Title: Anopheles and Malaria

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Objective.

The objective of this study is the investigation of those species of Anopheles responsible for the transmission of human malaria in Southeast Asia. Studies on the bionomics and population dynamics of anophelines have been undertaken in malarious regions of Thailand. Fluctuations in the density, species and age composition of anopheline populations are being measured in an effort to relate these factors to the incidence of malarial infections in both the human and mosquito populations. Specific factors being studied in the attempted definition of potential vector species in Thailand also include the determination of their flight range, longevity, patterns of biting activity, host preferences and susceptibility to infection with the plasmodia causing human malaria.

Description.

Important regional differences exist in Thailand with respect to the incidence of malaria and the species of Anopheles serving as vectors. At present, approximately 52 species of Anopheles have been recorded from this country, and about half of these species are known to feed on human beings. Anopheles minimus is responsible for much of the malaria occuring in the foothills of this country, and A. balabacensis has been recognized as an important vector species in many of the forested parts of Thailand. The vector status of other anophelines in Thailand, such as A. maculatus, A. sundaicus, A. campestris, A. aconitus and A. philippinensis, which are primary vectors of malaria in other parts of Southeast Asia, remains to be clarified. Permanent or recurrent studies of the anopheline populations in Nakornrajisima, Chantaburi, Phatumthani, Narathiwas, Songkla and Satun provinces have been established in areas representative of the ecological associations existing in malarious regions of Thailand.

Phatumthani Studies—The central plains of Thailand surrounding Bangkok have until recently been considered free of all but introduced cases of malaria, and this region has not been included in the residual spray program of the national malaria eradication effort. In recent years it has become apparent that foci of low level malaria do exist in this region which have certain peculiarities distinguishing them from other malarious areas of Thailand—1) none of the proven vector species, such as Anopheles minimus or Anopheles balabacensis, occur there, 2) the majority of the malaria infections are caused by Plasmodium vivax rather than P. falciparum and 3) the peak in incidence is reported to occur in the dry season (January-May) rather than in the rainy season. Thus, in February 1960 in Nontaburi province the National Malaria Eradication Project reported that 3.4 per cent of 3830 persons examined were infected with P. vivax while another 0.3 per cent had P. falciparum infections. Anopheles campestris was the commonest anopheline caught in houses at the time, but the results of dissections of over 2000 females of that species were negative.

During April, August and November 1966 entomological surveys were carried out by SMRL in a further attempt to define the vector (s) of malaria in the Central Plain area. Malaria surveillance reports

indicated the presence of malaria in villages of Sam Kok district in Phathumthani province adjacent to the Chao Phrya river some 20 miles north of Bangkok. These villages extend alongside the river bank for as much as a kilometer or more but are no more than one or two houses deep at any point. Gardens and rice fields tended by the villagers are located along the backside of the villages. Examination of thick blood smears from 615 Sam Kok villagers in April showed 22 (3.6%) P. vivax infections. No cases of falciparum malaria were found.

Biting collections of anophelines were made at Sam Kok between 1900 and 2400 hours using human bait. Six species—A. aconitus A. annularis, A. campestris, A. philippinensis, A. tesselatus and A. vagus—were predominant in these collections. Anopheles campestris was the species most frequently caught indoors, while the other five species were collected with significantly greater frequency outside houses than indoors. The results of dissection of approximately 3000 anophelines collected during this period are shown in table 1.

Table 1. Results of salivary gland and gut dissections of anopelines collected in Phatumthani Province-1966.

Species	Number infected/Number Dissected April	August	November
Anopheles aconitus	1/18	1/81	0/374
Anopheles annularis	0/3	0/57	0/70
Anopheles campestris	0/301	0/59	0/601
Anopheles philippinensis	0/15	0/218	0/181
Anopheles tesselatus	0/221	0/62	0/374
Anopheles vagus	0/139	0/77	———

The specimen of A. aconitus found infected during April had both sporozoites and oocysts and had partially fed upon the collector when captured. Eight days later the collector complained of headache and fever (102°F), but no parasites were seen in a thick bleod smear taken on that day. On the 20th day following his exposure this man had a topical paroxysm, and trophozoites and schizonts of P. vivax were observed in his blood. A second infected specimen of A. aconitus collected in August had oocysts but no sporozoites. The above circumstances strongly suggest that A. aconitus is one of species involved in malaria transmission in the Central Plain of Thailand. This mosquito has not been previously implicated in Thailand, but it has been considered an important vector in parts of Indonesia and Viet Nam.

