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Studies on Malaria and *Anopheles balabacensis* in Cambodia

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During the past few years Anopheles balabacensis *has come to be recognized as a very important human malaria vector in Thailand and the Indochinese area, but little has been published on its bionomics except from North Borneo.*

Studies of the feeding habits of A. balabacensis *in Cambodia showed it to be predominantly a forest mosquito. It was readily attracted to monkeys in the forest canopy but also readily attacked man on the ground. Very few of this species were attracted to domestic animals. Malaria infections were found more frequently in mosquitos captured in villages, but a significant number were infected from the forest beyond flight range of human habitation.*

The human population showed a high percentage of persons infected with malaria, Plasmodium falciparum *predominating. Cambodian monkeys were found also to be infected with* P. cynomolgi. *Although none of thirteen monkeys injected with sporozoites from wild-caught mosquitos came down with malaria, it was concluded that* A. balabacensis *probably was the vector of both human and monkey malaria and that the risk of cross-infection was considerable if monkey malarias infective to man exist in the area.*

Although *Anopheles balabacensis balabacensis* (as *A. leucosphyrus*) has been incriminated as a vector of malaria in Assam (Clark & Choudhury, 1941) and Burma (Macan, 1948; Kuitert & Hitchcock, 1948), as well as in Borneo (McArthur, 1947 and other papers), it has only been since the initiation of the malaria eradication projects that its real significance has become apparent. In Thailand as recently as 1957 it was stated that "*Anopheles minimus* is the only species known to be capable of maintaining the disease [malaria], though *A. leucosphyrus* (*A. b. balabacensis*) and *A. sundaicus* have shown sporozoites in a limited number of localities" (Ayurakit-Kosol & Griffith, 1962). In the Indo-Chinese countries naturally infected mosquitos had been found on several occasions (see summary in McArthur, 1947) but *A. b. balabacensis* was not universally considered to be an important vector.

As the malaria problem due to *Anopheles minimus* became less acute, the importance of *A. b. balabacensis* became more obvious, and Chow (1961) [3] briefly summarized the current situation as follows:

"*Anopheles balabacensis balabacensis* is a vector of malaria in almost all of the member countries (Burma, Thailand, Malaya, Laos, Cambodia and Viet Nam). This mosquito is prevalent in the rainy season and has highly exophilic habits. It is most active during the second and third quarters of the night."

A. b. balabacensis is a member of the *A. leucosphyrus* group of anophelines; in fact, the older literature refers to *A. b. balabacensis* as *A. leucosphyrus*. Henceforth in this paper it will be termed merely *A. balabacensis*. It has been reported from India (Assam), Burma, Thailand, Cambodia, Laos, Viet Nam, Malaya and northern Borneo. It is known or suspected to be a vector in all the countries except Malaya, where it has been recorded only in the vicinity of the western border with Thailand (Colless, 1956 and 1957).

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[3] Unpublished WHO document WPR/689/61, distributed at Anti-Malaria Co-ordination Board, Phnom, Cambodia, December 1961.

The *A. leucosphyrus* group consists of mosquitos which are found predominantly in the forest. The group has been of particular interest in our study of monkey malaria as we have demonstrated three species to be monkey malaria vectors in Malaya: *A. leucosphyrus*, *A. hackeri* and *A. balabacensis introlatus* (Wharton et al., 1962; Wharton & Eyles, 1961; Eyles et al., 1963). In addition we suspect that *A. pujutensis* is a vector.

As the picture of the importance of *A. balabacensis* emerged, we considered it essential that studies of the bionomics, particularly of the biting habits, of this species be made in an area in which it was an important vector of human malaria, to determine if it had a dual significance as a vector of both simian and human species.

Arrangements were made through Dr Keo Phann, Director of the Malaria Eradication Service of the Ministry of Health in Cambodia, to carry out a study in the Paillin area in October 1962. This paper reports the findings of that study, which was carried out by a team representing the United States Public Health Service, the Institute for Medical Research of the Federation of Malaya, and the Cambodian Malaria Eradication Service.

DESCRIPTION OF THE AREA

The study area was chosen on the basis of findings of the Malaria Eradication Service of Cambodia. It consisted of the village of Pangrolim and the adjoining village of Sur Sdei and surrounding forest. This area is located about 12 km (7½ miles) from the town of Paillin along the road from Battambang (see map, Fig. 1).

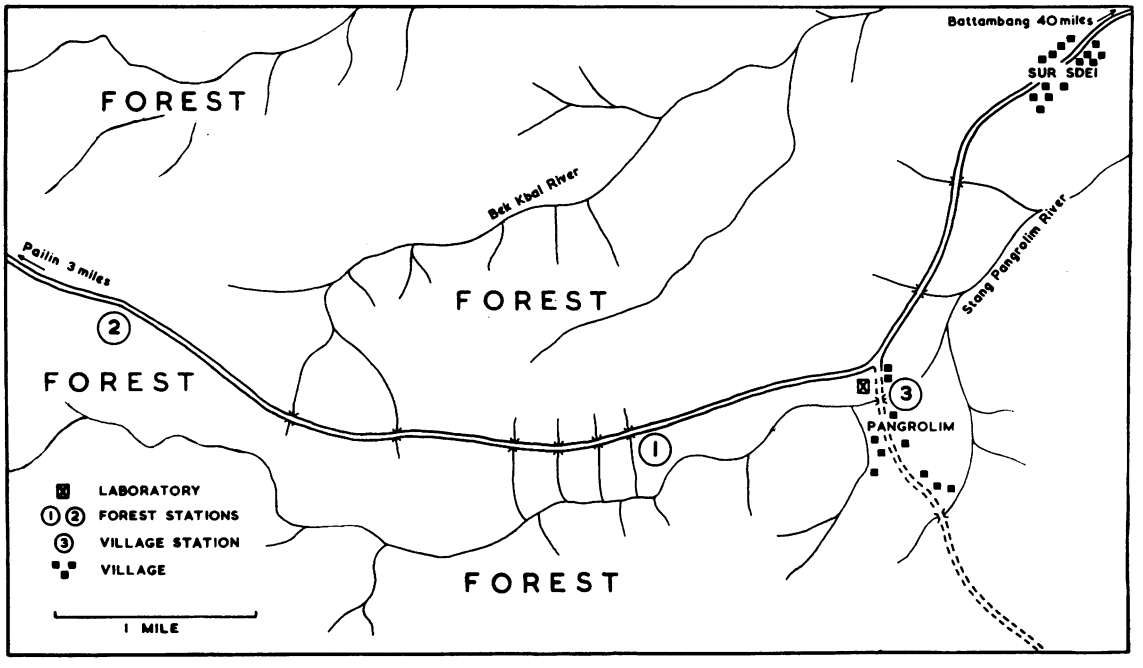
The country is rolling and forested, with a few high hills. The forest consisted of two types, a scrubby, more or less dry, sparsely wooded grassland forest intersected by a denser, more moist forest with high trees and characterized by bamboo thickets. Except for a few areas of upland rice and other crops near the villages, there was no cultivation.

