

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 51	Anopheles and Malaria
Project No. 3A 025601 A 811	Military Medical Research Program S.E. Asia
Task 01:	Military Medical Research Program S. E. Asia
Subtask 01:	Military Medical Research Program SEASIA (Thailand)
Reporting Installation:	US Army-SEATO Medical Research Laboratory APO 146, San Francisco, California  Division of Medical Research Laboratories  Department of Medical Entomology
Period Covered by Report:	1 April 1963 to 31 March 1964
Principal Investigator:	Major John E. Scanlon, MSC
Associate Investigators:	Dr. Udaya Sandhinand, MD
Report Control Symbol:	MEDDH-288
Security Classification:	UNCLASSIFIED

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ABSTRACT

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The object of this study is to determine the species of Anopheles which transmit malaria in various environments in Thailand. Most of the field work in the study was concentrated in the Khao Mai Kaeo area of Choburi province during the year. In this hyperendemic area the most abundant anthropophilic Anopheles species was A. balabacensis. In this area of tapioca plantation and remnants of jungle A. balabacensis is highly exophilic, resting among jungle vegetation during the day. Anopheles minimus also occurs in the area in some numbers, and also was found with sporozoite infections of the salivary glands. Extensive studies on the biology of A. balabacensis were summarized for this report, and are being prepared for publication. Additional observations on the Anopheles fauna were made in the malarious area of the Korat Plateau near

Pakchong, and in the tropical rain forest at Waeng on the Malaysian border. No infected mosquitoes were found in the former area, although A. balabacensis and A. innimus were present. At Waeng a single Anopheles karwari was found with a salivary gland infection. This species may be a vector in the Waeng area, but additional observations will be required at other times of the year to settle this point.

BODY OF REPORT

SEATO Medic Study No. 51                    Anopheles and Malaria

Project No. 3A 025601 A 311                Military Medical Research Program  
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Objective: To determine the role played by the Anopheles species of Thailand in the transmission of human malaria. Primary emphasis is placed on selected study areas, chiefly the Khao Mai Kaeo area where long term epidemiological studies are in progress. To determine the rates of sporozoite infection in various Anopheles species captured in nature, and specific infection rates for laboratory reared specimens fed on known gametocyte carriers. To make observations on the distribution, biting and breeding habits of the potentially important vectors of malaria in Thailand.

Description: Female Anopheles are captured individually in glass tubes while coming to feed on man or animals, or from vegetation, walls of dwellings, and other resting places. Other females are collected from human and animal baited net traps. The natural resting places of forest species are searched by means of nets and aspirators. Larval and pupae are collected to determine breeding sites. Living females are retained for colonization (Study No. 43) or dissected for detection of malaria parasites.

Progress: By far the greatest part of the effort in this study from May 1963 to March 1964 was devoted to the study of the Anopheles species in the study area at Khao Mai Kaeo in Choburi Province. A description of this area will be found in the report of Study 52. Briefly, it consists almost entirely of tapioca plantations surrounded by remnants of the forest from which the plantations have been cut in recent years. The ground is extremely sandy and in the rainy season the terrain is interlaced by innumerable channels and seepages. During most of the year mosquito collections were made from human residences from dusk to midnight. Human biting collections were made inside and outside the houses, and the place of collection of each Anopheles female was noted. A large (room size) mosquito net was suspended from bamboo poles several times during the year and used as a mosquito trap, baited with either a man or several macaque monkeys. The nets had two flaps which could be raised and lowered easily by the operator. The net traps were operated at ground level, even with monkey bait. The monkey catches were suspended after a few trials since no wild monkeys could be found in the area, and the original intent was to discover the natural habits of the Anopheles species in the area. In the discussion below, a few introductory remarks are given on the known vector status of Anopheles species in Thailand at the beginning of the study. The areas of collection are then discussed separately, and finally, notes are given on Anopheles balabacensis in Thailand.

#### Vectors of malaria in Thailand.

For a number of years it had been generally accepted that the only malaria vector of importance in Thailand was Anopheles minimus. This is a very important vector over much of SE Asia. It requires fairly clear and well oxygenated water for breeding, and thus is important chiefly in foothill areas. It was first found infected in Thailand in 1935. Subsequently a number of other species were found infected from time to time, but none of these (with the possibly exception of A. sondaicus in coastal areas) were thought to be important. As malaria due to A. minimus began to decrease in the face of control program in Thailand and neighboring countries, however, it was realized that other species might be even more important, especially in forested areas. Table 1 lists all of the

Anopheles species suspected or proven to be vectors as of May 1963.