Previous studies in the Pak Chong valley area, where dwellings have been sprayed with DDT for almost a decade as part of malaria eradication activities, demonstrated that A. minimus, A. maculatus and A. aconitus man-biting rates were consistantly higher outdoors than indoors. The question arises as to whether or not these differences represent adaptive changes on the part of these anophelines in response to the insecticide pressures exerted in that area. Because there have been no such pressures applied to the anopheline populations in the central plain area a similar comparison of indoor-outdoor biting rates was made at Phatumthani. Unfortunately, neither A. minimus nor A. maculatus are present in this region, but the observed biting rates for A. aconitus. A. phillipinensis, A. tesselatus and A. vagus were significantly

higher outdoors than indoors in Phatumthani (Table 2). On the other hand, A. campestris showed no such reluctance to enter and feed on the occupants of houses. These observations suggest that the exophilic behavior of species such as A. aconitus is inherent, and that outdoor transmission of malaria must be suspected where such anophelines are important vectors (see section below on biting activities of A. balabacensis at Sadao). In such situations malaria eradication efforts which rely solely upon application of residual spray to the interior of dwellings are not likely to interrupt transmission.

Table 2. Observed differences in numbers of anophelines collected biting indoors and outdoors in Phatumthani Province, 1966-67.

Species	November, 1966		January — March, 1967		Level of significance
	Indoors	Outdoors	Indoors	Outdoors	
A. aconitus	142	931	27	478	1%
A. campestris	269	254	37	58	n.s.
A. phillipinensis	127	593	0	9	1%
A. tesselatus	57	176	16	411	1%
A. vagus	8	30	0	23	1%

Sadao Studies-The Malayan border area has been the subject of several previous surveys by SMRL because the identity of the vector species in that region is not known with certainty. Anopheles minimus is known to be present only in Satun province. Previous studies in Narathiwas Province (1964, 1965) yielded some evidence of malarial infections in A. kurwari and A. maculatus. Evidence on the vector status of A. balabacensis in these areas was negative, as efforts to collect adults of this species on previous trips were fruitless. During June entomological and malaria surveys were made in Sadao district, Songkla Province, at the village of Ban Khao Roop Chang, near the Thai-Malayan border. Thick blood films from 171 villagers at Ban Khao Roop Chang were examined by personnel of the Department of Epidemiology on 13 June, and 43 (25%) were positive for P. falciparum, 10 (6%) for P. vivax and 1 (0.6%) slide demonstrated parasites of P. malariae. Biting collections were made on 13 nights between the 1800-2400 hours. The results of these collections are summarized in Table 3. On two nights collectors were also placed inside village houses, but no anophelines were collected biting indoors. Attempts to find resting anophelines during early morning hours (0600-1000) in village huts were also negative. Three of 202 A. balabacensis dissected during this period were found infected with malarial parasites—one with sporozoites in the glands, one with infected glands and oocysts and a third with oocysts only. This mosquito was the dominant man-biting species collected during these studies. Ovarian dissections revealed that 80 per cent of the balabacensis were parous, and that 10 per cent of the parous females had two or more previous blood meals. Approximately 14 per cent of the parous females collected were in a gravid or pregravid condition, and it was impossible to determine the number of dilatations present. The occurence of such a substantial number of gravid females in these biting collections suggests that the taking of a second blood meal during the course of an ovarian cycle may occur with significant frequency. The vector potential of such females is of course much greater. In contrast the other species of anopheles—especially A. philippinensis—had a

