SOME ENTOMOLOGICAL OBSERVATIONS ON MALARIA TRANSMISSION IN A REMOTE VILLAGE IN NORTHWESTERN THAILAND[1,2]

RALPH E. HARBACH, JOHN B. GINGRICH AND LORRIN W. PANG

U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences, APO San Francisco 96346-5000

ABSTRACT. Anopheline mosquitoes and their relation to malaria transmission were studied during the months of March, May and July in the Karen village of Mae Tha Waw located in the northwestern mountains of Tak Province. Thirteen species were captured on human bait during 80 man-nights of collecting. Four additional species were collected during routine larval surveys. *Anopheles minimus* and *An. maculatus* comprised 92.5% of the specimens captured biting man. *Anopheles minimus* and *An. nivipes* were implicated as vectors based on the detection of sporozoite infections using enzyme-linked immunosorbent assays for *Plasmodium falciparum* and *P. vivax*. *Anopheles dirus* was rarely encountered and probably played little part in transmission in Mae Tha Waw during the period of study. Information is provided on nightly biting activity, incidence of disease, infectivity and larval bionomics.

INTRODUCTION

This report is the result of entomological studies which were made in March, May and July 1986 to collect basic epidemiological and vector data relevant to malaria transmission at the end of the dry season and start of the wet season in a village-forest setting. A primary objective of the study was to determine sporozoite innoculation rates for congruous serological investigations related to antimalarial drug efficacy trials performed by the Department of Medicine of this institution. The studies were conducted in the Karen village of Mae Tha Waw located in the northwestern mountains of Tak Province some 480 km northwest of Bangkok. The village rests in a very narrow valley between forested hills which rise to elevations of 200 to 400 m. Small intermittent streams that are dry during most of the dry season drain the hills and feed into a larger stream that is divided into a network of channels within the village. Small rice fields at the north and south ends of the village are planted during the rainy season which extends from June to November. The fields are flooded at the beginning of July in preparation for planting.

Mae Tha Waw is an isolated village containing about 300 houses and a population of 2,500, mostly women and children. Houses are built on stilts, constructed of split bamboo with the front side largely or completely open and thatched with teak leaves. There are few animals in the village other than chickens, dogs and a few water buffalo.

The village is highly endemic for both falciparum and vivax malaria. A prestudy survey conducted in December 1985 showed a 30.4% monthly incidence of *Plasmodium falciparum* among school children. Transmission of falciparum malaria continued at lower levels during the study period with an average monthly incidence of 5.5%. The prevalence of vivax malaria at the start of the study was 36.6%.

METHODS

Human bait collections were made between 1830 and 0500 hours by two men sitting beside houses located near the north and south ends of the village. The men worked in shifts which changed at midnight. A rest period was taken during the last 10 minutes of each full hour. Mosquitoes were collected on a total of 20 nights during March, May and July. All anopheline mosquitoes were captured and the time of collection recorded. Specimens were identified and examined for parity the following morning. Parous individuals were bisected behind the second pair of legs, frozen in a non-specific protein blocking buffer (1% BSA, 0.5% casein, 0.01% Thimersol, 0.002% phenol red and PBS at pH 7.4) and transported to Bangkok for sporozoite analysis. Sporozoites of *Plasmodium falciparum* and *P. vivax* were detected and identified using the enzyme-linked immunosorbent assays (ELISA) developed by Burkot et al. (1984) and Wirtz et al. (1985). A marked positive ELISA (optical density equal to or greater than twice the lowest positive reference control which exceeded the highest negative control) of the head and anterior thoracic segments containing the salivary glands was defined as evidence that a mosquito was infectious.

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[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views or endorsements of the Department of the Army or the Department of Defense.

[2] Address for international mail: Rajvithi Road, Bangkok 10400, Thailand.

The incidence of falciparum malaria was determined from weekly blood smears done on a cohort of approximately 140 school children. As cases occurred, they were treated and removed from the cohort. Incidence was calculated from the weekly occurrence of new cases divided by the number of subjects in the cohort at midweek. The children were the control group (administered daily vitamin tablets) during a malaria prophylactic study conducted continuously between March and August 1986. Two hundred microscopic fields on a thick blood film (Giemsa stained) were examined before counting a slide as negative. The investigators supervised all malaria treatment: mefloquine + Fansidar (single dose) for falciparum malaria and chloroquine (four doses) + primaquine (14 doses) for vivax malaria.

Larval collections were made in and around the village to determine the types and abundance of habitats where anophelines occur. Other species of mosquitoes found in association with anopheline larvae were also collected and identified.