The population of the area consisted of less than 200 persons, all or nearly all Cambodians, living in elevated thatch houses. Cattle, water buffalo and a few pigs were kept by many of the people.

The area had not been sprayed with DDT since about 1958. It was located within the area of the medicated-salt field-research project of WHO; chloroquine was being given in the salt used by the people of the area. Malaria was known to be intense

FIG. 1

STUDY AREA IN CAMBODIA, SHOWING POSITION OF VILLAGES AND CATCHING STATIONS



in the general area and believed to be intense in the specific study zone, as proved to be true. The nearest villages to those in the study area were 9 km (6 miles) or more away, well beyond the flight range of *Anopheles* mosquitos.

In the specific study area, it was our observation that the population did not expose itself outside the houses after dark, and particularly during the peak activity hours of *A. balabacensis*. During numerous trips to trapping sites at all hours of the night, virtually no Cambodians were observed along the roads, and when catching was done in villages, all the people seemed to remain in the houses after dark.

The only animals seen in the area were monkeys (*Macaca irus* subspecies), a gibbon and an occasional rabbit, but wild pig, squirrel, deer, tiger and elephant were reported to occur. In addition to the macaques, a species of leaf monkey was known to occur in the area. Hoof marks of deer and wild pig were frequent.

Climatically, the area is tropical with sharply defined rainy and non-rainy seasons. Rain falls mostly during the period April to October, after which dry conditions prevail. During 1962, mean temperatures ranged from 73°F (23°C) in January to 83°F (29°C) in May.

A laboratory was established in a house provided by the Direction d'Eradication du Paludisme, with electric lights and sleeping accommodation, so that all field work and laboratory work could be conducted without leaving the area, and supervision was continuous and thorough. An animal house for rhesus monkeys (*Macaca mulatta*), which were brought from Malaya, was created in a well-screened tent. Although the period of study was less than three weeks, there were involved in the intensive work six professionally trained scientists, three thoroughly trained laboratory assistants, and eight well-trained collectors.

Reconnaissance of the area began on 6 October 1962. Active collecting was done from the 8th to the 23rd of the same month.

METHODS

A complete laboratory for dissection, blood-smear examination, identification of mosquitos and inoculation of monkeys was established within the study area. This was manned continuously with personnel who had had experience in all phases of malariology and had been engaged in similar ecological studies in Malaya.

For trapping of adult mosquitos the following methods were used:

1. Human-bait net trap (HBT), introduced by Gater in the Philippines and used widely in Malaya for many years (Hodgkin, 1956). These traps consist of nets 10 ft long, 7 ft wide, and 6 ft high (about $3 \times 2 \times 1.8$ m) with openings in two sides (3 ft wide—0.9 m) which had flaps that could be closed by the operators. Two persons slept on cots within the trap protected by small nets. The operators at specified intervals arose, closed the flaps and captured all anophelines within the net.

2. Monkey-bait net traps (MBT) 6½ ft long, 5 ft wide and 4 ft high ($2 \times 1.3 \times 1.2$ m) and similar to the HBT except that the side openings, which were 22 inches wide (55 cm), were not closed with flaps. A wire-mesh cage, accessible to mosquitos, was placed in the centre and contained four Cambodian long-tailed macaques (*Macaca irus* subspecies) as bait (Wharton et al., in press). Cambodian monkeys were obtained from an area about 150 km (93 miles) from the study zone. The MBTs were operated both on the ground and on platforms erected in trees at canopy level. The operators inspected the traps at prescribed intervals and caught all anophelines found resting within.

3. House catches, in which two men worked for three-hour periods throughout the night catching all anophelines found within and outside specific houses, were made on several occasions to obtain mosquitos for dissection. Catches around cattle were made as well, to determine the degree to which anophelines were attracted to them.

4. Bare-leg biting catches, in which operators captured all mosquitos biting throughout the night within and outside a house, were done through two nights. Two teams of three men each were involved, three men inside the house and three outside. One man reclined (and slept, if he wanted to); one man sat and caught mosquitos; and the third man (who had the primary responsibility for the catch) stood and caught any mosquitos biting him or the reclining person. The positions were alternated at hourly intervals. This was a standard procedure used in a study of the biting habits of Malayan mosquitos (Wharton, 1963).

5. Fortuitously it was found that *A. balabacensis* were attracted to a Jeep station wagon parked along a road in the forest, and some catches were made in this way.

6. Catches were made through two nights using a calf-baited net trap similar to the HBT and compared with an HBT operated simultaneously.

Blood surveys were made in both Sur Sdei and Pangrolim in which smears were taken from more than half the population. These were stained immediately so that the smears would not deteriorate. Smears were also made of the twenty Cambodian macaques used for trapping. Even though monkeys were numerous and were seen or reported all about the study area, efforts to shoot them were unsuccessful and no parasitological sample was obtained.

Larval surveys were made on two occasions but were not extensive, as the main purpose of the experiments was to study biting habits.

Nearly all the mosquitos caught by all of the methods were dissected and examined. After identification, the ovaries were removed and dried on a slide to determine whether or not the mosquitos were parous. If nulliparous, the mosquitos were discarded, but both glands and midguts were removed from the parous individuals. These were examined carefully for oocysts and sporozoites. Permanent preparations were made of all positives so that the morphology of the parasites could be studied. Filter-paper preparations were made of some of the blood-fed mosquitos for precipitin test work (to confirm that they had fed on the host to which they had been attracted). Midgut preparations were not examined from the small percentage that was fully blood-fed.

