et al., 1970).

Arruda et al. (1989), during a malaria survey in indian tribes in Pará state, used the IRMA with monoclonal antibodies for the presence of CS antigen of plasmodia in 755 *A. darlingi* and 110 *A. triannulatus:* 3 *A. darlingi* tested positive for what they refer as *P. brasilianum/malariae*. If *P. brasilianum* is a synonym of *P. malariae* these results would mean that *A. darlingi* could be regarded as a vector of the monkey parasite. However, its geographical distribution and frequency often do not coincide with those of simian malaria. Only in few places *A. darlingi* is found in appreciable numbers in the canopy, and so it could not explain the high rate of primates with simian plasmodia in great part of the Amazon. Terefore we believe that the anopheline species reponsible for the transmission of at least a great proportion of the monkey plasmodia in the Amazon remains to be identified.

We should remind that although acrodendrophily is an important condition for a mosquito to be an usual vector of simian plasmodia, several species of acrodendrophilic anophelines are not important for the transmission in nature. As seen above (Table XI), no sporozoites were found in numerous specimens dissected of two wery acrodendrophilic anophelines, *C. bonneae* and *C. fajardoi*, from enzootic foci of simian malaria in the states of Amazonas and Santa Catarina, respectivelly. *A. mediopunctatus* was 96% acrodendrophilic in Porto Mauá, where simian malaria was rare. In the surroudings of Belém, Pará state, with no simian malaria, two anophelines showed a marked preference for feeding in the canopy: *A. mediopunctatus* and *A. shannoni* (Table X) (Deane et al., 1953).

ENVIRONMENTAL CONDITIONS

As we mentioned in a previous paper, in Brazil simian malaria is to be expected in tall humid forests with a dense monkey population, while there is litle chance of finding it in drier bushy areas, even those harbouring many marmosets but few monkeys of the larger species. This can be exemplified by comparing the rates of simian malaria in the Amazon and the mountainous coastal forests of Southeastern and Southern Brazil with that of the Northeast. The environment may influence to a high degree the presence and density of the simian hosts and of anopheline vector species. In the Southeastern and Southern regions conditions are propitious for a high density of bromeliad plants which hold water where the immature stages of *A. cruzi* develop, so that the same species of monkey in which malaria in enzootic may be free of plasmodia in forests where bromeliads and *A. cruzi* are rare or absent. In Santa Catarina State infected howlers are numerous along the coastal mountains where bromeliads and *A. cruzi* are abundant, but inexistant in the same species of monkeys in the western forests devoid of the plants and of that mosquito. In the neighbour states of Minas Gerais and Espírito Santo a similar condition occurs: while nearly all howlers were infected in one area of the latter, where bromeliads and *A. cruzi* abound, all howlers of the same species were negative in forests of Minas Gerais, free from both.

TABLE VII

Anopheline mosquitoes captured in the State of Espírito Santo, Brazil, on baits placed on the ground and on plattforms in the forest canopy (1967-1968) in two areas: Sooretama, where monkey malaria was not found, and Santa Leopoldina, where 31.2 percent of the monkeys habored plasmodia[a]

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

a: from Deane et al., 1968.

In the areas of equatorial climate the high level of temperature throughout the year enables transmission to be continous, but in temperate climates infection rates are higher during the late summer and lower in the cooler winter months, this being clearer for *P. simium* than for *P. brasilianum*, whose parasitemia is more durable. In the winter months not only rainfall is lower, but also the vector's larvae take longer to pupate and the feeding and reproductive activities of imagoes decrease.

In the Amazonian foci the seasonal fluctuation of simian malaria has yet to be evaluated.

RELATION TO HUMAN MALARIA

Now we get to a crucial question concerning simian malaria: the possibility of simian parasites infecting humans in nature, in other words, the chances of malaria being a zoonosis, a situation of paramount importance in a malaria eradication campaign. Can malaria be a zoonosis in particular areas in Brazil?

Malaria could be a zoonosis if: a) man is susceptible to the simian parasites; b) humans live in or close to forests inhabited by infected non-human primates and c) susceptible vectors have the opportunity of frequently feeding on both, monkeys and man.