RESULTS

Six hundred and seven anopheline females were captured during 80 man-nights of collecting. The collections contained 13 species of *Anopheles: An. (Anopheles) barbirostris* van der Wulp, *An. (Ano.) peditaeniatus* (Leicester), *An. (Cellia) aconitus* Dönitz, *An. (Cel.) culicifacies* Giles, *An. (Cel.) dirus* Peyton and Harrison, *An. (Cel.) karwari* (James), *An. (Cel.) maculatus* Theobald, *An (Cel.) minimus* Theobald, *An. (Cel.) nivipes* (Theobald), *An. (Cel.) splendidus* Koidzumi, *An. (Cel.) tessellatus* Theobald, *An. (Cel.) vagus* Dönitz and *An. (Cel.) varuna* Iyengar (Table 1). *Anopheles minimus* and *An. maculatus* were the dominant species, accounting for 92.5% of the specimens. *Anopheles culicifacies* was next in abundance, representing 3.1% of the catch. The other 10 species were collected in very low numbers and represented only 4.4% of the total.

The overall patterns of biting activity for *An. minimus, An. maculatus* and *An. culicifacies* are shown in Fig. 1. *Anopheles minimus* were caught biting throughout the night with peak activity between 2100 and 2200 hours. The biting activity of *An. maculatus* commenced at a high rate immediately after dark, diminished sharply after 2000 hours, gradually declined further between 2000 and 2300 hours and continued at a low level until 0500 hours. *Anopheles culicifacies* appears to bite all night with possible periods of higher activity occurring shortly after dark and during the early morning hours. The three specimens of *An. dirus* were collected between the hours of 2200–2300 (March 27), 2400–0100 (May 28) and 0400–0500 (May 29).

More than 60% of all the specimens captured were parous based on the coiling pattern of ovarian tracheoles (Table 1). Specimens were not dissected and examined for the presence of oocysts but positive ELISAs on the thorax-abdominal portions of six *An. minimus* collected in May (1 specimen) and July (5 specimens) indicated the presence of intact or ruptured oocysts of *P. vivax*. Sporozoites were not detected in the head-thoracic portions of these specimens so they probably were not infective at the time of capture.

Sporozoites were detected in the head-thoracic portions of six specimens which had negative thorax-abdominal portions. Two specimens of *An. minimus* collected in May were strongly positive for *P. falciparum*. Three specimens of *An. minimus* collected in May (1 spec-

Table 1. Actual numbers of female anophelines captured during human bait collections made outside houses in Mae Tha Waw (March to mid-July 1986).

Species	Mar. 3–7	Mar. 24–28	May 5–9	May 26–30	July 7–11	Total
An. minimus	32 (75.0)[a]	9 (44.4)	73 (50.7)	183 (68.9)	44 (72.7)	342 (65.2)
An. maculatus	67 (56.7)	27 (66.7)	12 (50.0)	41 (65.9)	73 (45.2)	220 (55.5)
An. culicifacies	0	1 (0)	5 (60.0)	11 (63.6)	2 (100)	19 (63.2)
An. dirus	0	1 (100)	0	2 (100)	0	3 (100)
An. nivipes	0	0	0	1 (100)	3 (33.3)	4 (50.0)
An. karwari	1 (0)	0	0	0	0	1 (0)
An. varuna	2 (0)	0	0	0	0	2 (0)
An. splendidus	1 (100)	0	0	0	0	1 (100)
An. aconitus	0	0	7 (71.4)	0	0	7 (71.4)
An. tessellatus	0	0	0	1 (0)	1 (0)	2 (0)
An. vagus	0	0	0	0	4 (50.0)	4 (50.0)
An. barbirostris	1 (100)	0	0	0	0	1 (100)
An. peditaeniatus	1 (0)	0	0	0	0	1 (0)
Totals	106 (60.4)	38 (60.5)	97 (52.3)	239 (68.2)	127 (55.1)	607 (61.1)

[a] Numbers in parentheses are percent parous.