When sporozoites were found they were washed into a watch glass, then drawn into a hypodermic syringe for injection into uninfected rhesus monkeys. Dissections were done in 20% serum in physiological saline to avoid the deleterious effect of simple saline. The rhesus monkeys were examined for presence of infection. One strain of *Plasmodium cynomolgi* was isolated from one of the Cambodian macaques and returned to Malaya for study.

Throughout the study an attempt was made to use the same methods previously used with the established monkey malaria vectors in Malaya, so that accurate comparison might be made between the habits of *A. balabacensis* and the mosquitos studied previously.

COMPARISON OF THE ATTRACTIVENESS OF MAN AND MONKEY TO *A. BALABACENSIS* AND OTHER *ANOPHELES* SPECIES

A series of comparisons was made to determine the relative attraction of mosquitos to man and monkey exposed in different areas, on the ground and, in the case of the monkeys, in the canopy. For these studies the net traps described in the previous section were used.

Collecting stations were selected as follows:

1. Two monkey-bait net traps (MBT) were established about 150 yd (135 m) from habitations in the forest within the village of Pangrolim (see Fig. 1). One was situated on a platform constructed 25 ft (about 7.5 m) above the ground in a tree, and the second on a low platform (to keep the cage with the bait monkeys off the wet ground) at the base of the tree.

2. Similar MBTs were established in dense forest 1.2 miles (2 km) from the village a few hundred yards south of the road to Paillin (Fig. 1). One was situated on a platform 40 ft (about 12 m) above the ground, and the second, as before, at the base of the tree. All the MBTs were baited with cages containing four small (4-5 lb; about 2 kg) *Macaca irus* monkeys of Cambodian origin.

3. A human-bait net trap (HBT) was established within the village of Pangrolim near an occupied house. Bait was furnished by two collectors who rested within the trap.

4. A second HBT was established in the dense forest 1.2 miles (2 km) from the village about 150 yd (135 m) from the tree with the platform MBT.

5. Another HBT was operated for a few nights in dense forest 3.5 miles (about 5.5 km) from the village (Fig. 1), and an MBT on the ground was operated about 20 yd (18 m) away in the same area.

The four MBTs at the village and the 1.2-mile station were operated simultaneously for eight nights from 18.00 (approximately sunset) until midnight, catches being made every two hours. The results of the operation are summarized in Table 1.

Anopheles balabacensis predominated in all the catches using monkey bait. A total of 713 were taken, as compared with 33 of all other species combined, both on the ground and in the canopy. *A. barbirostris* and *A. kochi* were the only other *Anopheles* caught consistently, and their total numbers, 12 and 13 respectively, were small.

TABLE 1

COMPARISON OF THE NUMBERS OF *ANOPHELES* CAUGHT IN MONKEY-BAIT NET TRAPS (MBT) ON THE GROUND AND IN THE CANOPY IN THE VILLAGE OF PANGROLIM AND IN THE FOREST 1.2 MILES FROM THE VILLAGE [a]

Species	Pangrolim village		Forest	
	Ground	Canopy	Ground	Canopy
A. balabacensis	10	70	48	585
A. barbirostris	7	1	1	3
A. jeyporiensis candidiensis	0	1	0	1
A. kochi	4	5	3	1
A. maculatus	0	1	0	2
A. philippinensis	1	0	1	1
Total	22	78	53	593

[a] Collections made from 18.00 to 24.00 for eight nights.

Many more *A. balabacensis* were taken from the forest station than from the village (633 as compared with 80), whereas the other species were caught more frequently in the village. The total number in both places was very large as compared with populations of forest *Anopheles* previously studied in Malaya using the same method (Eyles & Wharton, 1963).

A. balabacensis was caught in significant numbers both on the ground and on the platform, but approximately eleven times as many were caught in the canopy. Thus a large proportion of the population was either in the canopy or was disproportionately attracted to it by the monkey. In comparison slightly more *A. barbirostris* and *A. kochi* were taken on the ground than in the canopy.

The observations based on the MBT captures seemed to indicate, first, that the population of *A. balabacensis* in the forest was much larger (eight times) than that in the segment of forest within the village; and second, that with identical attraction, the bulk of the population appeared to prefer to feed in the canopy. The conclusion was in part confirmed by the behaviour of the other *Anopheles* species, which predominated in the village and seemed to prefer the ground.

For comparison, studies were made with the HBT operated simultaneously in the forest and village. The HBTs at the 1.2-mile forest station and the village station were operated for four nights from 18.00 until midnight, with catches made every two hours. The results are presented in Table 2. Again the number of *A. balabacensis* caught in the forest was much greater than the number caught in the village—nearly five times as many in the forest, as compared with approximately eight times as many with the MBTs. The number of *A. balabacensis* taken (193) was large compared with the total of all other *Anopheles* (15).

TABLE 2

COMPARISON OF THE NUMBERS OF *ANOPHELES* CAUGHT IN HUMAN-BAIT NET TRAPS (HBT) IN THE VILLAGE OF PANGROLIM AND IN THE FOREST 1.2 MILES FROM THE VILLAGE [a]

Species	Pangrolim village	Forest
A. balabacensis	33	160
A. barbirostris	1	4
A. karwari	0	1
A. kochi	4	0
A. maculatus	1	0
A. philippinensis	3	1
Total	42	166

[a] Collections made from 18.00 to 24.00 for four nights.

It appeared, then, that the HBT traps confirmed the conclusion that the *A. balabacensis* population in the forest was much larger than in the village, but the additional information was obtained that man on the ground is highly attractive to this species, which is attracted so readily to monkeys in the canopy.

A further comparison was made at the 1.2-mile forest station, where an HBT was run for the entire night (catches every two hours) simultaneously with the two MBTs (ground and canopy) at the same station. The results are summarized in Table 3.

It is seen that the ground HBT baited with two men attracted nearly as many *A. balabacensis* as the four small monkeys on the platform, and the monkeys on the ground attracted comparatively few mosquitos. Again *Anopheles* spp. other than *balabacensis* were few in number, and the only other finding of slight interest was that *A. kochi* seemed more attracted to monkeys than to man.

