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STUDIES ON SIMIAN MALARIA IN BRAZIL[1]

by

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1. Introduction

Recent work on the possibility of transmitting monkey malaria to man has increased the interest in studies on simian plasmodia. Under the auspices of the World Health Organization a research on the subject is being performed now in Brazil.

In this country, two species of plasmodia had been previously found in monkeys: the quartan-like Plasmodium brasilianum, discovered by Gonder & Berenberg-Gossler (1908), in a Cacajao calvus from the upper Amazon (North Brazil) and P. simium, which is closer to P. vivax, and described by Fonseca (1951), from a howler-monkey Alouatta fusca, from the State of São Paulo (South Brazil). P. brasilianum has never been re-encountered in Brazil, but has been found several times in other countries of South and Central America (Clark, 1931; Clark & Dunn, 1931; Dunn & Lambrecht, 1963). In spite of being extensively studied abroad several aspects of its ecology remain unsettled (Taliaferro, 1932; Taliaferro & Taliaferro, 1934; Contacos et al., 1963). Of P. simium, only the morphology of the blood forms was known.

The aims of the present work, started in São Paulo, are to ascertain the prevalence of malarial infection of monkeys and its seasonal variation, the infectivity of simian plasmodia to species of monkeys other than the original hosts and, eventually to man; their vectors and the possibility of their transmission to man, in nature.

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Field work is being performed in a Government forest reservation where howler-monkeys are relatively abundant (Horto Florestal da Cantareira), in the outskirts of the city of São Paulo. It includes the capture and examination of monkeys and the capture of mosquitos on baits placed on the ground and on platforms in the forest canopy, their identification and examination for sporozoites. Since the capture of living howlers proved to be rather difficult (by means of traps or anaesthetizing bullets), most of them have been shot; while moribund their heart blood is obtained for the preparation of thick and thin films and for preservation for future inoculations; pieces of viscera are taken for smears and histological sections. The monkeys captured alive are splenectomized and submitted to daily blood examination and other observations, and used for transmission experiments.

2. Prevalence of infections and behaviour of P. simium in Alouatta fusca

Up to the end of August 1964 a total of 35 Alouatta had been examined. Plasmodia were found in the blood of 12 and three others, although negative for parasites, revealed malarial pigment in the spleen and other viscera - evidence of previous infection. Nearly all the positives harboured P. simium, but in one the parasites were quartan-like and another showed a mixed infection by P. simium and a quartan-type plasmodium; in two the forms were few and inconclusive as to species or type. Five Alouatta were captured alive, all others were shot. Only two of the living specimens were adults, the others being babies, the mothers of which were killed. Both adults caught alive were positive (for P. simium) and were kept in the laboratory for 42 and 60 days respectively. Their blood was examined for a short period two to four times a day and subsequently daily. They originally showed a very light infection (as did nearly all other howlers), but after splenectomy the parasitaemia increased extraordinarily; in one case from six to a peak of more than 225 000 parasites per cubic millimetre of blood on the 24th day. In both parasitaemia decreased after three to four weeks, but in one it rose again soon before death. Fever, anaemia, weight loss, hair loss and diarrhoea were observed. Blood examination revealed schizogeny to be of the tertian type, very regular after the first weeks, and accompanied by high fever (up to 41.5°C). In spleen smears, schizonts were much more numerous than in the peripheral blood.

3. Experimental infection of other monkeys with P. simium

In order to verify the susceptibility of other species of monkeys to P. simium and eventually isolate strains of this parasite, inoculations of blood of heavily infected Alouatta were performed (intraperitoneally and subcutaneously) in capuchin-monkeys (Cebus sp.), rhesus monkeys (Macaca mulatta), squirrel-monkeys (Saimiri sciureus) and marmosets (Callithrix jacchus).

No evidence of infection was obtained in two splenectomized Cebus sp. or in two rhesus (one splenectomized), but results were different with the other species inoculated. One splenectomized adult Saimiri sciureus developed a severe infection, parasitaemia reaching 212 000 per cubic millimetre of blood on the twentieth day, but decreasing thereafter. Its blood was injected in a young, splenectomized specimen of the same species, which also became intensely parasitized and died on the eighth day; and in two splenectomized adults one showed plasmodia in blood smears only from the second to the fourth days after the inoculation; in the other parasitaemia rose to 270 000 per cubic millimetre on the eleventh day, gradually decreasing afterwards. So, of four S. sciureus inoculated, all showed patent parasitaemia; one young specimen died, heavily parasitized; in one adult the parasitaemia was very low and transient, disappearing after the fourth day; in the remaining adults blood parasites reached a very high level but decreased later. Studies are being carried out on the periodicity of schizogony and course of infection in this experimental host.