higher percentage of nulliparous females than did balabacensis—indicating that the daily survival rate of these species was significantly lower than that of balabacensis. Furthermore, no infected mosquitoes were found among these other species. The pattern of biting activity (Table 4) exhibited by balabacensis during the Sadao studies was also remarkable in that the peak occurred between 1900 and 2000 hours while most of the villagers were still active. Elsewhere in Thailand (Cholburi Province) the observed peaks in biting activity by balabacensis have occurred between midnight and 0300 hours when human activity has ceased. Finally, the question of the identity of the malarial parasites found infecting the balabacensis at Sadao poses a problem, for it cannot be determined on morphological grounds whether the parasites are human or simian. Anopheles balabacensis is known to serve as the natural vector of several simian malarias, and the forests surrounding the village in Sadao abounded with both monkeys and gibbons. The possibility exists also that some of the villagers there may have had infections of simian malaria.

Experimental infection of Anophelines with human malaria—Since these studies were begun a total of 40 cases of falciparum malaria and 16 of vivax malaria have been exposed to laboratory reared Anopheles balabacensis and/or A. stephensi. A. balabacensis and A. stephensi were equally susceptible to both species of malaria. On the average, falciparum cases were more infective than vivax cases. The proportion of mosquitoes infected with falciparum gametocytes rose rapidly with increasing numbers of gametocytes ingested, reaching a peak at about 3000 gametocytes per cubic millimeter; thereafter it declined somewhat and stabilized at gametocyte levels of about 5000-6000 per cubic millimeter (Fig. 1.)

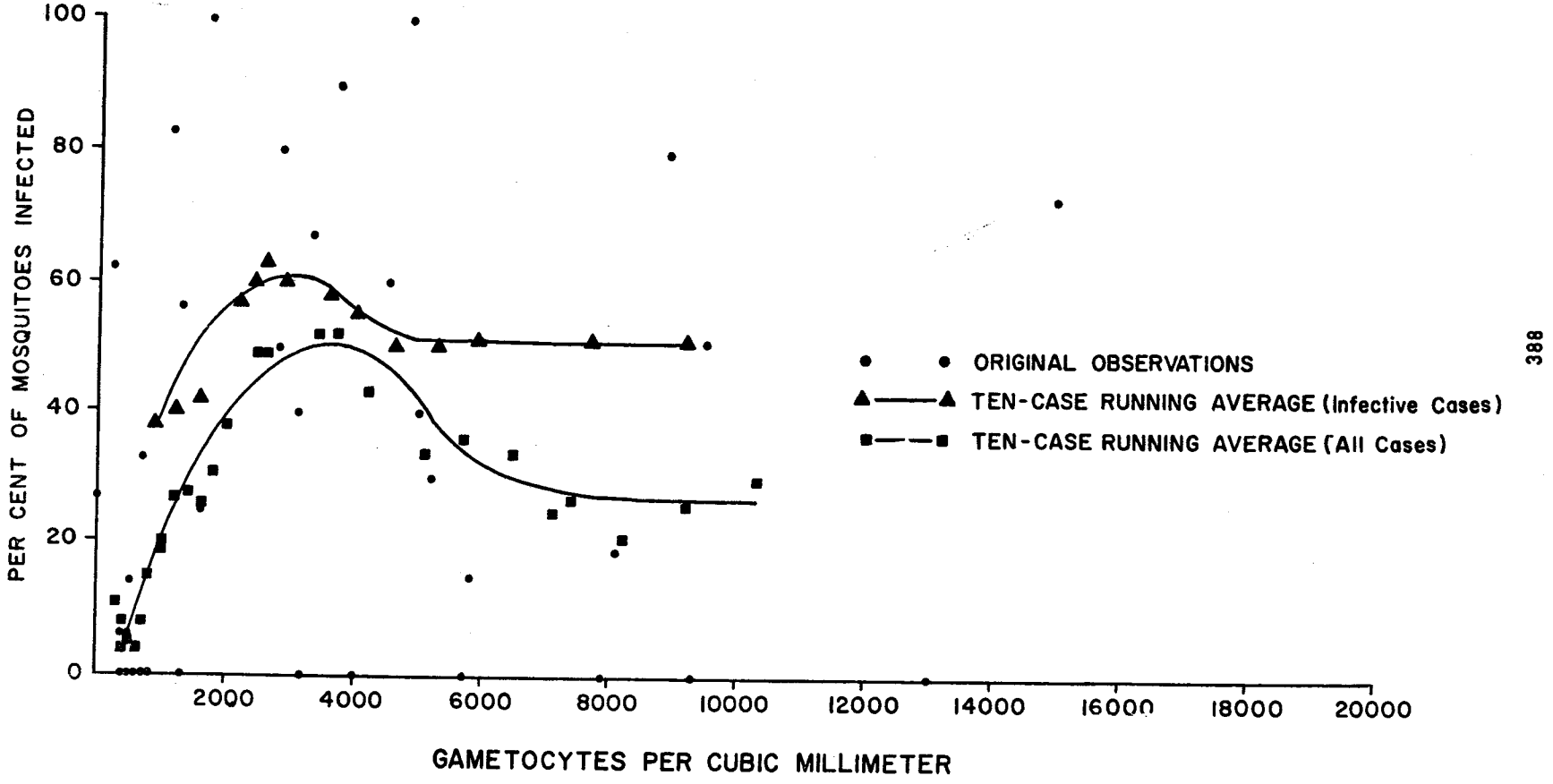
Table 3. Anopheles mosquitoes collected biting man outdoors between 1800-2400 at Sadao District—6-20 June 1966 (13 nights)

