STUDIES ON TRANSMISSION OF SIMIAN MALARIA AND ON A NATURAL INFECTION OF MAN WITH PLASMODIUM SIMIUM, IN BRAZIL

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For the last two years we have been studying simian malaria in Brazil. A station was established in a forest reservation - Horto Florestal da Cantareira - in the outskirts of the city of São Paulo, State of São Paulo, and observations have also been made in the State of Santa Catarina. Both places are on the coastal forested mountains of Southern Brazil and, in both, howler-monkeys (Alouatta fusca) are numerous, while other primates, like capuchin-monkeys (Cebus apella) and marmosets (Callithrix aurita) are less common. During our studies, malaria parasites of at least two species, Plasmodium simium and P. brasilianum, have been frequently found in A. fusca and never in the other species.

Transmission studies were concentrated in the Horto Florestal station where human malaria has never been detected. Since howlers are arboreal and very rarely come down, platforms were built on trees, 10 and 15 metres above the ground and mosquito captures were regularly made on them. Simultaneous captures were performed at ground level, so that the vertical distribution of mosquito species could give an indication as to the probable vectors. Humans were used as baits for most captures.

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due to the difficulty in obtaining living howlers, but these were employed when available. The mosquitoes caught near the canopy and some of those obtained at ground level were dissected and examined for sporozoites in the salivary glands.

From the start the bromeliad-breeding mosquito Anopheles (Kerteszia) cruzi became a suspected vector for being the only abundant anopheline and the only mosquito to be caught in large numbers in the forest canopy (Deane et al. 1965). From February 1964 to March 1966, at the Horto Florestal, 3887 mosquitoes of more than thirty species were caught: 1156, or almost 30% were Anopheles cruzi, 100 A. lutzii, 8 A. antunesi, 2 A. parvus, 11 A. strodei, 1 A. intermedius and 4 Chagasia fajardoi, the remaining being non-anophelines. In the platform captures the numbers were: 1410 mosquitoes, among which 1146, or 81.9% A. cruzi, 16 A. lutzii, 1 A. strodei, 3 Ch. fajardoi, the rest being non-anophelines.

During the year 1964 and most of 1965, the density of mosquitoes in the station was rather low, due to a severe drought. In the present summer (December 1965 to March 1966), however, rains and mosquitoes became abundant and malaria infection rates in howlers rose to a very high level: the quarterly rates, ranging from 20 to 47% until last December, grew to 82% in the first quarter of this year. We decided, therefore, to intensify the dissection of mosquitoes for sporozoites and, on 19 January 1966 we found the first infected specimen - one A. cruzi caught the previous afternoon on a human bait, 15 metres above the ground. Other infected mosquitoes of the same species were caught on 8, 15 and 24 February and on 11, 15, 23, 25 and 30 March. At the end of March we had 12 sporozoite-carrying A. cruzi out of 698 examined, while 500 mosquitoes of over eighteen other species were negative. From January to March 1966, when all infected specimens were found, we dissected 502 A. cruzi, the infection rate being 2.4% for that period.

Since no howlers were then available at the laboratory, the sporozoites were inoculated in splenectomized squirrel-monkeys Saimiri sciureus, which we had previously demonstrated to be susceptible to the plasmodia of local howlers through blood inoculation (Deane, 1964; Deane & Okumura, 1965).

Mosquitoes caught in the late afternoon or in the first hours of the night were kept in a portable ice-box up to the moment of handling them for dissection, next morning. Under slight etherization they were identified and dissection of the
salivary glands was made on a drop of chilled 10% squirrel-monkey serum in Locke's solution. Microscopical examination followed directly and the glands found positive were suspended in small amounts of chilled serum-Locke up to the moment of inoculation.

One Saimiri sciureus was inoculated into the heart with sporozoites from one *A. cruzi* on 9 February and died of pneumonia eleven days later while still negative to the daily examination of thick blood smears. Another monkey received intra-cardiac inoculation of the pooled sporozoites from 3 specimens of *A. cruzi* on 12 March, and in a third monkey, three consecutive inoculations each with one pair of infected glands were made by the subcutaneous route, on 16, 24 and 26 March. The daily thick blood examination of the second and third monkeys was still negative 46 days after the inoculation of the former and 38 to 42 days following the three inoculations of the latter.

With these results we cannot entirely rule out the possibility that the sporozoites are non-simian. But it is also conceivable that some detail in the manipulation of the parasites for inoculation be responsible for the failure to infect the monkeys. At present we are inclined to believe that the sporozoites found in *A. cruzi* do belong to plasmodia of *Alouatta fusca*, on the basis of the following facts: the sporozoites are long, slender and with pointed tips, resembling those of primate origin and not those of bird plasmodia; human malaria has never been recorded in the area; howler-monkeys show a high rate of infection, while other arboreal mammals, such as capuchin-monkeys, marmosets, sloths, opossums and rodents have been examined always with negative results.

Moreover, the natural infection of a human with *P. simium* in the Horto Florestal during this summer proves that monkey malaria was actually being transmitted there. On the other hand, the facts related to mosquito captures using this man as bait, as discussed below, support our belief that *A. cruzi* is the vector of simian plasmodia in the area.

