

a 52296



WHO/Ma.1/332 ✓
31 January 1962

ORIGINAL: ENGLISH

AN ENZOOTIC FOCUS OF SIMIAN MALARIA IN MACACA RADIATA RADIATA
GEOFFROY OF NILGIRIS, MADRAS STATE, INDIA¹

by

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In view of the recently revived interest in simian malaria and its transmissibility to man (Eyles et al., 1960), a malaria survey of monkeys² was carried out in the foot-hills of the Nilgiris. The location for the survey was chosen because the only existing records of natural simian malaria (in brown monkeys) in India was from the Nilgiris (Donovan, 1920; Mulligan and Swaminath, 1940). In 1942, a few monkeys purchased from a dealer in Gudalur showed natural infection with P. inui (Mohan, 1961). Besides, the examination of blood smears from nine monkeys captured in February 1961 by the Pasteur Institute, Coonoor,³ for their virological work showed six positive to malaria. A preliminary report has already been made by the authors (1961) of their finding natural infection in 13 out of 19 monkeys captured in the Kallar area in February 1961. This paper reports the results of a detailed survey of the incidence of simian malaria in the Nilgiri Hills, establishing the presence of an enzootic focus.

¹ The work was mainly financed from the regular budget of the Malaria Institute of India and partly from grants of the Indian Council of Medical Research and the World Health Organization.

² The permission of Shri C. A. R. Bhadrán, I.F.S., Chief Conservator of Forests, Madras State, to capture a limited number of monkeys in the Nilgiris is acknowledged.

³ The authors are indebted to the Director, Pasteur Institute of South India, Coonoor, for his permission to examine the monkeys at the Pasteur Institute.

The identity of the infections (P. inui and P. cynomolgi) mentioned in the preliminary report was tentative. Infected monkey blood from the Nilgiris has since been inoculated into clean M. mulatta monkeys at Delhi. Detailed studies have confirmed the presence of natural infections of P. inui (Halberstädter & Prowazek, 1907) and P. cynomolgi (Mayer, 1907) in Macaca radiata radiata¹ Geoffroy in the Nilgiris area. Description of the morphology of the parasites, their asexual erythrocytic periodicity and immunological status with reference to other strains of P. cynomolgi will form the subject of another paper.

While trapping, it was noted that the younger monkeys in the group were driven away by the group leader. Usually it was the group leader - a fully-grown adult - or the "second in command" that was captured. Shooting monkeys in the Nilgiris is prohibited.

Between February and December 1961, 43.4 per cent. of the monkeys were found positive to infection out of a total of 53 captured in different localities in the Nilgiri mountains (vide map). Table I gives the captures altitude-wise, and the presence or absence of infection in them. Unfortunately the weight of all the monkeys was not recorded and, therefore, it is not possible to classify the infection rate according to weights which could be approximately correlated to the age of the monkeys. Four of the bigger monkeys did not show parasites in the blood till after splenectomy. This indicated that the infections were chronic in them.

In general, the infection in the positive slides was scanty except in those from splenectomized monkeys. Positive monkeys were found at all altitudes of capture between 457 and 1707 metres. To fix an exact altitude beyond which transmission would cease may not be easy, in view of the well-known migratory habits of monkeys.

The predominant patent infection was seen to be P. inui. Sixteen (70 per cent.) out of the 23 positive monkeys showed this infection. Pure P. cynomolgi infection was not encountered in the course of the survey. The remaining seven showed mixed infection. In view of the fact that of the seven monkeys splenectomized five showed mixed infection, the true prevalence of P. cynomolgi infection may have been higher than actually seen.

¹ The authors are indebted to the Director of Zoological Survey of India, Calcutta, for the identification of the monkeys as Macaca radiata radiata.

TABLE I. NUMBER OF MACACA RADIATA RADIATA CAPTURED AT DIFFERENT ALTITUDES IN THE NILGIRIS AND FOUND INFECTED WITH P. CYNOMOLGI AND P. INUI (vide map)

Region	Altitude range above mean sea-level in metres at which monkeys were trapped	Number of monkeys		Number showing		
		trapped and examined	found infected	<u>P. inui</u>	<u>P. cynomolgi</u>	Mixed
Kallar-Coonoor Road	457-1235	19	13 ¹	9	-	4 ¹
Coonoor town	1707	21	3	3	-	-
Mettupalayam-Kotagiri Road	792-924	9	5 ²	3	-	2 ²
Gudalur town	1066	4	2 ³	1	-	1
TOTAL		53	23	16	-	7

¹ Three monkeys were splenectomized. In one the latent infection became mixed patent infection. In the second the scanty mixed infection was confirmed. The third, which was negative prior to splenectomy, showed mixed infection after splenectomy.

