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HYPERENDEMIC MALARIA IN A FORESTED, HILLY MYANMAR VILLAGE

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ABSTRACT. A 1-year longitudinal study of hyperendemic malaria was carried out at Tha-bye-wa village, Oktwin township, situated in the forested Bago mountain range in south-central Myanmar. Mosquito infectivity was assayed using specific, sporozoite enzyme-linked immunosorbent assays. *Anopheles dirus* was the predominant vector in the postmonsoon season (October); during the cool-dry season (January), both *An. dirus* and *Anopheles minimus* were vectors. Members of the *Anopheles culicifacies* complex were caught in the hot-dry season (April) but none was infective. The entomological inoculation rate was estimated to be at least 13.7 infective bites/person/year. Infective *An. dirus* were caught feeding on cattle as well as on humans. Three of the 4 positive *An. dirus* and both positive *An. minimus* were caught biting humans indoors in the second quarter of the night when most people were sleeping. This suggests that use of insecticide-impregnated bednets in this area could interrupt transmission.

INTRODUCTION

In Myanmar, malaria has been identified as one of the most important public health problems in every national health plan since 1978. Since 1984, the number of confirmed malaria cases has been consistently above 60,000, with >80% due to *Plasmodium falciparum.* Since 1986, annual mortality has been >8/100,000 population (>3,000 deaths), with most being nonimmune adult males who became infected working in the forests (Tin and Tun 1991).

About 60% of all malaria cases in Myanmar are reported from the forest and forest fringe areas. Dense forests cover an area of 324,288 km^2 or about 48% of the total area of the country; 20% of the population live in these forested areas, which are characterized by difficult terrain and poor accessibility. Studies on forest malaria transmission have been carried out in neighboring countries such as Bangladesh (Rosenberg and Maheswary 1982) and Thailand (Gingrich et al. 1990, Rosenberg et al. 1990b). In Myanmar, preliminary studies were carried out in Tha-bye-wa village, which is situated in a hyperendemic area deep inside a forest. From October 1992 to July 1993 we studied transmission in the village more intensively. Our objective was to obtain the first seasonal baseline data on malaria transmission in the deep forest of Myanmar, including vector incrimination, sporozoite inoculation rates, seasonal vector behavior and abundance, and larvae habitats. The ultimate goal of these studies was to help in formulating an improved malaria control program.

MATERIALS AND METHODS

Study area: The study site—Tha-bye-wa village, Oktwin township, Bago division—is situated on the eastern slopes of the Bago mountain range (18°35′N, 96°10′E) at about 210 m above sea level. It lies in a valley on the Oktwin Paukkaung–Prome road, which is the only road going through the Bago Mountains, and is completely surrounded by upwards of 15 km of teak forests (Fig. 1). There is no public transportation, except trucks carrying teak logs along the mountainsides, and few public services. For example, there are no volunteer health workers. The area is highly endemic for malaria. Previous surveys found the spleen rate in 2–9 year olds to be 68.9%, with an average enlarged spleen index (AES) of 1.87. The combined parasite prevalence was >30%. Tha-bye-wa had not received interior spraying of DDT or other insecticides for more than 10 years.

The study village consisted of 50 households with a population of 220 (109 females and 111 males). There were 10 infants at the start of the study and 52 children between 2 and 9 years of age. More than 50% of the households said they had bednets and used them. Visitors are common (about 10% of the population studied); generally they stay from 1 wk to several months.

Entomology: Quarterly longitudinal surveys were done in October 1992 (postmonsoon), January 1993 (cool-dry season), April 1993 (hot-dry season), and July 1993 (monsoon season). The duration of each survey, which required approximately 40 man-nights each, was 2 wk. One cattle-bait and two human-bait catching stations were used. Mosquitoes were collected by the following methods:

1) Human-bait landing catches (indoor and outdoor collections) in which the collector caught all landing mosquitoes with a mouth aspirator.