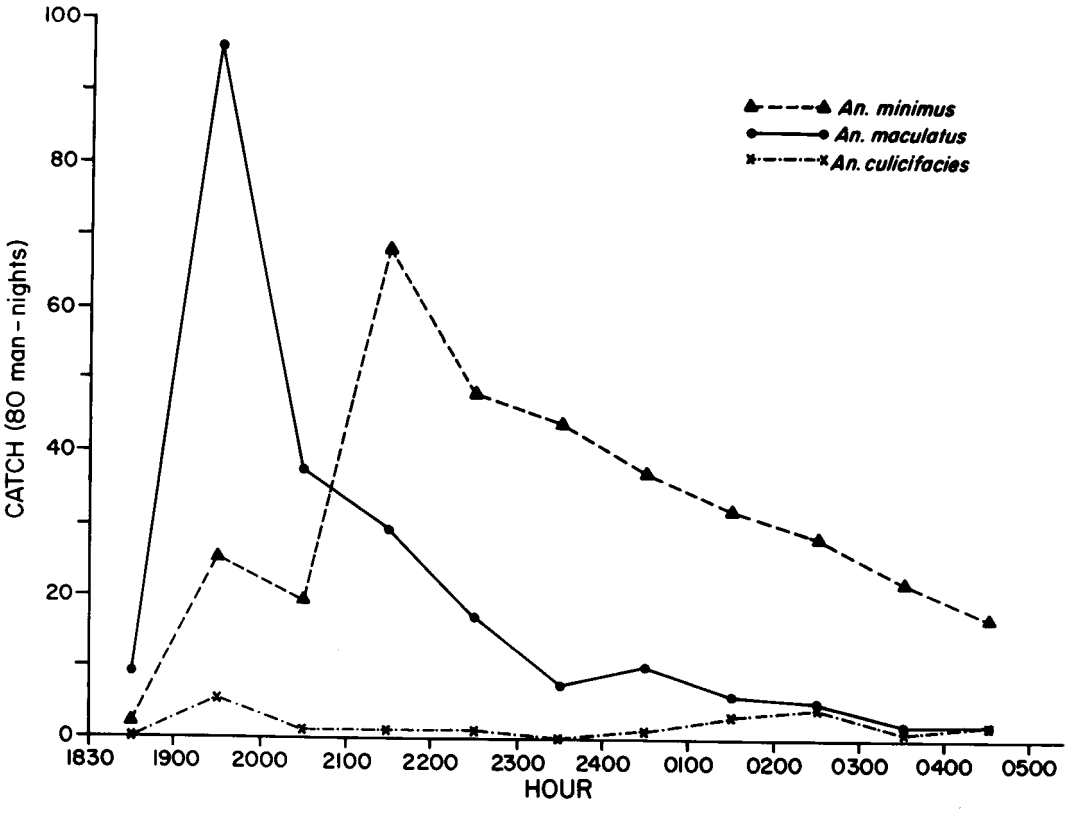

Fig. 1. Nocturnal biting activities of *Anopheles* females captured on humans outside houses in Mae Tha Waw (March to mid-July 1986).

imen) and July (2 specimens) and a specimen of *An. nivipes* collected in July were strongly positive for *P. vivax*. The infectivity rate was 1.0% for all anophelines collected and 1.6% for the parous specimens. The inoculation rate was 0.09 per man-night during May and 0.19 during July. Based on this data (Table 2), unprotected residents at Mae Tha Waw were likely to have received an average of three infective bites in May and six in July, assuming, of course, that indoor biting was the same as outdoor biting. There seems to be no direct correlation between the number of infective bites calculated from the data and the monthly occurrence of malaria among villagers. No infective mosquitoes were captured in March although the prevalence of vivax malaria was high.

Forty-one larval collections were made in and around the village during the periods when the biting collections were made. Thirty-two of these contained anopheline larvae. The species collected, their associations and the breeding places where they were found are listed in Tables 3 and 4. Larvae of *An. dirus* were not collected near the village, but a collection containing this species was made in July at a location about 2 km away. The larvae were found in a small partially shaded pool in soil without vegetation at the edge of forest.

DISCUSSION

Anopheles dirus is considered to be the primary vector of human malaria in the hilly, forested areas of Thailand. Although this is probably true in many areas, particularly during the rainy season (Wilkinson et al. 1978, as *An. balabacensis* Baisas), there is no published information which shows that this is true throughout its distribution or during all seasons. The results of the present study suggest that *An. minimus* may be the principal vector species in some areas during the dry season and early part of the rainy season when populations of *An. dirus* are low. *Anopheles minimus* was considered to be the primary vector of malaria in Thailand until Scanlon and Sandhinand recognized *An. dirus* as a major vector in 1965. Natural infections in *An. minimus* were first found in Thailand by Payung-Vejjasastra (1935) and the capacity of this species to carry sporozoites of human plasmodia is well documented. This is the first time

Table 2. Summary of malariometric and entomological findings made at Mae Tha Waw (March to July 1986).

Month	Incidence of falciparum malaria	Prevalence of vivax malaria	Man-nights	Mosquitoes caught	Infective mosquitoes	Percent infective	Infective bites/man-night
March	4.8	36.6	32	144	0	0 (0)[a]	0
April	4.3	—	—	—	—	—	—
May	6.5	—	32	336	3[b]	0.9 (1.4)	0.09
June	8.0	—	—	—	—	—	—
July	3.7	—	16	127	3[c]	2.4 (4.3)	0.19
Total	—	—	80	607	6	1.0 (1.6)	0.08

[a] Percent of total catch (percent of parous specimens).
[b] *An. minimus* (2 positive for *P. falciparum*; 1 for *P. vivax*).
[c] *An. minimus* (2 specimens) and *An. nivipes* (1 specimen) positive for *P. vivax*.