² Two of the monkeys showed patent parasitaemia only after splenectomy; one showed P. inui and the other mixed infection.

³ Two monkeys were splenectomized; one showed a patent P. inui infection and the other showed a mixed infection.

In the course of search for the natural vector for the simian malaria, night catches of mosquitos were made around two huts (only two huts exist) in an areca-nut grove in Kallar. It was in this area that many of the positive monkeys (Table I) were captured. A total of 878 female mosquitos were caught between March and December 1961, and the results of dissection of their salivary glands are shown in Table II.

Most of the mosquitos were caught from the vegetation around the huts. None was found at any part of the night in a cage with a monkey at ground level or in another with a rabbit about 20 feet above the ground. With the exception of three specimens of C. vishnui, which showed sporozoites in the salivary glands, all the others were negative. The sporozoites in the salivary glands of C. vishnui were confirmed by staining. The source of the infection, however, could not be identified for want of clean monkeys. The finding merits further investigation in view of Mulligan's (1935) record that, in the course of experimental feeding of mosquitos on monkeys with P. cynomolgi gametocytes, the occurrence of oocysts in the stomach of C. vishnui was encountered on one occasion. This mosquito is not a listed vector of any of the avian malaria parasites (Boyd, 1949).

Female A. fluviatilis and A. stephensi mosquitos obtained from the colonies maintained at the Coonoor Branch of the Institute were experimentally fed on different lots on monkeys which showed good infections, predominantly of P. cynomolgi or a mixed infection of both P. inui and P. cynomolgi. The mosquitos were dissected ten days later. Batches of fed mosquitos were dissected separately for oocysts and sporozoites. The presence of oocysts in many was confirmed. The results in Table III, however, indicate only the proportion of mosquitos that showed sporozoites, which were confirmed by staining. In most of the infected mosquitos the sporozoite infection of the glands was scanty in comparison to the degree of oocyst infection in the mid-gut. Whether this was due to the partial suitability of the vector or the time interval between feeding and dissection can only be determined by further work.

Fifteen specimens of A. stephensi, which had an infective blood meal 13 days previously, were fed on a clean radiata monkey. That the monkey was clean was proved by the fact that its blood was negative to infection after splenectomy. Two of the mosquitos were dissected immediately after engorgement and sporozoites were seen in their salivary glands. That the sporozoites were of both P. cynomolgi and P. inui was shown by the monkey developing a patent erythrocytic infection of both species 11 days after the inoculation.

TABLE II. NUMBER AND SPECIES AND RESULTS OF DISSECTION
OF THE 878 FEMALE MOSQUITOS CAUGHT AROUND THE
TWO HUTS AT KALLAR IN 1961

Mosquito	Number caught and dissected	
<u>A. culicifaciens</u>	1)
<u>A. fluviatilis</u>	4)
<u>A. hyrcanus</u>	6)
<u>A. jeyporiensis</u>	1)
<u>A. maculatus</u>	21)
<u>A. pallidus</u>	1)
<u>A. splendidus</u>	6)
<u>A. subpictus</u>	38) Negative
<u>A. tessalatus</u>	317)
<u>A. vagus</u>	17)
<u>A. varuna</u>	4)
<u>Aedes spp.</u>	36)
<u>Mucidus spp.</u>	1)
<u>Culex gelidus</u>	2)
<u>Culex vishnui</u>	208	Three specimens found infected with sporozoites, presumably of avian origin
<u>Culex spp. (other than <u>gelidus</u> and <u>vishnui</u>)</u>	215	Negative

TABLE III. RESULTS OF DISSECTION OF LABORATORY-BRED MOSQUITOS FED ON MONKEYS SHOWING INFECTION OF P. CYNOMOLGI AND/OR P. INUI

Number of monkeys used for feeding	Spleen: intact/removed	Source of infection	Parasite species	A. stephensi		A. fluviatilis	
				Number dissected	Number showing sporozoites	Number dissected	Number showing sporozoites
1	intact	Infective blood	Mixed ¹ infection	22	6	43	15
1	removed	Sporozoites	Mixed ¹ infection	60	13	6	2
1	removed	Infective blood	<u>P. cynomolgi</u>	16	11	91	12
1	intact	Infective blood	<u>P. cynomolgi</u>	9	3	48	11
TOTALS and percentage				107	32 (31%)	188	40 (24%)

¹ Mixed infection: P. cynomolgi and P. inui.