The two known species of simian plasmodia in Brazil are infective for humans. The quartan-like *P. brasilianum* may be a synonym of the human *P. malariae*, but even if it is a distinct species it has been shown to infect man experimentally through mosquito bite (Contacos et al., 1963), and one human infection due to *P. simium* contracted in nature (although not exactly in a "natural" way) has been reported by us (Deane et al., 1966). The patient was one of our assistants, a forest guard who performed most of the canopy mosquito captures in the highly enzootic Cantareira forest reservation. One afternoon he complained of chills and fever two days previously and the same symptoms occurred while he was doing a canopy capture in my presence. His blood showed typical *P. simium* parasites and, taken to the hospital he had a third paroxysm two days after and cured spontaneously. He was born and always lived in the forest reservation where human malaria has never been detected and he had never received a blood transfusion. He was almost illiterate, but a very enthusiastic helper. When I first called him to work in the project and told him he should take a prophylactic drug, he refused with the argument that one aim of the

TABLE VIII

Anopheline mosquitoes captured in the State of Amazonas, Brazil, on baits placed on the ground and in the forest canopy (1966-1967) in two localities: Porto Mauá, where only 1.8 per cent of the monkeys showed plasmodia, and the Manaus-Itacoatiara Road, where the simian malaria infection rate was 15.8 per cent[a]

Species of anopheline	Porto Mauá		Manaus-Itacoatiara Road	
	Ground	Canopy	Ground	Canopy
Anopheles (Kerteszia) neivai	–	–	8	77
Anopheles (Nyssorhynchus) darlingi	–	1	–	–
Anopheles (Nyssorhynchus) arbitarsis	–	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 (Anopheles) mediopunctatus	28	706	2	22
Anopheles (Anopheles) shannoni	4	48	–	9
Anopheles (Anopheles) intermedius	3	19	–	–
Chagasia bonneae	1	3	2	118
Total	313	1054	17	244
Hours spent	33	33	141	141

a: from Deane et al., 1971.

experiment was to know if the monkey parasites would infect humans; and when he caught malaria and I wanted to treat him right away, he said he did not feel too bad, so we should wait to see how that monkey malaria would develop in a human; he would be ready to receive treatment if his conditions worsened, as I would detect through the examination of his blood every six hours. His attitude enabled us to learn that humans can acquire *P. simium* infection through mosquito bite and that it can cure spontaneously within few days (Deane, 1988). So, we know that both plasmodia of Brazilian monkeys are infective for man.

In some parts of Brazil, like the Amazon and the Southeastern and Southern coastal forests, infected monkeys are plentiful and live and wander quite close to human settlements or places where people work in wood chopping, gathering of forest products, mining or fishing. Indian tribes in the Amazon live in close proximity to monkeys carrying their own plasmodia which are infective for humans.

TABLE IX

Anopheline mosquitoes captured in the State of Amapá, Brazil, on baits placed on the ground and on platforms near the forest canopy (1968-1969) in two areas, Serra do Navio and Tracajatuba, where, respectively, 17 per cent and 12.5 per cent of the monkeys harbored plasmodia[a]

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 (Anopheles) mediopunctatus	11	47	51	678
Anopheles (Anopheles) shannoni	–	–	62	603
Anopheles (Anopheles) intermedius	4	–	63	13
Anopheles (Anopheles) minor	–	–	2	–
Total	513	140	1643	4938
Hours spent	101	101	121	121

a: from Deane et al., 1971.

TABLE X

Vertical distribution of some species of Anopheline mosquitoes captured on human baits, simultaneously on the ground and on platforms at 5, 10 and 15 meters above the ground in a forest, Utinga, in the outskirts of Belém, State of Pará[a]

Species of anopheline	Ground	5m	10m	15m
Anopheles (Nyssorhynchus) darlingi	86	31	33	20
Anopheles (Anopheles) peryassui	16	1	2	1
Anopheles (Anopheles) shannoni	13	24	81	211
Anopheles (Anopheles) mediopunctatus	7	12	63	122
Total	122	68	179	354

a: from Deane et al., 1953.

Being so, the chances of human infection of simian origin may depend on the susceptibility and the feeding habits of the mosquito vectors.

There is not much information on the susceptibility of Brazilian species of anophelines to simian plasmodia, although it is known that *A. aquasalis* is susceptible to *P. brasilianum* (Clark & Dunn, 1931), and *A. cruzi* to both *P. brasilianum* and *P. simium* (Deane et al., 1970a; 1971a).

I have no adequate quantitative data comparing the anthropophily versus the simiophily of Brazilian mosquitoes, although several species of Brazilian anophelines do bite humans and monkeys under experimental conditions, and on several ocasions we caught *A. cruzi* feeding on humans and on howler-monkeys. But even if some vectors of human malaria do feed on monkeys experimentally, they may not have many chances of so doing in nature, because of their preference for biting near the ground. Species of anophelines which are strongly acrodendrophilic may be the vectors of simian malaria but not of the human disease, while species that rarely or never go up in the forest may transmit human but not the simian parasites. However, many species of anophe-lines are rather eclectic as to their feeding at different levels and they would obviously be the most plausible vectors of simian plasmodia to man and vice-versa.