Anopheles aconitus	1
Anopheles balabacensis	214
Anopheles barbirostris	50
Anopheles indiensis	5
Anopheles karwari	10
Anopheles kochi	52
Anopheles maculatus	19
Anopheles philippinensis	129
Anopheles tessellatus	51
Total	531

Table 4. Pattern of human biting activity of A. balabacensis at Sadao District (13 Nights)-June, 1966.

Hour	No. Mosquitoes Collected	Per Cent Total
1800-1900	8	4%
1900-2000	121	56%
2000-2100	40	19%
2100-2200	20	9%
2200-2300	21	10%
2300-2400	4	2%

Fig. 1. Proportion of mosquitoes infected following engorgement on falciparum malaria carriers.



Falciparum cases with high densities of asexual parasites tended to infect higher proportions of mosquitoes (Table 5). The mechanism of this effect is unknown, since asexual parasitemia was not correlated with gametocytemia. Insufficient data was obtained from vivax cases to allow for a similar comparison. A tendency of the older falciparum carriers to infect higher proportions of mosquitoes than the younger carriers was observed, but this was not due to higher gametocytemias since gametocytemia was not correlated with age. (Table 6). In falciparum malaria where was a distinct trend for cool season cases to be more infective for mosquitoes than hot or rainy season cases (Table 7). It appears that this can be explained on the basis of carries gametocytemia levels, for in nine out of ten falciparum cases encountered in the cool season gametocyte levels were higher than the median gametocytemia (2250/mm^3) for falciparum cases. The analogous tabulation for vivax cases was inconclusive; however, all four vivax cases encountered in the cool season had gametocytemias higher than the median level (700/mm^3) for vivax cases.

Table 5. Relation of asexual parasitemia to infection of mosquitoes in falciparum malaria.

Asexual Parasites per cmm	Cases		Mosquitoes: Infective Cases			Mosquitoes: All Cases		
	Infective	Total	Diss.	Pos.	%Pos.	Diss.	Pos.	%Pos.
<1000	10	15	122	44	36	222	44	20
>1000	7	14	90	62	69	192	62	32

Table 6. Relation of carrier age to infection at mosquitoes in falciparum cases.

Carrier Age	Cases		Mosquitoes: Infective Cases			Mosquitoes: All Cases		
	Infective	All	Diss.	Pos.	%Pos.	Diss.	Pos.	%Pos
<20	12	21	164	62	38	256	62	24
>20	12	19	130	74	57	263	74	28

Table. 7 Relation of season to infection of mosquitoes in falciparum malaria.