This comparison seemed to indicate that *A. balabacensis* is highly attracted to man on the ground and

TABLE 3

COMPARISON OF THE NUMBERS OF *ANOPHELES* CAUGHT IN HUMAN-BAIT NET TRAPS (HBT) WITH THE NUMBER CAUGHT IN MONKEY-BAIT NET TRAPS (MBT) ON THE GROUND AND IN THE CANOPY IN THE FOREST 1.2 MILES FROM THE VILLAGE OF PANGROLIM [a]

Species	HBT	MBT (ground)	MBT (canopy)
A. balabacencis	203	12	295
A. barbirostris	4	0	1
A. jeyporiensis candidiensis	0	0	1
A. kochi	0	2	2
A. karwari	1	0	0
A. maculatus	0	0	1
A. philippinensis	1	1	1
Total	209	15	301

[a] Collections made from 18.00 to 06.00 for four nights.

monkeys in the canopy. Since there is no objective way to compare the attractiveness *per se* of two men (in a large net) versus four monkeys (in a small net), it can only be concluded that *A. balabacensis* is quite prepared to bite either host in large numbers. It also is not clear whether or not competition was involved in the trapping at the forest station. It is believed that it was not, as the canopy catches when the HBT was used were very similar in magnitude to those when the HBT was unoccupied.

As a measure of control, in three instances, at a period of the night when peak activity would be expected, the unoccupied HBT in the forest station was inspected for mosquitos. No *A. balabacensis* appeared to have blundered into the net in spite of the large forest population.

As pointed out below, the net traps measure an attraction to bait rather than biting activity, although there is obviously a relationship. The HBT is so operated that the human subjects are largely protected from the bites of the mosquitos; however, on a number of occasions *A. balabacensis* did manage to enter the protective nets and bite the subjects.

On the other hand, the monkeys were not protected from the mosquitos, unless the chicken-wire mesh had a deterrent effect. A proportion of the mosquitos taken from the MBTs were blooded, this proportion being lower prior to midnight (12%) and higher after midnight (42%). This accords well with the cyclic biting behaviour described in a later section of this paper.

Strangely, there appeared to be a higher proportion of blood-fed mosquitos from the MBTs on the ground (52%) compared with the canopy (15%). The significance of this finding is not understood.

The population of *A. balabacensis* in the forest at 1.2 miles was huge, and it was considered that this was so far from human habitation that there could not be any material influence by man upon the mosquitos. To verify this point and to determine whether populations were similarly large in more remote forest areas, an HBT and one MBT on the ground were operated at a station ecologically similar to the 1.2-mile station but located 3.5 miles from the village (close to the half-way point between Pangrolim and Paillin) for three nights from sunset to dawn.

Because of unconfirmed rumours of dangerous animals in the vicinity, four men went to this station, two remaining in a Jeep station wagon parked on the road and two operating the traps some hundred yards within the forest. Surprisingly, it was found that even more mosquitos were attracted to the two persons in the Jeep than to the net trap, and these were caught in self-defence as there was no protection. Therefore Table 4 includes not only data from the MBT and HBT but also data from the Jeep catches.

As is seen in this table, the *A. balabacensis* population at this remote spot was also very great. No direct comparison could be made, as no other traps were operated on the same night at other stations, but the magnitude of the catches seemed intermediate between those of the 1.2-mile station and the village. The HBT outcaught the MBT on the ground

TABLE 4

COMPARISON OF THE NUMBERS OF *ANOPHELES* CAUGHT IN HUMAN-BAIT NET TRAPS (HBT), A MONKEY-BAIT NET TRAP ON THE GROUND (MBT) AND IN A JEEP STATION WAGON IN THE FOREST 3.5 MILES FROM THE VILLAGE OF PANGROLIM [a]

Species	HBT	MBT	Jeep
A. balabacensis	49	9	125
A. barbirostris	1	0	0
Total	50	9	125

[a] Collections made from 18.00 to 06.00 for three nights.

as at the 1.2-mile station, and the only *Anopheles* sp. caught other than *A. balabacensis* was a single *A. barbirostris*. The factors involved in the large catch in the Jeep are not understood, but the location of the vehicle in the open and perhaps heat or some chemical stimuli may have had an influence.

STUDIES OF CYCLIC BEHAVIOUR OF *A. BALABACENSIS*

Previous studies in Cambodia, for the most part not published, had indicated that *A. balabacensis* entered houses and bit man. The consensus was that the major activity was rather late in the evening. To verify these findings, cyclical studies were made in which biting catches were made inside and outside a house in the village of Sur Sdei at two-hour intervals, and these findings were compared with data from the net-trap catches tabulated at two-hour intervals.

The biting-catch procedure was carried out throughout the night for two nights as described in the methods section. The house used was a representative Cambodian dwelling. The outside catch was made in the open air near the house.

Table 5 summarizes briefly the total biting catches for the two nights. As in the net collections summarized in the previous section, *A. balabacensis* predominated, 35 individuals being caught as opposed to 12 of all other species combined. About twice as many mosquitos were caught outside the house as inside, and the variety of mosquitos caught outside was much greater. The number of *A. balabacensis* caught were of the same order of magnitude as those caught in the village HBT (average of 8 per night with catches terminated at midnight).

TABLE 5

SUMMARY OF ALL *ANOPHELES* CAUGHT DURING BITING-CYCLE CATCHES INSIDE AND OUTSIDE HOUSE AT THE VILLAGE OF SUR SDEI [a]

Species	Inside of house	Outside of house
A. aconitus	2	2
A. annularia	0	1
A. balabacensis	13	22
A. barbirostris	0	2
A. jeyporiensis candidiensis	0	1
A. peditaeniatus	0	1
A. philippinensis	0	2
A. tessellatus	0	1
Total	15	32

[a] Catches made from 18.00 to 06.00 for two nights.