The marmoset (Callithrix jacchus) has also shown to be susceptible to P. simium: one splenectomized adult specimen inoculated with blood from the first experimentally infected S. sciureus showed patent parasitaemia from the second day until the twenty-second day, always, however, of a low or moderate degree.

Thus two species of primates have been found susceptible to P. simium besides its original host: one, the squirrel-monkey (Saimiri sciureus) might be a convenient laboratory animal for the study of this plasmodium, because it is a common species (in North Brazil), relatively easy to obtain in São Paulo, easy to keep in the laboratory, small (about 500 grams), good-tempered, easy to handle and not very expensive. The marmoset (Callithrix jacchus) is even smaller (about 300 grams), easier to get in

Southern Brazil and much less expensive, but is too restive, more aggressive and difficult to handle. The other monkeys tested - Cebus sp. and rhesus - are much larger, more expensive and difficult to deal with and could not be infected so far. The original host, Alouatta fusca, is difficult to catch alive and usually does not live long in captivity.

4. Search for vectors of P. simium in nature

This part of the study has been hampered by an unusual drought followed by a severe winter, leading to a very low density of mosquitos. Forty-four captures were performed, all on human bait, 28 at ground level and 16 on the platforms in the forest canopy. Most mosquitos caught were sabethines (genera Trichoprosopon, Phoniomyia and Weycomyia) and culicines (Aedes, Culex and few Haemagogus); the few anophelines belonged to species Anopheles (Kerteszia) cruzii, A. (Myzorhynchella) lutzi and A. (Nyssorhynchus) strodei, only the first of which was obtained on the platforms. Dissections for the search of sporozoites have been negative, so far.

5. Summary

In Brazil, two simian plasmodia had been previously reported, each only on one occasion: Plasmodium brasilianum in the Amazon Region and P. simium in São Paulo. The present studies, performed in a forest reservation in the outskirts of the city of São Paulo, revealed a high prevalence of malaria infection among howler-monkeys (Alouatta): in 35 examined, 12 had positive blood smears and three others were negative for plasmodia but showed malarial pigment in the viscera. Nearly all infections were due to vivax-like P. simium but in two the parasites were quartan-like. Schizogony in P. simium was seen to be of tertain periodicity. Splenectomized squirrel-monkeys, Saimiri sciureus, proved to be highly susceptible to P. simium through blood inoculation. A splenectomized marmoset (Callithrix jacchus) was also successfully inoculated, but showed a lighter infection.

The natural vectors of P. simium are being sought for through examination of mosquitos caught on baits at ground level and in the forest canopy.

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RESUME

Au Brésil, on n'avait signalé jusqu'ici que deux plasmodiums simiens qui n'avaient été observés qu'une fois : Plasmodium brasilianum dans la Région de l'Amazone, et P. simium dans l'Etat de São Paulo. Les études relatées ici ont été faites dans une réserve forestière de la périphérie de São Paulo et ont permis de constater que les cas d'infection étaient nombreux chez les singes hurleurs (Alouatta) : sur 35 animaux examinés, 12 donnaient des frottis de sang positifs et trois autres, chez qui on ne trouvait pas de plasmodiums, avaient du pigment paludéen dans les viscères. Presque tous les cas d'infection étaient dus à des P. simium ayant un comportement analogue à celui de P. vivax mais dans deux cas la schizogonie du parasite avait la périodicité quarte. On a constaté que la schizogonie de P. simium présentait la périodicité tierce. Des saïmiris, Saimiri sciureus ayant subi une splénectomie, se sont montrés très sensibles à P. simium lorsqu'on les infectait par inoculation sanguine. On est également parvenu à infecter un ouistiti Callithrix jacchus splénectomisé, mais il a présenté une infection plus bénigne.

On recherche actuellement les vecteurs naturels de P. simium en examinant les moustiques capturés sur des appâts au niveau du sol et dans le dôme de verdure.

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