DISCUSSION

The survey revealed for the first time that a considerable amount of natural simian malaria, at least in one focus, is prevalent in India. It also has recorded for the first time the presence of P. cynomolgi as a natural infection in India. The extent of simian malaria in the country, its natural vector(s), susceptibility of man to the simian infections and related information are being investigated.

Natural infection in the brown monkeys has seldom been recorded from India previously. The general impression was that natural simian malaria was extremely rare in India. This was mostly due to the fact that not a single natural infection has been

encountered in the thousands of Macaca mulatta monkeys that have been utilized for research at the Malaria Institute of India since about 1930, when Sinton and Mulligan commenced research on simian malaria in India. Mulligan & Swaminath (loc. cit.), while recording a natural infection of P. inui in one monkey, stated that they had not encountered any natural malaria infection in the many hundreds of sinicus and rhesus monkeys examined by them previously.

The first record of a natural simian infection in a brown monkey from India was made by Donovan (loc. cit.). He reported negative results of the examination of blood smears from 76 Silenus sinicus (synonyms: Macaca sinicus; Macaca radiata radiata) monkeys shot in some of the valleys of the Nilgiri Hills - the same locality as the present survey. As an addendum to the paper, however, he recorded the finding of a Plasmodium in a blood slide from a sinicus monkey which was sent to him subsequently and that the parasites resembled P. cynomolgi. Sinton & Mulligan (1933) critically reviewed the identity of simian malaria parasites and, from the meagre data in Donovan's publication, placed the parasite he recorded in the P. inui group with which P. cynomolgi was at that time confused.

The second record of a natural infection by Mulligan & Swaminath (loc. cit.) was in a young S. sinicus captured from Kallar, also in the Nilgiris. They identified the parasite as P. inui.

The present survey showed that in the valleys of the Nilgiris simian malaria is enzootic. Out of 53 monkeys captured (Table I) a total of 23 were found to be infected, but in four of them the infection was patent only after splenectomy. The parasite rate, therefore, was 19/53 or 35.4 per cent. The high parasite rate, the scanty parasitaemia in general, and the chronicity of infection revealed by post-splenectomy patency in all the four animals which had negative pre-splenectomy blood smears, showed the enzootic nature of the infection in the valleys of Nilgiris.

Mulligan & Swaminath (loc. cit.) drew attention to the fact that Kallar, the place of origin of the strain of P. inui recorded by them was hyperendemic to human malaria and that the prevalent human infection was P. malariae. The human parasite rate was 51.2 per cent. and 76.7 per cent. of the positive blood smears showed the presence of infection with the human quartan parasite. The chief, or probably the sole vector of human malaria in the Kallar area was A. fluviatilis.

The human malaria situation has completely changed since 1953 in the Kallar area, as elsewhere in India, on account of the National Malaria Eradication Programme. For several years there has been no evidence of human malaria transmission in that area. As stated earlier, between March and December 1961 night catches of mosquitos in Kallar resulted only in the capture of four specimens of A. fluviatilis (Table II). All of them were caught in the vegetation around the two huts. The point of interest, however, was that A. fluviatilis was found to be susceptible to the simian infection (Table III).

The search for the natural vector of the simian malaria has not yielded results so far. The continuation of the work during the year was not possible due to the monsoon in the area and is to be taken up from January 1962 onwards.

The previous impression that natural simian infection is rare in India probably arose due to the fact that Macaca mulatta monkeys utilized for research at the Malaria Institute of India, Delhi, were chiefly captured in "North India" in the Delhi and Lucknow areas. This is confirmed by Schmidt et al. (1961), who reported that from 1945 to July 1960 they had used 5600 M. mulatta monkeys from India for their research and that not one was found to be naturally infected. They found that, out of 228 M. mulatta monkeys imported from East Pakistan ("probably Dacca") in 1960 between October and December, 22 were found to be naturally infected with Plasmodium. The identification of the infection has been tentatively made by them to be P. inui and P. cynomolgi, the former being more prevalent than the latter.

The discovery by Shortt et al. (1961) of a Plasmodium infection in Macaca radiata monkeys captured around the campus of Osmania University, in Hyderabad, reveals an additional focus of simian malaria. They have tentatively designated the infection to be due to Plasmodium osmaniae, a new species.

In view of the above and the results of the present survey, an intensive search in Indian monkeys for natural malaria infection is indicated. Mulligan & Swaminath (loc. cit.) drew attention to the possibility of natural simian malaria foci only in some foot-hill jungles.

From the point of view of Macaca mulatta as the animal of choice in experimental malariology, greater caution has to be exercised in future to detect natural infections, particularly with regard to monkeys obtained from certain localities.