Table 6 presents the biting-cycle data at two-hour intervals and compares the catches with the catches of *A. balabacensis* attracted to the MBTs and the

TABLE 6

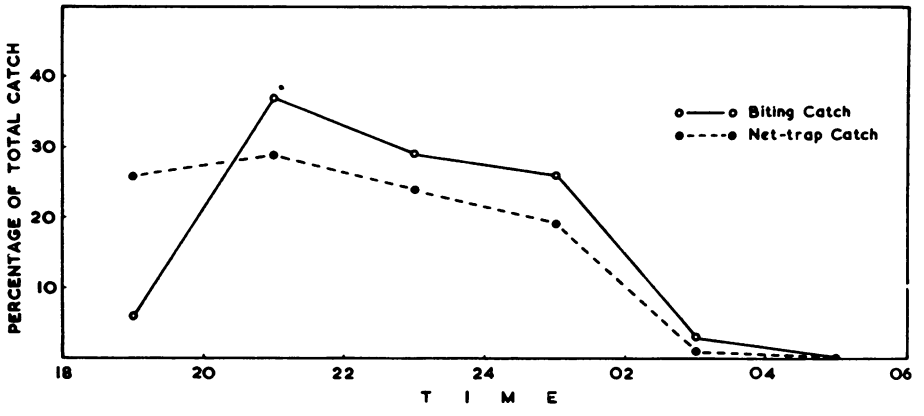
COLLECTIONS OF *ANOPHELES BALABACENSIS* RELATED TO HOUR FROM BITING CATCHES INSIDE AND OUTSIDE HOUSE AT SUR SDEI AND FROM HUMAN-BAIT NET TRAPS AND MONKEY-BAIT NET TRAPS IN THE FOREST 1.2 MILES FROM THE VILLAGE OF PANGROLIM [a]

Hour	Biting catches (Sur Sdei)			Forest net-trap catches			
	Inside house	Outside house	Total	HBT	MBT (ground)	MBT (canopy)	Total
18.00-20.00	0	2	2	53	4	67	124
20.00-22.00	6	7	13	59	1	69	129
22.00-24.00	5	5	10	48	4	61	113
24.00-02.00	2	7	9	39	1	48	88
02.00-04.00	0	1	1	3	2	49	54
04.00-06.00	0	0	0	1	0	1	2
Whole night	13	22	35	203	12	295	510

[a] Collections made every two hours from 18.00 to 06.00.

FIG. 2

COMPARISON OF BITING CATCHES AND HUMAN-BAIT NET-TRAP CATCHES RELATED TO TIME OF CAPTURE



HBT in the forest. Fig. 2 summarizes the information and shows graphically the percentage of mosquitos biting or attracted through the night. There was very little biting activity during the first two hours after sunset. Activity was maximal from 20.00 until 02.00, then fell to a very low level. There was a distinct indication that biting started earlier and continued later outside the house, but the numbers were not sufficiently large to be certain.

When the biting catches are compared with the net catches, an interesting contrast is seen. The catches from the nets during the two hours immediately after sunset were nearly as large as any 2-hour catch from 20.000 to 02.00. This may be explained on the basis that the nets measure attraction and not biting activity. It is likely that mosquitos attracted to the vicinity of the bait may remain there for some time before biting. Similar findings have been made with some of the Malayan *Anopheles*.

Significant numbers of *A. balabacensis*, both fed and unfed, continued to be captured from the monkey-baited trap in the canopy from 02.00 to 04.00, after human biting activity and activity around human bait had practically ceased. The factors responsible for this persisting activity in the canopy are not understood.

ATTRACTION OF *A. BALABACENCIS* TO NON-PRIMATES

Previous observers (unpublished work) had asserted that in Cambodia *A. balabacensis* was primarily anthropophilic, and our results, while showing that it bites monkeys as readily as it does man or more readily, also showed a strong inclination to bite man. It was considered of interest to determine to what extent this species was attracted to domestic animals. particularly bovine species.

Catches were made outside in the vicinity of animals. No *A. balabacensis* were seen near roosting chickens (in contrast to Malaya, where it is quite easy to find *A. letifer* around chickens). Surprisingly, a number of *A. balabacensis* were seen or caught resting on grass or on a fence near an area in which both water buffalo and cattle were kept. Some of these mosquitos were blood-fed.

Because of this observation, which was unexpected, an experiment was conducted in which an HBT was operated simultaneously with an identical net trap in which a calf was tethered. The results of the study, which was carried out for three nights, are summarized in Table 7.

It is seen that, as in other trials, the HBT attracted *A. balabacensis* primarily, only a few individuals of other species being caught. A great variety of *Anopheles* were attracted to the calf, including many *A. barbirostris*, *A. kochi*, *A. philippinensis*, *A. tesselatus*, and even *A. maculatus*. Despite this preponderance of zoophilic *Anopheles*, 12 *A. balabacensis* were caught as compared with 64 in the HBT, an attraction ratio, as defined by Reid (1961), of 5.3 : 1 in favour of man. This indicates some slight measure of deviation of this species to cattle in the area.

Attention should be drawn to the results with *A. maculatus*. Fifteen were caught on the calf bait and none on human bait. In Malaya extensive experience has shown that this species is attracted to

TABLE 7
COMPARISON OF THE NUMBERS OF *ANOPHELES* CAUGHT IN IDENTICAL CALF-BAIT NET TRAP (CBT) AND HUMAN-BAIT NET TRAP (HBT) OPERATED SIMULTANEOUSLY IN VILLAGE OF PANGROLIM [a]

Species	HBT	CBT
A. annularis	0	1
A. argyropus	0	1
A. balabacensis	64	12
A. barbirostris	0	152
A. karwari	0	2
A. kochi	1	191
A. maculatus	0	15
A. peditaeniatus	0	1
A. philippinensis	3	46
A. tesselatus	0	31
A. vagus	0	6
Total	68	458

[a] Collections made from 18.00 to 24.00 for three nights.

cattle more than to man (ratio of 1.3 : 1 in favour of calf), but that a significant proportion of the *A. maculatus* population feeds on man even in the presence of cattle. It seems likely that *A. maculatus* is more zoophilic in Cambodia than in Malaya, and that differences in the feeding preferences may help to explain why it is a vector in some areas and not in others.

RESULTS OF MOSQUITO DISSECTIONS

Except for the zoophilic mosquitos caught from animal bait, virtually all the anophelines caught were dissected and parous individuals were examined for the presence of oocysts and sporozoites. Table 8 summarizes the dissection results by species.