### Field Studies

a. Khao Mai Kaeo. Khao Mai Kaeo was chosen as a study site because of its consistently high rate of malaria, in the face of attempts to control the vector population by residual spraying. A list of the Anopheles species collected during the year is given in table 1. The collection totals do not include several collections from cattle, and net traps, nor the very large numbers of females (over 15,000) from ten special all night collections, which will be discussed below. The cattle collections produced large numbers of certain Anopheles species (such as jamesii) which were not attracted to man in numbers. Perhaps more importantly, neither of the vector suspects, A. balabacensis and A. minimus were attracted to cattle at all. This reemphasizes the anthropophilic nature of these species, and the lack of any protection by diversion to domestic animals. Table 2 includes the species of Anopheles species taken biting cows. The pattern of biting in the net trap, baited human and monkey is given in table 3. Monkeys were included in the study because of the interesting findings of Eyles and his associates in Cambodia, in which A. balabacensis fed on monkeys. The implications for a possible simian cycle of malaria involving man are most important. The traps were not run simultaneously with human and monkey bait, so the figures given can be used only as a rough guide. However, it is apparent that A. balabacensis is attracted to monkeys. Most of the specimens captured were engorged, but the blood was not saved for precipitin testing. Almost all of the A. balabacensis from the study area collections, in addition to some of the other species, were saved for production of eggs for colonization (Study No. 43). However, a number were dissected for examination for sporozoites. The results of these dissections are given in table 4. The high sporozoite rate in A. balabacensis and A. minimus and the lack of natural alternate hosts for these species in the survey area leads to the conclusion that these are the vector species. The higher infection rate, and the very much higher attack rate on man also lead to the conclusion that A. balabacensis is a more important vector at Khao Mai Kaeo than A. minimus. The collections of Anopheles are continuing in the area, and all seasonal data will be tabulated at the end of the second year of observation. With the preliminary observations indicating the importance of A. balabacensis and A. minimus in malaria transmission at Khao Mai Kaeo, a series of ten all night collections was made at a laborers' houses in an isolated areas of tapioca plantation and jungle where the population of these species was found to be particularly high. Six collectors participated in these collections, and rotated duties among them. Data from these collections are being prepared for publication, and some details regarding A. balabacensis will be given in the remarks on that species at the end

of this report. In all, over 15,000 Anopheles were taken in these collections, a remarkable number for the time and number of collectors involved. These species were distributed as follows:

<u>Anopheles balabacensis</u>	11,748
<u>Anopheles minimus</u>	1,953
<u>Anopheles maculatus</u>	1,838
	15,539

Collection data relating to these 15,539 Anopheles females have been tabulated by place of collection (inside wall, outside wall, vegetation, etc.), time of collection, state of feeding and time of biting. These data are still being analyzed for publication.

b. Khao Yai National Park and Vicinity. Reports were received in September 1963 that a considerable amount of malaria was occurring in this newly opened National Park. The Park proper is largely covered with evergreen tropical forest, with large open meadows of grass. There is a large and varied animal population, but relatively few humans live in the Park. This area is on the edge of the Korat Plateau and receives a considerable amount of rain. The foothills of Khao Yai (literally "Big Mountain") have been notorious for malaria for many years. Anopheles were collected from around the homes of forest workers during the evening in the Park, and from a work camp and several villages in the foothill area. The list of species collected is given in table 5. None of the females were positive on dissection. It is postulated that A. balabacensis and A. minimus are the vectors in the foothill villages and perhaps for the small number of malaria cases which were found in the Park, but this question remains open for the moment. Neither of the suspected vectors were taken in the Park, and almost all of the specimens listed came from the foothill villages. It was somewhat surprising that none of the A. balabacensis were taken biting man. However, the biting collections were suspended somewhat early (2300 hours), and it is possible that feeding activity begins a little later at Khao Yai than at at Khao Mai Kaeo.

c. Waeng District, Narathivas Province. Collections in this area were severely hampered by heavy rains and high wind during the survey period in January. Records of the Ministry of Health had indicated that sporozoite infections of Anopheles maculatus had been found in the nearby villages, although A. maculatus is generally not though to be an important vector of malaria in Thailand. In some parts of Malaysia A. maculatus is an important vector. A list of the Anopheles mosquitoes collected in six nights (1900 hours to 24000 hours) is given in table 6. Eighty five of the Anopheles karwari were dissected and one specimens was found with sporozoites in the salivary glands. This specimen was collected from a