Season	Cases		Mosquitoes: Infective Cases			Mosquitoes: All Cases		
	Infective	All	Diss.	Pos.	%Pos.	Diss.	Pos.	%Pos.
Hot (Feb.-May)	3	8	42	20	48	101	20	20
Rainy (Jun.-Oct)	13	22	139	58	42	265	58	22
Cool Nov.- Jan)	8	10	113	58	51	153	58	38

Experimental malaria in lower primates—During this period 42 attempts were made to infect mosquitoes by allowing them to feed on splenectomized gibbons with falciparum gametocytemias. Gametocyte concentrations in these gibbons ranged from less than 50 to 160,000 per cubic mm. A total of 23 individual gibbons have been used; five of them were used two or more times, at different stages of their individual infections, including consecutive daily feedings on three animals and seven feedings covering a 24-hour period at four hour intervals on one of them. Six strains of P. falciparum were involved, and these had been passed in gibbons from 1 to 20 times. Special procedures tried included post-feed incubation of mosquitoes at low and high temperatures, prefeeding of mosquitoes on normal blood and in vitro feeding of infected gibbon blood diluted with normal blood or with the plasma replaced with normal plasma. Both A. balabacensis and A. stephensi from laboratory strains were fed, and each of these species were known to be fully susceptible to falciparum malaria through concurrent experiments with human cases. All the above attempts have been unsuccessful.

As the result of the failure of attempts to infect mosquitoes on gibbons infected with falciparum malaria, experiments were undertaken to determine the stage at which the parasite fails to complete its development following ingestion by the mosquito. The exflagellation of microgametocytes was demonstrated in four of six falciparum-infected gibbons, but none of these animals were infective for mosquitoes fed upon them. Exflagellation was also observed, incidentally, in an ordinary thin smear prepared at the time of another feeding. Three series of attempts to find ookinetes in the guts of mosquitoes fed on gametocytemic gibbons, made at two hour intervals up to the 24th following blood meal ingestion, were negative. In the case of a single human infection, ookinetes were found in the mosquito gut within the first two hours of searching. Deformed gametocytes were more common in gut contents of mosquitoes fed on gibbons, and gametocytes became very difficult to find within 24 hours following ingestion.

A third gibbon (S-27) inoculated with falciparum sporozoites became patently positive after an incubation period of approximately 70 days. Two earlier successful attempts became patent after incubation periods of 44 and 45 days, respectively. Parasite levels in gibbon S-27 were highest on day 126 following inoculation, reaching 1500 parasites per 500 leucocytes. No parasites have been seen in this animal since day 147.

Summary—Entomological and malaria studies conducted in the vicinity of vivax malaria foci in Phatumthani Province in the central plain of Thailand indicated that Anopheles aconitus is a vector in that region. That species, as well as A. philippinensis, A. tesselatus and A. vagus exhibited pronounced exophilic biting behavior; on the other hand, A. campestris was frequently collected biting indoors in that area. Studies in Sadao district, Songkla Province, near the Thai-Malaysian border implicated A. balabacensis as the principal vector of malaria there. Biting activity of balabacensis was greatest between 1900 and 2000 hours in the Sadao district, whereas elsewhere in Thailand this mosquito exhibits much later (2400-0300) peaks in biting activity.

Anopheles balabacensis and A. stephensi were fed on falciparum and vivax gametocyte carriers in central Thailand. In falciparum cases the highest rate of infection of mosquitoes occured when the gametocyte density was 3000 per cubic millimeter. Falciparum carriers were more infective than vivax carriers. Cases tended to be more infective during the cool season, or if they high asexual parasitemias or were older than the median age.

Feedings of anophelines on splenectomized gibbons with blood-induced falciparum gametocytemias gave uniformly negative results. Gametocytes in gibbon infections are apparently immature and short-lived. The microgametocytes are capable of exflagellation, but the ookinete stage is not achieved.

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