Except for *A. barbirostris*, of which there were 45, only small numbers of mosquitos other than *A. balabacensis* were dissected. None (including *A. barbirostris*) were found infected; whereas the infection rate in *A. balabacensis* was quite high. Over all, 3.0% of 1218 dissected were infected, and 1.9% of the mosquitos were carrying sporozoites. All the sporozoite infections were heavy (several hundred or more sporozoites observed), and all appeared to be similar to those of the primate malarias (slender and fairly long, compared with the short, fat sporozoites of *Plasmodium traguli*); however, we are ignorant of the morphology of the sporozoites of some of the animal malarias.

Significant numbers of *A. balabacensis* were dissected from the stations at the villages and the 1.2-mile and 3.5-mile stations in the forest. Table 9 summarizes the results when the mosquitos are grouped by station. The infection rate from the villages was extremely high. From Sur Sdei over 9% of the mosquitos were infected, and from Pangrolim

TABLE 8
SUMMARY OF MOSQUITO DISSECTIONS BY SPECIES

Species	Number dissected	% parous	Number infected		% infected	
			Glands	Total	Sporozoites	Total
A. balabacensis	1 218	63	23	36	1.9	3.0
A. barbirostris	45	60	0	0	0	0
A. jeyporiensis candidiensis	2	0	0	0	0	0
A. karwari	6	33	0	0	0	0
A. kochi	15	40	0	0	0	0
A. maculatus	11	52	0	0	0	0
A. philippinensis	4	50	0	0	0	0
A. peditaeniatus	1	0	0	0	0	0
A. tessellatus	1	100	0	0	0	0

TABLE 9

SUMMARY OF DISSECTIONS OF *ANOPHELES BALABACENSIS* RELATED TO CAPTURE STATIONS

Capture station	Number dissected	% parous	Number infected		% infected	
			Gland	Total	Sporozoites	Total
Village:						
Sur Sdei	131	66	10	12	7.6	9.2
Pangrolim	207	62	5	9	2.4	4.3
Both	338	64	15	21	4.4	6.2
Forest:						
1.2 miles	699	53	7	11	1.0	1.6
3.5 miles	181	69	1	4	0.6	2.2
Both	880	56	8	15	0.9	1.7

over 4%. Totalling the village data, which appeared logical since the villages adjoin, gave a figure of 6.2% of 338 mosquitos dissected. In contrast, the infection rate of mosquitos from the forest stations was much lower. From the 1.2-mile station 1.6% were infected, and from the 3.5-mile station, 2.2%. Combining these, 1.7% of a total of 880 mosquitos had parasites. When the total samples from the two stations were compared statistically (using chi-square test with correction for small samples) the difference of 4.5% was found to be highly significant (*P* less than 0.0002).

Two hypotheses were immediately forthcoming to explain this significant difference: (1) that the infections might be human, and there would be likely to be fewer far from the habitations; (2) that the infections were of animal, probably lower primate, origin. The second hypothesis would seem the more logical, as infection rates were similar in the close and the remote forest stations. Also, even the 1.2-mile station was beyond what is generally considered to be the effective flight range of most *Anopheles*. Finally, no night traffic was seen on the road to explain human infection at these distances from the village.

The mosquito-dissection data were also analysed to see if there was any relationship to the time of night when the mosquitos were captured. Some infected mosquitos were caught at all hours, and there appeared to be no temporal relationship to which any significance could be attributed. Similarly, the proportions of parous mosquitos seemed to be independent of the time captured.

RESULTS OF MONKEY INOCULATIONS

As stated earlier, 20% human serum in physiological saline was used for all dissections. Whenever sporozoite infections were found the salivary glands were washed off the slides, picked up in a 2-ml disposable plastic hypodermic syringe with 25-gauge needle and injected intravenously into an uninfected Indian rhesus monkey.

All available monkeys were inoculated, thirteen in all. Six were injected with sporozoites from village mosquitos, six with sporozoites from the 1.2-mile station and one with sporozoites from the 3.5-mile station.

None of the rhesus monkeys developed malaria infection. This was very surprising in view of the previous work on the *A. leucosphyrus* group (Wharton et al., 1962; Wharton & Eyles, 1961; Eyles et al., 1963), as monkey malarias had been isolated previously from *A. leucosphyrus*, *A. hackeri* and *A. balabacensis introlatus* in Malaya. This would indicate either that our reasoning was wrong and that the forest infections were in fact human, or that technical error produced a misleading result, or that the sporozoites came from some other forest-dwelling animal, probably a canopy-dwelling animal. Only additional work can give a certain answer to this question.

Perhaps related to this finding is the failure to produce malaria in either rhesus monkey or man in two instances in which sporozoites from *A. balabacensis introlatus* in Malaya were injected into both primate species (Eyles et al., 1963). In the Malayan

area and the Cambodian area as well, there are undoubtedly other animals with malaria. For instance, we have recently found a gibbon malaria in Malaya as well as a new species in the arboreal, jungle-dwelling non-primate, *Cynocephalus variegatus* (Eyles et al., 1962; Dunn et al., 1963). We have also found malarias in leaf monkeys (langurs) in Malaya that are only with difficulty transmissible to rhesus monkeys. Gibbons and leaf monkeys are present in the Cambodian area, and it lies within the range of *Cynocephalus*. Furthermore, there are some jungle animals which may harbour species not yet known to science, and there is also the mouse-deer species *Plasmodium traguli* (which produces sporozoites morphologically different from those of the primate species). It is hoped that a sequel investigation to this first study may provide an answer to this problem.

MALARIA INFECTION IN PRIMATES OF CAMBODIA

Although monkeys were observed frequently in the study area, none could be obtained for study except for one pet animal which was found to be without patent infection. A sample of twenty Cambodian monkeys, representative of a subspecies of *Macaca irus*, was obtained from a region about 150 km from the study area and probably similar ecologically. Six of the monkeys were infected, all with what appeared to be *Plasmodium cynomolgi*. One strain was isolated in a rhesus monkey and returned to the laboratory in Malaya for study. Morphologically it was similar to *P. cynomolgi*. It produced severe infections in the rhesus monkeys, more severe than the laboratory strains of this species previously studied. Gametocytes were produced readily, and a few *A. balabacensis introlatus* of Malayan origin were fed on the infection. Even though the numbers of mosquitos were small, it was determined that *A. balabacensis introlatus* was susceptible to this strain.

