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The Establishment of *Anopheles elegans* as the Natural Vector of Simian Malaria in Ceylon*

by

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SUMMARY The toque monkey, *Macaca sinica aurifrons*, of Udawattekelle, a small jungle in Kandy, was found to be infected with malaria parasites. Studies were carried out in this area to determine the vector involved in the transmission of these parasites. It has now been established that *Anopheles elegans*, a member of the *Anopheles leucosphyrus* group, is the mosquito involved in this transmission.

INTRODUCTION

Of the three species of monkeys in Ceylon, two, namely the toque monkey, *Macaca sinica* and the grey langur, *Presbytis entellus thersites*, have been shown to harbour malaria parasites, (Dissanaike, 1965). The parasites in *Macaca sinica* are *Plasmodium shortti*, *P. cynomolgi ceylonensis*, *P. fragile* and *P. simiovale*. *P. shortti* was first isolated in Ceylon by Dissanaike (1963) and described under the name of *P. inui shortti* by Jayawardene (1963). *P. c. ceylonensis*, *P. fragile* and *P. simiovale* were described by Dissanaike, Nelson and Garnham (1965 a and b). A *P. cynomolgi* type parasite has been isolated from the grey langur by Dissanaike (1965) and described by Nelson (1966).

As the mosquito involved in the transmission of these parasites was not known, the present study was undertaken to ascertain the vector of simian malaria in Ceylon.

Area of Study (Fig. 1)

Udawattekelle is a small jungle bordering the city of Kandy in the central hills of Ceylon. It is 257 acres in extent and at an elevation of 1,700 feet above sea level. The vegetation includes tall large trees with intervening dense undergrowth. At the periphery of the jungle there are several human habitations.

Macaca sinica aurifrons is the monkey found in this flora and fauna sanctuary, (Phillips, 1935 ; Eisenberg and McKay, 1970). Troops of these monkeys can be seen in the daytime

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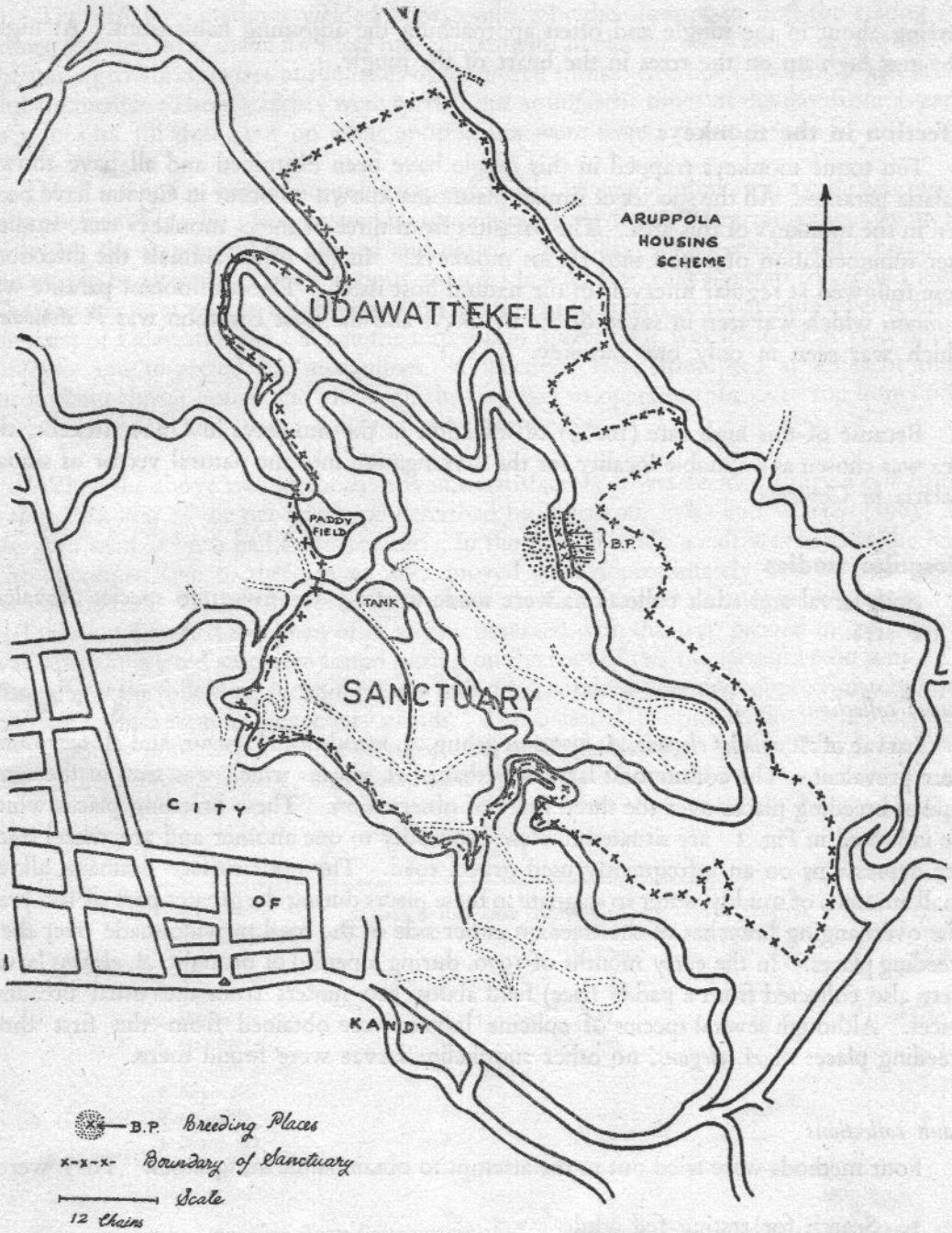