For the mosquito captures, one of the authors plus one helper acted as baits at ground level, while two other helpers worked on the platforms. None of us took any suppressive antimalarial drug. One of the platform baits, Mr Antonio Cassalho, reported to us on 22 March that he had suffered from headache, chills and fever on the evenings of 19 and 21 March. The first paroxysm was more severe, the
temperature reaching 39.5°C, and in the intervals he felt quite well. We took his
blood and found several plasmodia in the thick and a few in the thin films. The
parasites were contained in enlarged red cells which showed a coarse stippling and
resembled P. simium. In the evening of 23 March a third paroxysm occurred, confirming
the tertian periodicity. We were present then, and able to observe it. The patient
complained of chills and headache and his temperature rose to 38.6°C, remaining high
until about four hours later, when it was followed by sweating. In the same night
we took him to the Institute of Tropical Medicine in São Paulo, where he remained
until 26 March. During this period temperature and blood samples were taken each
day at 0300, 0900, 1500 and 2100 hours, and from 27 March on, once a day. On 24
March he complained of uneasiness under the left costal margin and we found his spleen
to be slightly enlarged, palpable on deep inspiration only; he also had headache and
loss of appetite. His blood was still positive, but showing very few parasites.
On 25 March the expected paroxysm did not materialize and the patient was feeling
quite well and still remains so. His blood has been negative since 2100 hours on
25 March.

On 23 March, during the third paroxysm (with moderate parasitaemia), 15 ml of the
patient's blood were collected and preserved with anticoagulant at 4°C. Another
sample of 10 ml was obtained on the next day, when parasitaemia was very low. The
two samples were pooled and centrifuged and about 10 ml of the sedimented erythrocytes
were injected subcutaneously in a splenectomized squirrel-monkey. Previous to the
inoculation, eight blood examinations of the animal had been negative. Daily
examinations were carried out after the injection and on the 8th day one parasite
was detected in a thick film; from then on all thick films were positive but showed
very few parasites until the 16th day, when parasitaemia started to rise and plasmodia
could be detected also in the thin films and their morphology was seen to be typical of
of P. simium. By the 34th day parasitaemia was still rising and had reached 24 200
per mm³. Since we have previously examined nearly 100 Saimiri sciureus from Brazil
(including 42 after splenectomy) and since P. simium has never been found in the
Amazon Region, where the squirrel-monkeys came from, we may rule out the possibility
of a previously unnoticed infection of the animal inoculated.
The patient was born in the Horto Florestal and to the best of his knowledge never had malaria before. During his present infection he suffered only three paroxysms, followed by spontaneous cure. In his blood the few plasmodia seen in thin smears were of the benign tertian type, but the erythrocytes harbouring some of the more developed forms showed the coarse Schuffner's dots seen in *P. simium*. In a squirrel-monkey inoculated with his blood a high parasitaemia developed and the organisms were typical of *P. simium*, differing from *P. vivax* by the heavy and very precocious stippling of the erythrocytes and the large proportion of grown forms in slightly enlarged or even normal sized red cells, also heavily dotted. Therefore, we are interpreting this as a case of naturally acquired malaria of simian origin in man and, to our knowledge, the second to be reported in the world (Chin et al. 1965).

It is of interest to remark that *Anopheles cruzi* is a proven important vector of human malaria, being the chief (and in some places the only) vector in coastal areas of the Southern States of Paraná, Santa Catarina and Rio Grande do Sul. At the Horto Florestal da Cantareira Station, São Paulo, the density of *A. cruzi* is moderate, while it is high or very high in some areas of the other mentioned States. In the Horto Florestal Station, however, *A. cruzi* usually feeds near the canopy of the forest and rarely at ground level. Of 1,156 specimens of this species, 1,146, or more than 99% were caught on baits at 10 or 15 metres above the ground and less than 1% at ground level. The averages per 10 hours of captures were 72 on the platforms and 0.5 at ground level. This may account for the absence of human malaria in the area, in spite of the high incidence of monkey malaria. In areas of the State of Santa Catarina where both simian and human malaria are prevalent, *A. cruzi*, which is very abundant, feeds both near the canopy and at ground level; in a series of comparative captures we performed there from June 1965 to February 1966, and in which a total of 4,871 *A. cruzi* were obtained, the averages per 10 hours of captures were 398.1 near the canopy and 390.7 at ground level. We would not be surprised if in such areas some of the human infections were of simian origin, since *Plasmodium simium* could easily be diagnosed as *P. vivax* by microscopists unaware of the morphological differences and of the possibility of occurrence of monkey plasmodia in man.
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REFERENCES


RéSUMé

Dans une réserve forestière de la banlieue de São Paulo, où le paludisme simien est fréquent et le paludisme humain inexistant, on a observé un indice sporozoïtique élevé chez Anopheles cruzi. C'est cette espèce qui est probablement l'espèce vectrice du plasmodium trouvé chez les singes locaux, bien que l'on n'ait pu apporter la preuve définitive de cette affirmation par l'infection expérimentale de singes.

Dans la même zone, un homme qui faisait la collecte de moustiques dans la partie haute des arbres a contracté le paludisme à Plasmodium simium. Il a été atteint d'une infection passagère à faible parasitémie, qui a guéri spontanément. Une inoculation de son sang à un singe non impaludé a provoqué chez l'animal une infection typique à Plasmodium simium.
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