More than half the total population of the associated villages of Sur Sdei and Pangrolim allowed us to take blood smears, and the results of the examination are tabulated in Table 10. In spite of the fact that the villages are located in the area of the chloroquinized-salt field project, more than half the smears showed infection. More than 20% carried *P. falciparum* and 15% *P. malariae*, this being perhaps a measure of the very large amount of malaria in the people. Many of the parasite counts were quite high, but not many of the *falciparum* carriers had large numbers of crescents. *Plasmodium vivax* was not very prevalent.

The smears were made at night, after 20.00, and an examination was made for microfilariae. The sample taken was 20 cm. Of the smears from Sur Sdei, 9%, and 2% of those from Pangrolim, showed *Wuchereria bancroftii*. No elephantiasis was seen.

Upon returning to Malaya, three of the seven persons participating in this investigation developed *Plasmodium falciparum* infection. Since the total exposure was 68 man-days, this represents an annual attack rate of 16 000 per 1000 population. Nets had been used for sleeping, but the collection activities had exposed the personnel freely to *A. balabacensis*.

The attacks developed after prophylactic chloroquine was discontinued, but there was question as to

TABLE 10

RESULTS OF SURVEY OF HUMAN MALARIA MADE IN VILLAGES OF SUR SDEI AND PANGROLIM, OCTOBER 1962[a]

Number examined	Number infected	% infected	Species				
			falciparum	*vivax*	*malariae*	Unidentified	Mixed
Sur Sdei:							
44	20	45.5	7	3	8	2	0
Pangrolim:							
45	30	66.7	14	1	7	7	1
Total:							
99	50	50.5	21	4	15	9	1

[a] Twenty cm of blood were taken for thick films. Surveys were made at night (after 20.00).

whether an adequate amount to prevent infection had been taken. One of the three patients relapsed twice about twelve days after intensive chloroquine therapy (1.8 g and 1.5 g base). Another patient relapsed after treatment with Darachlor (pyrimethamine and chloroquine), and the third patient has not relapsed.

Blood levels on the first patient taken at the time of relapse showed 50 μg per litre, indicating definite chloroquine resistance. The fact that the area was one in which chloroquine salt is administered may not be pertinent, as resistance is being observed currently in a number of other areas in near-by countries where chloroquine salt is not used.

DISCUSSION AND CONCLUSIONS

Although the question of *A. balabacensis* as a malaria vector has been widely discussed, there has been relatively little published on the habits of this mosquito, except in Borneo. Although more is known of the Bornean form, it may differ biologically and even morphologically from the Indo-Chinese form. One distinction was immediately apparent. Large numbers of *A. balabacensis* entered our net traps, but McArthur (1947) stated that the net-trap method was completely unsuccessful in North Borneo.

The consensus had been that *A. balabacensis* was primarily anthropophilic in Cambodia, judging by the rarity of the mosquito in catches from cattle and the frequency with which it was caught in houses and biting man. Our investigations confirmed the fact that it bites man readily, but showed very clearly that it also feeds readily on monkeys. The fact that the populations of *A. balabacensis* were so large in the remote forest would indicate that lower primates (and perhaps other canopy animals) are the natural hosts but that this species is able to adapt itself and modify its habits in the presence of man.

The behaviour of Cambodian *A. balabacensis* is very similar to that of members of the *Anopheles leucosphyrus* group which have been studied in Malaya. Table 11 presents data on several species, and it is clear that all members of the group studied so far feed readily on monkeys in the canopy but vary in the degree to which they are prepared to feed on man on the ground. The habits vary from the extremes of *A. pujutensis*, which has not yet been caught attracted to man on the ground, and *A. hackeri*, which predominates in the canopy, to *A. leucosphyrus* and the two subspecies of *A. balabacensis*, all of which are readily attracted to human bait on the ground or monkey bait in the canopy. Table 11, in addition to ratios, also shows actual numbers, which give an indication of the very large mosquito population in our study area, as the Cambodian data were derived from only three nights' trapping, whereas the other data are based on trapping for fifty or more nights.

TABLE 11

COMPARATIVE ATTRACTIVENESS OF MAN AND MONKEYS TO MOSQUITOS OF THE *ANOPHELES LEUCOSPHYRUS* GROUP (*A. MACULATUS* AND *A. SUNDAICUS* ADDED FOR COMPARISON)

Species	Proportion of mosquitos caught [a]		
	HBT	MBT (ground)	MBT (canopy)
A. b. balabacensis (this study)	100 (209)	7 (15)	144 (301)
A. balabacensis introlatus (Malaya)	100 (6)	33 (2)	50 (3)
A. leucosphyrus	100 (10)	60 (6)	210 (21)
A. hackeri	100 (11)	91 (10)	500 (65)
A. pujutensis	nil in HBT—predominantly in canopy		
A. maculatus	100 (1382)	22 (300)	4 (49)
A. sundaicus	100 (220)	5 (10)	2 (4)

[a] Index number of 100 arbitrarily set for HBT catch and catches of MBTs adjusted proportionally; actual catches given in parentheses after indices.

As a basis for comparison similar data are given for *A. maculatus* and *A. sundaicus*, which do not often enter the canopy and probably do not transmit monkey malaria.

As more and more is learned of the habits of the various members of the *A. leucosphyrus* group, it becomes evident that they are by and large feeders on primates. They vary in the extent to which they bite man as compared with monkeys, but it is unlikely that other animals are bitten frequently except possibly for some of the other small canopy mammals.

The observations using the calf as compared with man indicate that *A. balabacensis* is not greatly attracted to the bovine species, and this is confirmed by its relative scarcity in catches made around cattle. The behaviour of *A. balabacensis* in Borneo is stated by McArthur (1947) to be similar, but the quantitative approach using uniform bait traps should be attempted there and elsewhere.

The differences in infection rates in the forest mosquitos as compared with those caught in the village were discussed earlier, as well as the inexplicable finding that none of these sporozoites infected rhesus monkeys. In spite of these contradictory observations, it would seem that the general knowledge of the *A. leucosphyrus* group may be applied; that is, that these forest mosquitos are frequently the vectors of simian malaria and it is quite likely that the Cambodian representative, *A. b. balabacensis*, is the vector there.