SUMMARY

An enzootic focus of simian malaria in Macaca radiata radiata Geoffroy in the Nilgiri Hills, Madras State, India, is described. The two natural infections found were of P. cynomolgi and P. inui. The former is recorded for the first time in India and the latter confirms earlier records of many years ago.

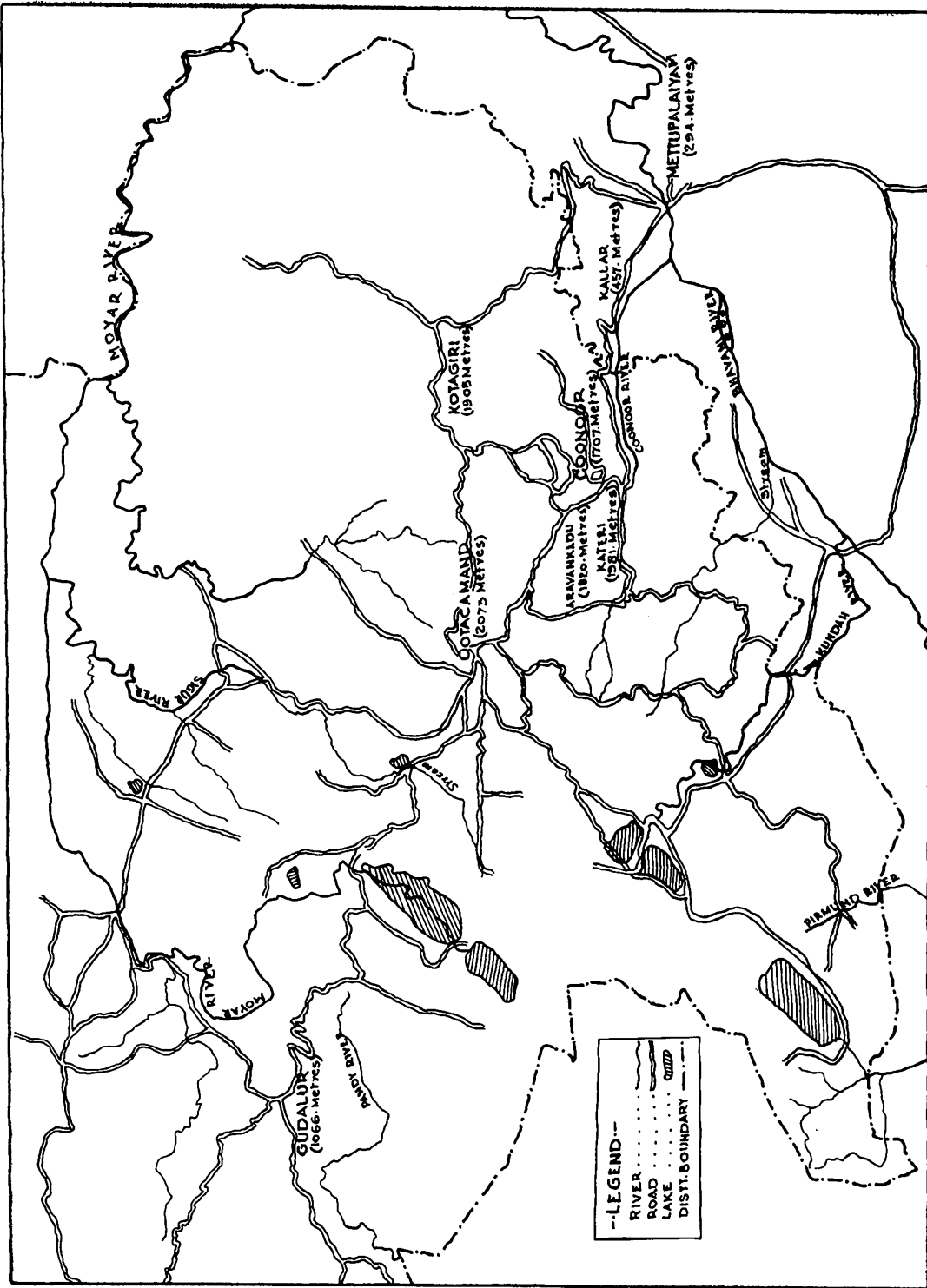
ACKNOWLEDGEMENT

Due credit is acknowledged for the help in the work in the field and laboratory rendered by Sarvashri Cornelius and Puran Singh, Laboratory Technicians, and the two animal attendants, Sarvashri H. M. Giriappa and K. Dorai Raju. Special mention is made of Shri M. A. Aruldoss, the van driver, who enthusiastically participated in the capture of the monkeys.

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Map of Nilgiris area where the simian malaria survey was carried out in 1961



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