Table 1. Anopheles females collected biting man or resting in or near houses, Khao Mai Kaeo, May 1963 to March 1964

<u>Species</u>	<u>Total Number Caught</u>	2
<u>A. balabacensis</u> Baisas 1936	3,159	
<u>A. maculatus</u> Theobald 1901	1,424	
<u>A. minimus</u> Theobald 1901	448	
<u>A. barbirostris</u> Van der Wulp 1884 1/	356	
<u>A. aconitus</u> Donitz 1902	310	
<u>A. hyrcanus</u> group 1/	43	
<u>A. vagus</u> Donitz 1902	40	
<u>A. tessellatus</u> Theobald 1901	32	
<u>A. philippinensis</u> Ludlow 1902	31	
<u>A. kochi</u> Donitz 1901	22	
<u>A. splendidus</u> Koidzumi 1920	20	
<u>A. jamesi</u> Theobald 1901	5	
<u>A. karwari</u> James 1903	1	
<u>A. letifer</u> Sandosham 1944		
<u>A. subpictus malayensis</u> Hacker 1921	1	
Total anopheles	5,793	
Total culicines	2,288	

1. Several species included under this name
2. Does not include mosquitoes from special all night collections referred to in text.

Table 2. Anopheles captured from cows, Khao Mai Kaeo Thailand, 1963.

Species	May	June	July	Total
<i>A. jamesi</i>	109	263	247	619
<i>A. maculatus</i>	0	124	4	128
<i>A. barbirostris</i>	9	74	3	86
<i>A. hyrcanus</i> group	15	193	25	233
<i>A. vagus</i>	237	61	36	334
<i>A. aconitus</i>	38	0	32	70
<i>A. tessellatus</i>	0	5	0	5
<i>A. kochi</i>	11	84	27	122
<i>A. karwari</i>	0	3	1	4
<i>A. splendidus</i>	0	29	24	53
<i>A. philippinensis</i>	7	33	42	82
<i>A. ramsayi</i>	0	3	0	3
Total	426	872	441	1,739

Table 3. Anopheles females attracted to human and monkey bait in net traps at ground level. Khao Mai Kaeo

Species	Females per trap night	
	Human (10 nights)	Monkeys (3 nights)
<i>A. balabacensis</i>	14.6	32.3
<i>A. minimus</i>	0.2	1.0
<i>A. maculatus</i>	2.8	4.3
<i>A. barbirostris</i>	1.1	0.3
<i>A. hyrcanus</i> group	0.4	-
<i>A. vagus</i>	0.3	-
<i>A. philippinensis</i>	0.2	-
<i>A. splendidus</i>	0.1	-

Table 4. Dissection of Anopheles females for malaria sporozoites, Khao Mai Kaeo, Cholburi.

Species	Number dissected	Positive	Rate
A. balabacensis	335	29	8.7%
A. minimus	81	1	2.5%
A. maculatus	139	0	0
A. barbirostris	4	0	0

Table 5. Female Anopheles, Khao Yai and vicinity, September 1963 (8 nights)

Species	Biting man	Biting cattle	Resting	Total
A. balabacensis	-	-	21	21
A. minimus	2	-	2	4
A. maculatus	9	-	9	18
A. barbirostris	2	3	2	7
A. hyrcanus group	4	6	2	12
A. vagus	1	36	3	40
A. philippinensis	-	2	-	2
A. tessellatus	1	1	5	7
A. kochi	-	1	1	2
A. karwari	1	-	-	1
A. aconitus	-	1	-	1
Total	20	50	45	115

Table 6. Female Anopheles, Waeng, Narathivas January 1964  
(6 nights - human biting)

Species	Number examined
A. karwari	313
A. maculatus	1
A. kochi	1
A. hyrcanus group	3
A. philippinensis	4
A. barbirostris	1
Total	323

house in which two children had enlarged spleens, and the younger had a blood smear positive for P. falciparum. The sporozoites were not inoculated in a human volunteer or experimental animal, and there is a possibility that the parasites were not those of human malaria. However, in view of the circumstances outlined there is a strong presumption that A. karwari was acting as a vector in the area. This requires additional investigation. Larval collections were made in a number of habitats, including foothill streams. The following species were collected: A. karwari, barbirostris, philippinensis, maculatus, aconitus and balabacensis. This is the first report of A. karwari as a possible malaria vector in Thailand.