On the other hand, it is equally clear that *A. balabacensis* is serving as a very efficient vector of human malaria in the same area. The village sporozoite rates, although matched by previous dissections of the same species in Thailand, Burma and India (previously cited), are among the highest reported for Asia for any species (except for small samples from isolated situations), and the intense malaria in the human population is evidence that the majority of these infections were human rather than simian. Even so, application of the sporozoite rates in statistical procedures must be qualified because of the possibility that not all the infections were human.

Another indication of the study in Cambodia is that there must be a very intimate monkey-and-man contact via mosquitos in this area. Monkeys are common, and the mosquitos bite monkey and man almost equally avidly. If simian malaria is transmitted to man in nature, it is likely that the Cambodian situation is the type of situation in which this would be most likely to occur. The same might apply also to the adjacent countries in which *A. balabacensis* is important. Clearly, follow-up studies are necessary and perhaps might be done most effectively in an ecologically similar area in which eradication procedures (residual insecticides) are being applied.

Prior to such a study it would be well to determine whether the Cambodian *Plasmodium cynomolgi* is in fact transmissible to man experimentally. A strain has been isolated, and it is hoped that this can be tested in the near future; however, up to the present all three strains of *P. cynomolgi* tried in man have been infective (*P. c. bastianellii*, the Mulligan strain of *P. cynomolgi* and a newly isolated strain from the pig-tailed macaque).

Quite aside from the simian malaria question, it is important to interpret the results of the present study from the point of view of human malaria eradication or control. It is unlikely that residual insecticides will have any perceptible effect on the tremendous *A. balabacensis* population. In contrast to *A. minimus* (the other important human malaria vector in the Cambodian area), the population as a whole will scarcely be touched by insecticides, as it is largely in the forest and there will probably always be a " spill-over " of mosquitos into the villages. Theoretically this should provide no obstacle, as the insecticide should break the chain of transmission, but with the exophilic species it will certainly be necessary to maintain extremely high standards of application. Likewise, data are needed on the entering of houses by *A. balabacensis* to determine whether they rest sufficiently long to be exposed to a lethal dose of insecticide. In our experience in the area, blood-fed *A. balabacensis* were often found under the low overhang of the thatch roofs outside the houses and were obviously leaving having satisfied their need for blood. How long they had exposed themselves within the house was not determined.

The extreme seasonal variation in rainfall in Cambodia and the adjacent areas poses another problem. The villages are most accessible for spraying during the dry season; whereas the peak of transmission is probably towards the end of the wet season. Therefore the maximal insecticidal effect is probably present when transmission is minimal. Some of the villages are practically impossible to reach by surface transportation during the rains, so the logistic phase of the work presents a real problem for those responsible for the application of anti-malaria measures.

From our view it would appear that a limited-scale but very intensive controlled pilot experiment in a carefully selected *A. balabacensis* area should be conducted independently of the regular antimalaria programme. Parasitological and entomological studies should be thoroughly integrated and on-the-spot supervision provided continuously by experienced personnel. It is our opinion that only in this way can adequate information be obtained on methods whereby malaria transmitted by this dangerous vector can be controlled.

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RÉSUMÉ

Ces dernières années, *Anopheles balabacensis* a été reconnu comme un vecteur très important du paludisme humain en Thaïlande et dans l'ensemble de l'Indochine, mais, si l'on excepte le Bornéo septentrional, peu de publications ont été consacrées à sa bionomie. Il est maintenant évident qu'il est difficile d'appliquer à cette espèce les méthodes conventionnelles d'éradication du paludisme; il est essentiel d'entreprendre des recherches sur son mode de vie.

La présente étude a été effectuée peu de temps avant la fin de la saison des pluies près de Paillin, au Cambodge, par une équipe de chercheurs représentant le service de la santé publique des Etats-Unis, le service cambodgien d'éradication du paludisme et l'Institut pour la Recherche médicale de la Fédération de Malaisie. La densité des moustiques et la transmission du paludisme sont probablement à leur acmé à cette période de l'année.

Les études portant sur les habitudes alimentaires de *A. balabacensis* montrent que les singes attirent davantage cette espèce dans la haute futaie qu'au sol. Elles montrent aussi que les populations anophéliennes de la forêt sont beaucoup plus grandes que celles des villages. Aucun moustique autre que *A. balabacensis* n'est attiré par les singes en nombre appréciable.

L'on a également trouvé que *A. balabacensis* vient facilement au sol et que deux hommes au sol attirent sensiblement autant ces anophèles que quatre petits singes dans la haute futaie. Des études du cycle des piqûres montrent que l'homme est surtout piqué entre 20 h. et 2 h. du matin. Un piège contenant un veau comme appât a attiré environ cinq fois moins de *A. balabacensis* qu'un piège identique avec l'homme comme appât.

L'on est parvenu à la conclusion que le piège à filet avec appât humain, tel qu'il est utilisé en Malaisie, est très efficace pour la capture de *A. balabacensis*, contrairement aux conclusions auxquelles sont parvenus les chercheurs au Bornéo septentrional.

En tout, 1218 *A. balabacensis* ont été disséqués; 3% d'entre eux étaient infectés (indice sporozoïtique: 1,9%). Les moustiques provenant des villages étaient infectés dans la proportion de 6,2% alors que ceux vivant dans les forêts situées en dehors de l'aire de vol des moustiques des villages n'étaient infectés qu'à 1,7 %. L'on en a déduit que les infections de forêt pourraient bien représenter une infection palustre simienne, mais 13 singes inoculés avec des sporozoïtes prélevés sur les moustiques capturés dans la forêt n'ont pas présenté de symptômes de paludisme.

Sur 99 villageois examinés, 50 (50,5%) étaient infectés par le paludisme, *Plasmodium falciparum* venant en tête des parasites identifiés. *P. cynomolgi* a été isolé chez des singes du Cambodge. L'on peut en conclure que le contact homme-singe par l'intermédiaire du moustique est très intime, d'où la possibilité d'une transmission croisée au cas où des souches de paludisme simien infectantes pour l'homme existeraient dans cette région.

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