

BODY OF REPORT

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Objective: To determine the role played by the Anopheles species of Thailand in the transmission of human malaria. Important regional differences exist in Thailand with respect to the degrees of malarial endemicity present and the species of Anopheles involved in transmission. Furthermore, the widespread use of residual insecticides in malaria control programs is exerting selective pressures on vector populations which may result in an alteration in the relative status of primary and secondary vector species. The clearing of large tracts of forest in the

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southern and southeastern parts of the country is bringing settlers into contact with dangerous forest anophelines such as An. balabacensis and at the same time deforestation creates new breeding sites for vectors preferring open habitats such as An. maculatus and An. minimus. The different types of ecological associations existing in malarious regions of Thailand must be studied in order to develop specific measures of control for each region. Primary emphasis is placed upon long term studies in selected areas where the species composition and density of the anopheline populations, their breeding habitats, flight range, feeding cycles, age composition, and oocyst and sporozoite rates can be correlated with the incidence of human malaria. Furthermore, anopheline surveys have been correlated with the Malaria Project's investigation of chloroquine-resistant malaria in an effort to determine whether resistant strains of P. falciparum are associated with transmission by particular vector species such as A. balabacensis.

Progress: The coincidence that Anopheles balabacensis is the principal mosquito vector in most of the localities of SE Asia from which chloroquine resistant falciparum malaria is known has been noted in the literature (1), and the question of whether transmission by that species is in some way associated with the appearance of the resistant strains has been raised. Because of this it was proposed that an anopheline survey be made in conjunction with the chloroquine-resistance reconnaissance undertaken by the Malaria Project. The area selected for the preliminary chloroquine-resistance studies was in the southern Thai provinces of Satun, Yala and Narathiwat which adjoin Malaya. It was from the Malayan state of Perlis immediately adjoining Satun province that one of the earliest reports of chloroquine resistance came. The principal malaria vector in the forested parts of Perlis is reported to be A. balabacensis. During November and December anopheline collections were made at the Kuankalong Self-Help Land Settlement near Satun, in February and March collections were made near Waeng in Narathiwat province and again in the Satun area.

As the result of the loss of the personnel who had been engaged in anopheline collecting, the entomological studies at Khao Mai Kaeo were largely terminated during the period of this report. Preliminary surveys were made at Khai Mai Kaeo, Ban Kram and at the Rayong Self-Help Land Settlement to establish the location of permanent collecting sites. Preparations were made to begin the detailed mapping of the study sites preliminary to undertaking long-term studies of anopheline bionomics can be resumed.

a. Kuankalong Settlement, Satun Province. This settlement is located approximately 30 km north of Satun and has a population of approximately 1,800 people. Some 11,425 rai are allocated to the settlement, of which about 8,460 rai are currently utilized. The terrain of the settlement is rolling and savannah-like, and it is surrounded by the primary rain forest that once covered the area but which now is largely confined to the hills surrounding the settlement area. Just north of the settlement is a steep range of hills which rise to 1000-2000 foot elevations and separate the Satun area from the Haadyai district to the north. The settlers at Kuankalong cultivate bananas, tapioca, rubber and rice. The

Table 1

ANOPHELES FEMALES COLLECTED BITING MAN OR IN NET TRAPS, SATUN,
NOVEMBER AND DECEMBER 1964

Species	Total Number Caught
<u>A. aconitus</u>	58
<u>A. argyropus</u>	1
<u>A. barbirostris</u>	10
<u>A. indiensis</u>	44
<u>A. karwari</u>	10
<u>A. kochi</u>	9
<u>A. maculatus</u>	34
<u>A. minimus</u>	6
<u>A. peditaeniatus</u>	19
<u>A. philippinensis</u>	261
<u>A. sinensis</u>	3
<u>A. subpictus malayensis</u>	1
<u>A. tessellatus</u>	12
<u>A. vagus</u>	16
TOTAL	483

Table 2

RESULTS OF BITING COLLECTIONS MADE AT SATUN DURING NOVEMBER
AND DECEMBER 1964

Species	November (1900-2200 hours - 6 collections)	December (1800-2200 hours - 12 collections)
<u>A. aconitus</u>	<u>8</u>	<u>41</u>
<u>A. barbirostris</u>	0	6
<u>A. indiensis</u>	15	10
<u>A. karwari</u>	10	0
<u>A. maculatus</u>	<u>17</u>	<u>5</u>
<u>A. minimus</u>	3	1
<u>A. philippinensis</u>	5	<u>241</u>
TOTAL	58	304