#### Notes on Anopheles balabacensis

Anopheles balabacensis was first recognized as an important malaria vector in Borneo by McArthur, Colless and others. Interest in the species accelerated after the discovery that this "leucosphyrus" species was probably an important vector in the forested areas of Cambodia and Thailand. However, an even more important series of findings has spotlighted the possible importance of the species in the last two years. During that time, forms of Plasmodium falciparum resistant to chloroquin (and certain of the other synthetic antimalarials) have appeared in Malaysia, Cambodia and Thailand. In each of these places Anopheles balabacensis appeared to be the principal or only vector. Whether this association is coincidental or causal can probably only be determined by controlled experiments. Since proven cases of drug resistant malaria have occurred in Khao Mai Kaeo, and since the A. balabacensis population there is unusually high, it is a particularly suitable area for field investigations of this phenomenon.

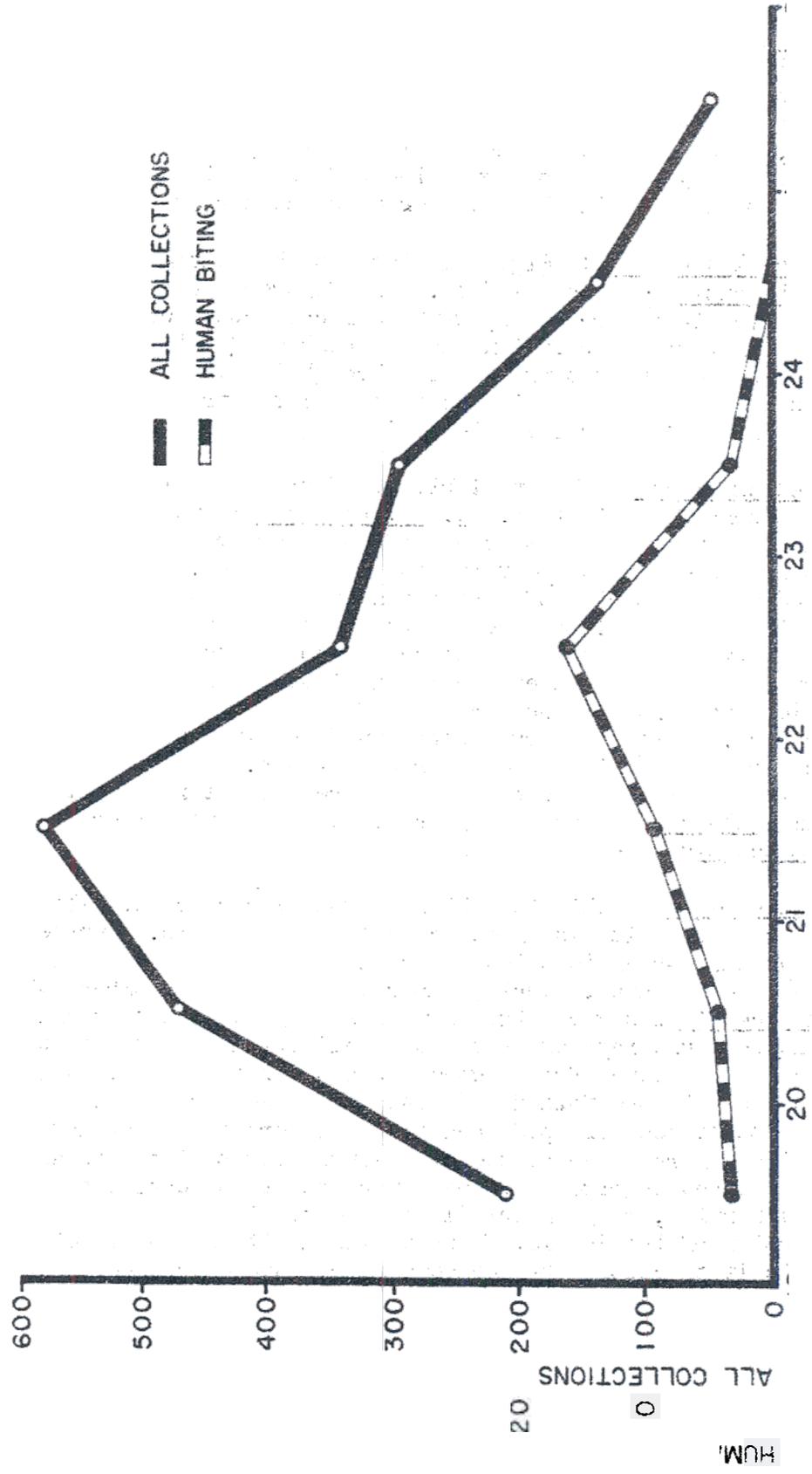
Biting habits: As noted in above A. balabacensis is a highly anthropophilic species. It will, however, feed readily on monkeys (Malaya, Cambodia and Thailand) and many feed on other forest animals. Fig. 2 shows the number taken biting at various times of the night at Khao Mai Kaeo, as compared with the total number captured. It appears to be in the vicinity of man for some time before it bites. A few specimens were taken biting man in the deep forest shade during daylight hours.