Fig. 1
Area of Study

moving about in the jungle and often approaching the adjoining habitations. At night they rest high up on the trees in the heart of the jungle.

Infection in the monkeys

Ten toque monkeys trapped in this jungle have been examined and all have shown malaria parasites. All the species of simian plasmodia known to occur in Ceylon have been seen in the monkeys of this area. The parasites from three of these monkeys were studied after subinoculation of blood into rhesus monkeys. In the other animals the infections were followed at regular intervals in the natural host itself. The commonest parasite was *P. shortti* which was seen in seven of the monkeys and the least common was *P. simiovale* which was seen in only one monkey.

Because of this high rate (100%) of infection in the monkeys in Udawattekelle, the area was chosen as a suitable locality for the investigation into the natural vector of simian malaria in Ceylon.

Mosquito studies

Both larval and adult collections were made to study the mosquito species prevalent in the area.

Larval collections

Larvae of *Anopheles elegans*, *A. hyrcanus* group, *A. maculatus*, *A. vagus*, and *A. barbirostris* were prevalent. The commonest larva was that of *A. elegans* which was seen in the same type of breeding places over the three years of observation. These breeding places which are indicated in Fig. 1 are situated in close proximity to one another and are wheel track like depressions on an infrequently used gravel road. The poor surface drainage allows small amounts of muddy water to stagnate in these places during the greater part of the year. The overhanging branches of the trees on either side of the road provide shade over these breeding places. In the early months of 1970, during a period of drought, *A. elegans* larvae were also collected from a paddy (rice) field about 500 meters from the usual breeding places. Although several species of culicine larvae were obtained from the first three breeding places of *A. elegans*, no other anopheline larvae were found there.

Adult collections

Four methods were tried out in the attempt to obtain adult mosquitoes. They were :

1. Search for resting fed adults
2. Monkey-baited trap
3. Calf baited net-trap
4. Net-trap over breeding places of *A. elegans* (Plate 1).

The first two methods yielded poor results. In the attempt to find the resting fed adults, searches were made for these mosquitoes with an electric torch and a sucking tube in the undergrowth, crevices at the bases of large tree trunks, tree holes and small pits in the embankments. These searches were carried out at different times of the day from as early as 3.30 a.m. till dusk, but no adult anophelines were seen.

Next a monkey-baited mosquito trap was tried. A single monkey in a cage was placed on a platform 3 feet by 4 feet covered by mosquito netting with a flap arrangement by means of which the trap could be opened and closed. A pulley system enabled the height of the trap to be altered. The collections were made after lowering the trap to ground level. The trap was set at various heights up to a maximum of 10 meters above ground level in different parts of Udawattekelle. When the trap was in operation it was lowered at two hourly intervals and inspected for mosquitoes. Collections were attempted at different times throughout the 24 hours, and although the trap was in operation for over 100 hours only one *A. maculatus* and some culicines were trapped.