incidence of malaria among the settlement population was approximately 40 per cent based upon an examination of blood smears taken by the Malaria Project Team; the majority of the infections were due to P. falciparum. During November and December anophelines were collected by biting-collections and by use of human-baited net traps. In over 60 man hours of collecting between 1800 and 2200 hours only 483 anophelines were collected (Table 1); this was probably a reflection of the reduction in the number of available oviposition sites produced by lack of rainfall during October and throughout the period of the Satun studies. A comparison of the results of biting collections made at the same site on the Kuan-kalong settlement during November and December indicate that the maculatus population was on the decline, while there is an apparent rise in the populations of both aconitus and philippinensis (Table 2). The three months drought resulted in the disappearance of most of the small hill side streams and seepages favored by maculatus, but there still remained water in rice fields and swamps suitable for breeding-places for latter two species. Of the 14 species of Anopheles represented in these collections only two (A. minimus and A. maculatus) are considered dangerous. While minimus is one of the principal vector species in Thailand, maculatus has not yet been incriminated as important vector in this country. It is known to be a major vector in Malaya. Since maculatus favors open, hilly terrain it can be anticipated that this species will extend its range in southern Thailand as more of the forested hills are cleared. The most abundant anophelines at the time of these collections were: A. philippinensis, A. aconitus, A. indiensis, and A. maculatus. No A. balabacensis were collected in the Satun area during November or December, but it is possible that this species is present in the area during other seasons, for Colless (2) has reported that balabacensis is most abundant in North Borneo during the period of greatest rainfall. A total of 145 anophelines (37 A. aconitus, 10 A. karwari, 22 A. maculatus 3 A. minimus and 73 A. philippinensis) were dissected for evidence of malarial infection, but no oocysts or sporozoites were seen. Ovarian dissections were also made in an effort to determine what percentage of the anophelines attacking humans in the Satun area had had previous blood meals and therefore the opportunity to acquire malarial gametocytes. The appearance of the terminal tracheoles on the ovarian sheath was used to distinguish parous (one or more previous blood meals) from nulliparous (no previous blood meals) females. The ingestion of blood stimulates ovarian development in mosquitoes, and as the eggs mature the ovaries undergo tremendous expansion in size. The terminal branches of the tracheoles are tightly coiled in skeins at the onset of the first ovarian cycle, but as the ovary enlarges these skeins uncoil, and the tracheoles are stretched out. Following ovulation they remain uncoiled, and the presence of tracheolar skeins is thus indicative of the nulliparous state. The results of the ovarian dissections made at Satun were given in Table 3. Unfortunately, only small numbers of A. maculatus were collected in December when the ovarian dissections were made, so the significance of the higher proportion of parous maculatus to either aconitus or philippinensis may be more apparent than real.

(1) Sandosham, A. A. et. al. Med. J. Malaya 18: 172 (1963)

(2) Colless, D. H. Med. J. Malaya, 6: 234 (1952)

Table 3

RESULTS OF DISSECTIONS OF OVARIES OF ANOPHELES COLLECTED AT SATUN, NOVEMBER-DECEMBER, 1964.

Species	Number Dissected	Number Parous	Number Nulliparous
<u>A. aconitus</u>	32	10	22
<u>A. maculatus</u>	11	7	4
<u>A. minimus</u>	2	1	1
<u>A. philippinensis</u>	73	32	41
TOTAL	118	50	68

An entomological team revisited the Satun area in March 1965 for the purpose of observing whether significant changes in the anopheline population had occurred there since the studies of the previous November and December. Because of a continuing lack of rain at the lower elevations occupied by the Kuankalong settlement the hillstreams and hillside seepages favored by A. minimus and A. maculatus had dried up. The anopheline breeding there was confined to stagnant pools in dried stream beds and to other bodies of standing water. Immature stages of A. barbirostris, A. hodgkini, A. kochi, A. aconitus and members of the hyrcanus group -- all non-vector species-- were collected in the settlement area. Collecting activities were then shifted into the higher forested hills on the northern boundary of the settlement. Immature stages of A. balabacensis balabacensis and the closely related A. riparis macarthurii and A. hackeri were collected from pools on the margins of streams in the forest, but no evidence of biting activity by these species was encountered. A few specimens of A. maculatus, A. minimus and A. barbirostris were collected in this area while biting man, but as the lower elevations the anopheline population was apparently at a low level. The presence of breeding sites of balabacensis at higher elevations in the dry season may represent foci from which this species can expand when the rainy season produces more oviposition sites at the lower elevations.

b. Waeng District, Narathiwat Province. During February and March, an anopheline survey was conducted in the vicinity of Waeng, Narathiwat province in association with a reconnaissance for chloroquine resistance by the Malaria Project in the same region. Waeng district is located in the southeastern part of