Seasonal abundance: Figure 1 diagrams the seasonal abundance of A. balabacensis at Khao Mai Kaeo. The figures given are for two hour periods of three nights per month, with four collectors working. The September-November period population was the highest for the year, but some adults were present and biting man all through the year. The relationship of this population to the intensity of malaria transmission in the study area is still under investigation.

Breeding places: The availability of breeding places is directly related to seasonal distribution. A. balabacensis larvae are found in seepages and shallow pools at the sides of the many small streams which intersect the Khao Mai Kaeo area during the rainy season. The number of larvae found after a series of very intensive searches however, seemed disproportionately small compared to the number of adults in the area. The larval breeding sites were so scattered and so numerous that a complete survey of the area is extremely difficult. In the early part of the field investigation another type of larval habitat was examined in Chantaburi Province. Here the larvae were found in pits dug along streams for the recovery of sapphires and other gem stones. In both situations, and in several other places where larvae were encountered a spiny fruit-bearing palm (Salacca sp.) which appears to be a useful marker organism for A. balabacensis.

Resting places: This is a highly exophilic species. Although it enters houses to feed it also feeds readily out of doors. Furthermore, those individuals which feed indoors rapidly leave the house and return to the forest before dawn. During a period (July to September) when the A. balabacensis was very high as measured by human biting only 1 specimen could be found resting around the collection site during the day. This specimen was taken from a wooden box under the house. Eighteen specimens were found in the folded bed nets of the workers in the house, these had undoubtedly been trapped when the nets were folded at dawn. Several specimens were found by sweeping jungle vegetation with nets during the daytime, and engorged specimens were observed leaving the houses in the early morning, resting briefly on the vegetation and then flying in the direction of the jungle. The resting habits of the species thus make it difficult to control by the usual technique of residual spraying of houses.

# TIME OF COLLECTION OF ANOPHELES BALABACENSIS



Summary: A very large number of Anopheles females were collected in the malaria study area at Khao Mai Kaeo during the year. The most abundant species attacking man was A. balabacensis, and over 8% of the specimens of this species examined had salivary gland infections. Infected A. minimus were also found in the study area, but the human attack rate for this species was much lower than for A. balabacensis. Both of these species were highly anthropophilic, neither of them were taken biting cows. However, both were attracted to monkeys in a net trap at ground level. A. balabacensis proved to be highly exophilic, feeding on man outdoors as well as in, and very rarely resting around houses during the day. It was found resting in the jungle in daylight hours, and also fed on man in the deep jungle shade during the day. A. minimus and A. balabacensis were also found in villages in the foothills of the Khao Yai area of the Korat Plateau, but no infected individuals were found among the small number dissected. At Waeng, on the Malaysian border, Anopheles karwari was by far the most abundant species in houses with malaria infected inhabitants, and a single specimen with sporozoites was found among over three hundred examined. This is the first report of this species as a potential malaria vector in Thailand, although it has been suspected to be a vector in Malaysia on epidemiological grounds.

Conclusions: In the highly malarious area of Khao Mai Kaeo, Choburi Province, Anopheles balabacensis was by far the most abundant mosquito species attacking man. This, combined with the high sporozoite rate in this species indicates that it is the most important vector of malaria in the area. A. minimus which has been regarded as the primary vector in Thailand, was much less abundant, had a lower rate of sporozoite infection, and probably plays a much less important role in the transmission of the disease at Khao Mai Kaeo. The same two species are probably involved in malaria transmission at Khao Yai, but this requires additional study. On the Malaysian border evidence was obtained which may indicate that A. karwari is a locally important vector, but this too requires confirmation.