When the above two methods proved unsatisfactory it was decided to try a calf-baited trap. This was of the net-trap type described by Wharton, Eyles and Warren (1963) for use with man as both bait and operator. In the present study a calf was used as the bait. The operators, two to three in number moved in at approximately hourly intervals to inspect the trap. This trap was found to attract a fair number of anophelines as shown in Table 1. The first specimen of *A. elegans* obtained with this trap proved interesting. It was a previously fed specimen found resting on the roof of this trap around 8.00 p.m. On dissecting it the following morning it was found to contain sporozoites, approximately 15μm long in the fresh state, in the salivary glands. This material, in normal saline, was inoculated intravenously into a clean rhesus monkey after an initial delay of half an hour. Unfortunately, this monkey failed to show any infection.

TABLE 1

MOSQUITO SPECIES	CALF BAITED TRAP (106 hours)	NET-TRAP OVER BREEDING PLACES (52 hours)	NO. POSITIVE GUT OR GLAND INFECTION
A. vagus	90	—	—
A. elegans	2	81	15
A. maculatus	70	—	—
A. hyrcanus group	62	—	—
A. barbirostris	10	—	—
A. tessellatus	2	—	—
A. jamesi	2	—	—

Seven months later the second specimen of *A. elegans* was obtained with this trap, after the trap had been in operation for a total of 106 hours. On this occasion too the specimen was a previously fed one found resting in the trap around 7.30 p.m. This mosquito on dissection showed no oocysts or sporozoites. It was now felt that the calf bait in no way

attracted the *A. elegans* but that the trap had merely served to intercept these mosquitoes which were in flight soon after dusk. As the other species of anophelines did not show any evidence of malaria infection, it was decided to change the method of trapping.

Net-trap over breeding places of A. elegans (Plate I)

In this method the net-trap used earlier was suspended immediately over one of the breeding places of *A. elegans*. It was hoped that this trap would intercept some of the mosquitoes coming to oviposit. The trap was suspended with the flaps open and the operators moved in every 15 minutes to inspect the trap. Although no other species of anophelines were attracted by this trap, the catches of *A. elegans* by this method were striking. Eighty one specimens of *A. elegans* were obtained by this method over a collection period totalling 52 hours. None of the mosquitoes attempted to feed on any of the operators. Seventy two of these mosquitoes were in various stages of ovarian development, including 24 with mature eggs. The other nine were newly emerged ones. Fourteen of the *A. elegans* showed evidence of malaria infection, four with sporozoites in the salivary glands and ten with oocysts in the midgut. Few discrete dark brown pigment granules were seen in the immature oocysts. The number of oocysts in each midgut varied from 1-30 and the largest oocyst seen was a mature one, 70 μm in diameter, with formed sporozoites in it.

It is difficult to explain what factors attract the *A. elegans* mosquitoes to the breeding place. These mosquitoes which show nocturnal activity, appear to start moving from their daytime resting places at dusk. The resting places are not known but the breeding places seem to be in the path of flight of the mosquitoes from their resting places to other parts of the jungle in pursuit of different activities. A few mosquitoes, however, may have actually sought out the breeding places to oviposit, as in the instance of the mosquitoes with mature eggs.

Sporozoites in serum saline (10% inactivated monkey serum in normal saline) from the salivary glands of one of the infected mosquitoes were inoculated intravenously into a clean rhesus monkey. In fourteen days the monkey showed an infection with *P. shortti* and a week later a mixed infection with *P. shortti* and *P. fragile*, thus establishing that *A. elegans* is a natural vector of simian malaria in Ceylon. A third parasite as yet unidentified was also seen.

DISCUSSION

A. elegans is a member of the *Anopheles leucosphyrus* group (Colless, 1956) and probably the only member of the group occurring in Ceylon. Other members of this group, namely *A. hackeri* (Wharton and Eyles, 1961). *A. leucosphyrus* (Wharton, Eyles, Warren and Moorhouse, 1962) and *A. balabacensis introlatus* (Eyles, Warren, Guinn, Wharton and Ramachandran, 1963) have been incriminated as vectors of simian malaria in Malaya. *A. elegans* itself has been shown to be the vector of simian malaria in the Nilgiris, South India (Choudhury, Wattal and Ramakrishnan, 1963). It is also interesting to note that in our observations in the Upper Hantane area of the University Campus, which is another enzootic focus of monkey malaria, larvae of *A. elegans* have been seen.

There is only one record of malaria in wild-caught Ceylon anophelines (other than *A. culicifacies*, the vector of human malaria) and that is the finding by Carter (1944-45) of immature infections in two mosquitoes of the *A. hyrcanus* group. The source of this infection was not known. In the present study there was no evidence of malaria infection in the 62 mosquitoes of the *A. hyrcanus* group which included four specimens of *A. nigerrimus*.

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