Table 4

ANOPHELES COLLECTED BITING HUMANS AT WAENG, FEBRUARY
AND MARCH, 1965

Species	Number
<u>A. aconitus</u>	3
<u>A. barbirostris</u>	15
<u>A. hyrcanus</u> group	4
<u>A. karwari</u>	2
<u>A. kochi</u>	8
<u>A. letifer</u>	2
<u>A. maculatus</u>	95
<u>A. philippinensis</u>	1
<u>A. tessellatus</u>	1
TOTAL	131

Table 5

RESULTS OF DISSECTIONS OF OVARIES OF ANOPHELES COLLECTED
AT WAENG, FEBRUARY AND MARCH, 1965

Species	Number Dissected	Nulliparous	Parous
<u>A. barbirostris</u>	11	6	5
<u>A. maculatus</u>	70	13	57

Narathiwat province adjoining the Malaysian state of Kelantan. The boundary between the two countries in that region runs along the Sungei Golok river which empties into the Gulf of Siam to the northeast. The eastern portion of the district consists of flat to undulating terrain devoted to rice and rubber cultivation which extends ten to twelve miles westward from the Sungei Golok to a range of hills which rise to about 3,000 feet. On the other side of this range is a smaller valley formed by tributaries of the Saiburi River. Beyond that point is a continuous stretch of mountains extending without break to the Betong Valley-- 50 miles to the west. These mountains are covered with dense tropical rain forest and constitute one of the wildest and most inaccessible regions in Thailand. Along the lower slopes of the hills facing Waeng the forest has been cleared for rubber plantations, and there is a lot of logging activity at the higher elevations.

Anopheline collections were made principally at the main section of the Waeng Self-Help Land Settlement (population 2,000) 6 km south of Waeng, at the new section of this settlement located in the Saiburi Valley 17 km west of Waeng and at several logging and road-construction camps on the road between Waeng and the new settlement. Thick film blood smears were taken from the inhabitants at all of these collection sites by Malaria Project personnel, and 50 to 80 per cent of them were positive for malaria. The majority of the infections seen were due to P. falciparum. Adult anophelines were collected between 2000 and 0100 hours while biting humans inside and outside dwellings. During daylight hours larval mosquito collections were made in the vicinity of the adult collection sites, and the adults from the previous night's collection were dissected. The density of the anopheline population during the period of these studies was very low, for only 131 adults were collected at the rate of approximately one mosquito for man-hour of collection (Table 4). Ninety-five (70 per cent) of the anopheline collected biting humans were A. maculatus. Smaller numbers of A. barbirostris, A. kochi members of the hyrcanus group accounted for most of the balance of the adults collected. Peak biting activity for maculatus occurred between 2100 and 2400 hours. These mosquitoes apparently showed no reluctance to enter habitations, for a majority of those collected were taken while biting the sleeping occupants of huts in logging and road-construction camps on the fringes of the forest. Seventy maculatus and 11 barbirostris were dissected, and one maculatus was found with oocysts on its gut. Ovaries of the dissected mosquitoes were also examined (Table 5), and 57/70 (80 per cent) of the maculatus were found to be parous. The fact that such a high percentage of that species had taken at least one previous blood meal together with the fact that maculatus was the predominant species biting man in Waeng area increased the likelihood that it was serving as a vector in that region. It is worthwhile noting that no adult A. balabacensis were collected in Waeng district. A majority of the collecting sites in the Waeng studies were located within or at the fringes of primary rain forest -- a habitat which balabacensis favors. Immature stages of A. balabacensis introlatus and the closely related A. riparis macarthuri and A. pujutensis were collected from forest pools in the vicinity of the adult collection sites, but the adults of none of these species were observed biting man. The question of whether the subspecies balabacensis introlatus plays a significant role in the transmission of human malaria

remains to be clarified. These findings are puzzling because balabacensis has been reported (3) responsible for the transmission of chloroquine-resistant strains of P. falciparum in Perlis just across the Malayan border from Satun. A further note of interest is that during a survey of the Waeng area in January 1964, SMRL personnel reported that the majority of the malaria cases seen were due to P. vivax, while the anopheline species implicated as the vector at that time was A. karwari. These various contradictory observations serve to emphasize how complicated the whole picture of malaria ecology in the southern peninsular region appears to be.

Summary and Conclusions: Anopheline surveys were conducted in two regions of southern Thailand near the Malayan border-- in Satun province on the west coast and in Narathiwat province on the east coast. While evidence of the presence of chloroquine-resistant strains of P. falciparum in these areas was produced by the studies of the Malaria Project personnel, there was no evidence that A. balabacensis was connected with the transmission of malaria in either region. The results of these studies suggested, instead, that A. maculatus is the principal vector there.