Table 3. Larval associations from collections made at Mae Tha Waw.

	An. barbirostris	*An. barbumbrosus*	*An. annularis*	*An. culicifacies*	*An. indefinitus*	*An. jamesii*	*An. karwari*	*An. maculatus*	*An. minimus*	*An. nivipes*	*An. vagus*	*Ur.* sp.	*Cx. bitaeniorhynchus*	*Cx. fuscocephala*	*Cx. mimeticus* subgr.	*Cx. vishnui* subgr.	*Cx. nigropunctatus*	*Cx. (Lop.)* sp.
An. barbirostris	■							X				X						
An. barbumbrosus		■						X										
An. annularis			■						X	X	X		X			X		
An. culicifacies				■		X		X	X		X							
An. indefinitus					■			X										
An. jamesii				X		■	X		X		X					X		
An. karwari							■	X										
An. maculatus	X	X		X	X		X	■	X		X	X	X	X		X	X	X
An. minimus			X	X		X	X	X	■		X	X			X	X		X
An. nivipes			X							■	X		X			X		
An. vagus			X	X		X	X	X	X	X	■		X			X		

that ELISA methodology has been used to incriminate *An. minimus* as a vector of malarial parasites.

Both *Anopheles nivipes* and its sister species, *An. philippinensis* Ludlow, occur in Thailand (Klein et al. 1982, Klein et al. 1984). *Anopheles philippinensis* has been found infected with human malaria parasites in India (Covell 1944, Ganguli 1947), Bangladesh (Quraishi et al. 1951) and Burma (Feegrade 1926), and is considered a suspected or potential vector in Thailand (Harinasta [*sic*] 1966, Harinasuta et al. 1976). *Anopheles nivipes* was not recognized as a distinct species until Reid (1967) distinguished it from *An. philippinensis* and it has never before been implicated as a malaria vector. Reid (1967) attributed the infections found by Feegrade in Burma to *An. philippinensis*, but it is possible that this record, as well as those for India and Bangladesh, actually refer to *An. nivipes*. These species occur together from India to Vietnam (Reid 1968).

Adult females of *An. nivipes* and *An. philippinensis* are morphologically very similar and the characters used to separate them are sometimes unreliable. The specimens captured during

Table 4. Breeding places of *Anopheles* larvae collected at Mae Tha Waw.

	stream margin	stream pool	pit in gravel on stream shore	ditch (streamlet)	marshy depression	ground pool	flooded pool (in rice field)	animal track	seepage
An. barbirostris	X								X
An. barbumbrosus	X								
An. annularis							X		
An. culicifacies	X	X		X		X		X	
An. indefinitus				X					
An. jamesii	X				X				X
An. karwari	X						X	X	X
An. maculatus	X	X	X	X		X		X	
An. minimus	X	X	X				X	X	
An. nivipes							X		
An. vagus				X			X		

this study were readily identified as *An. nivipes* by the presence of a long presector dark spot on wing vein R (Reid 1967, 1968; Harrison and Scanlon 1975). Recent cytological analyses of ovarian polytene chromosomes have shown that two distinct biological species are confused under the name of *nivipes* in Thailand (Baimai et al. 1984; C. A. Green, personal communication). These species must be differentiated before it will be possible to assess their individual roles in the epidemiology of malaria.

Anopheles maculatus is the nominotypical member of a sibling species complex which consists of seven morphologically and genetically distinguishable species in Thailand (Rattanarithikul and Green 1987;[3] C. A. Green, personal communication). Three of these species, species A, B and I, were collected at Mae Tha Waw. Species A was the most abundant (98/220), species I was second in abundance (86/220) and species B was least abundant (36/220). Species B (= *maculatus*) is a major vector of malaria in peninsular Thailand, but it is thought to be of little or no importance elsewhere in the country. There is no evidence from this study to indicate which, if any, of these species are malaria vectors in Mae Tha Waw, but too few specimens were collected to conclusively determine their relationship to malaria transmission.

Infections were not detected in any of the other species collected at Mae Tha Waw although most of them, including *An. barbirostris, An. aconitus, An. annularis, An. culicifacies, An. splendidus, An. tessellatus, An. vagus* and *An. varuna*, have been found naturally infected with malarial parasites in areas outside of Thailand. However, the numbers of females of these species assayed for sporozoites (*An. annularis* was collected in the larval stage only) were too small to determine their involvement in malaria transmission. Further investigation is needed.

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[3] NOTE ADDED IN PROOF: The article by Rattanarithikul and Green (1987) was published while this paper was in press. Species A is a new species, *An. sawadwongporni*, species B is *An. maculatus* and species I is *An. pseudowillmori*, a species previously regarded as a junior synonym of *An. maculatus*.

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