In the Southeastern and Southern Brazil *A. cruzi* is the only proven vector of simian malaria. But in those regions south of parallel 25°S all human malaria is also transmitted by the bromeliad-breeding mosquitoes of sub-genus *Kerteszia*, the most widely distributed and abundant being *A. cruzi*.

Concerning the level of the forest where it feeds, *A. cruzi* behaves differently in different places. In the inland plateau forests it is decidedly acrodendrophilic, as we have seen in Cantareira, São Paulo state (Table V) and in such areas malaria occurs only in monkeys, not in man. In the coastal mountains of Santa Catarina state subject to high humidity due to winds from the sea, *A. cruzi* is eclectic, feeding near the ground and at distinct forest levels (Table VI). In Joinville, for instance, both simian and human malarias occur and both are transmitted by *A. cruzi*. In Campo Alegre where *A. cruzi* is acrodendrophilic there was only simian malaria. Some time ago we believed on the possibility of *A. cruzi* being a complex of sibling species, one acrodendrophilic and the other feeding at ground level, the former responsible for the transmission of simian malaria and the latter conveying the human disease. But in an experiment performed in Santa Catarina state, by staining with different coloured fluorescent powders, females of *A. cruzi* caught in the canopy and at ground level, releasing them and recapturing specimens feeding at

TABLE XI

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

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 (Nyssorhynchus) antunesi	13	4	–
Anopheles (Nyssorhynchus) lutzi	46	–	–
Anopheles (Nyssorhynchus) oswaldoi	–	–	5
Anopheles (Nyssorhynchus) strodei	14	–	–
Anopheles (Nyssorhynchus) nuneztovari	–	–	1
Anopheles (Anopheles) midiopunctatus	–	–	17
Anopheles (Anopheles) shannoni	–	–	7
Chagasia fajardoi	4	539	–
Chagasia bonneae	–	–	100
Total	1307(24)	1677(8)	202(1)

a: from Deane et al., 1971.

the opposite level, and searching for stained specimens with ultra-violet light, we could see that this mosquito moves in appreciable numbers from the canopy to the ground and *vice-versa* (Deane et al., 1984). This would suggest that in areas where *A. cruzi* is a vector of human and simian malarias, natural infection of man with monkey plasmodia might be expected. But this has not been confirmed up to the present in Southern Brazil. Before the anti-malaria compaign was established in Santa Catarina state in the 1940s, about 40 thousand cases of human malaria occurred yearly in a population of about 1 million, and the three human plasmodia were present. *P. malariae* was frequent, corresponding to 4.4% of the cases in the state, but reaching up to 55% among the species in one locality, Tijucas (Ferreira & Rachou, 1945). One might suppose that this high rate could be due in part to human infections with *P. brasilianum*. However, with the control compaign, *P. malariae* and *P. falciparum* soon disappeared and *P. vivax* was also later eradicated from the state. As it is not to be expected that the measures used to fight human malaria would affect the simian infections, there is no evidence of zoonotic malaria in the area.

The Amazon would be another region we would suspect to be propitious for zoonotic malaria.

As afore mentioned, the usual local vector of simian malaria has yet to be discovered. The specimens of *A. darlingi* found infected with *P. brasilianum/P. malariae* by Arruda et al., (1989), were caught not in the forest canopy but in indian huts; it is, therefore, more plausible that they had fed on humans, because this anopheline prefers to bite near the ground although, as stated above, it can feed at different fôrest *strata*, including at the canopy (Deane et al., 1953) (Table X).

According to Arruda et al. (1989) patent parasitemia was very low among the indian tribes they examined in Pará, but serological tests detected surprizingly high positivity for antibodies against blood stages of *P. vivax* and *P. falciparum* and as high as 56% of *P. brasilianum/P. malariae*. In two of the tribes, 90% and 100% of the individuals had circulating antibodies against *P. brasilianum/P. malariae* sporozoites. Concerning the simian anti-malarial response, of 13 pet monkeys examined 4 had *P. brasilianum* in blood smears, while antibodies against blood stage of *P. brasilianum* were present in 100% of the pet and in all 28 wild monkeys examined.

These results lead to the conclusion that malaria can be a zoonosis among Amazonian indian tribes.

However, in a recent paper Cochrane et al. (1990), reported that among two of the indian tribes there was a *P. vivax* variant strain identical to the one first observed in Thailand and that some of the variant positive sera reacted with *P. brasilianum* sporozoites and with the *P. brasilianum/P. malariae* CS repeat.

To what extent may such findings shaken the attractive and seemingly justified hypothesis of malaria being a zoonosis among those indians?

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