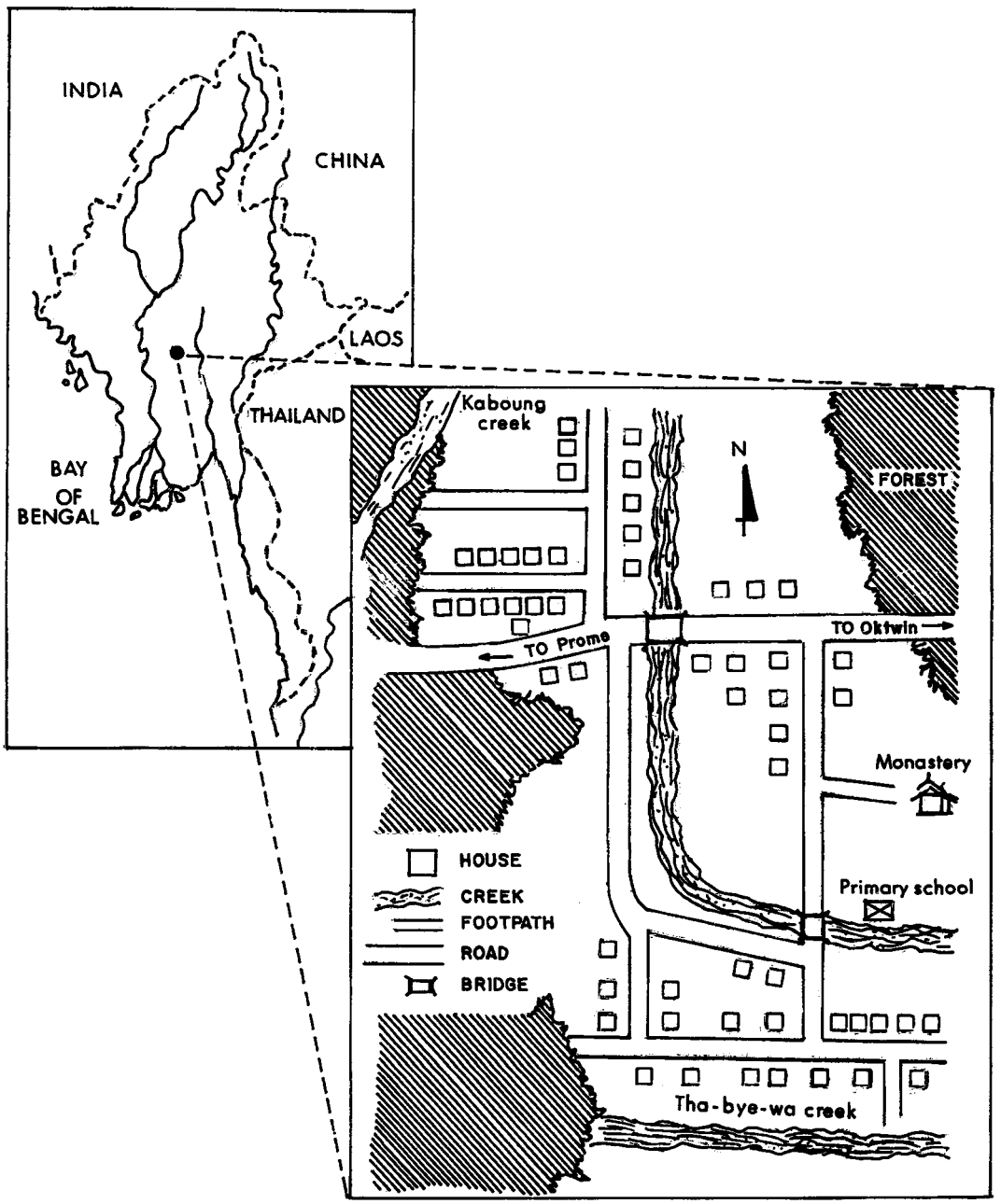

Fig. 1. Schematic diagram of study area (Tha-bye-wa village), Oktwin township, Bago division, 1992–93.

2) Large bednet (330 × 330 × 180 cm) catches with 2 adult humans sleeping in the net as bait. *Anopheles* found inside the net were collected hourly.
3) Large bednet (330 × 330 × 180 cm) hourly catches with a cow as bait, to estimate the zoophilic behavior of the *Anopheles* collected.

To eliminate bias, the sites between the second human-bait catching station and the cattle-bait catching station (together with their respec-

Table 1. Quarterly age-specific parasite rates and cumulative gametocyte rates by surveys in Tha-bye-wa village, Oktwin township, 1992–93.

Age-group (years)	Age-specific parasite rate (no.)			
	Oct. 92	Jan. 93	Apr. 93	Jul. 93
<1	60% (5)	67% (6)	0% (5)	33% (6)
1–<2	0% (4)	57% (7)	60% (5)	100% (3)
2–4	53% (17)	50% (14)	58% (19)	21% (14)
5–9	54% (24)	42% (24)	26% (23)	35% (20)
10–14	20% (15)	14% (22)	19% (21)	13% (15)
≥15	20% (81)	27% (109)	10% (92)	24% (110)
Mean	30.1% (146)	31.3% (182)	20% (165)	25.6% (168)
Gametocyte	0.68% (146)	1.65% (182)	0% (165)	1.19% (168)

tive large bednets) were interchanged midway during each survey period. Occasional catches were made at other village sites to gain additional information on vector distribution. The human-bait subjects were given mefloquine prophylaxis regimen on a weekly basis for 6 wk, beginning 1 wk before deployment. Larval surveys were carried out randomly every alternate day within a 3-km radius of the study village.

Vector and malaria parasite identification: Enzyme-linked immunosorbent assays (ELISAs) for circumsporozoite protein were performed according to Wirtz et al. (1987). *Plasmodium vivax*-variant (VK247) and *Plasmodium malariae* could not be detected with the ELISA kits available. A Titertek Multiscan® MCC/340 MK II ELISA plate reader was used. The *Anopheles* collected were identified according to Peyton and Scanlon (1966), Reid (1967), Harrison (1980), and Myo-Paing et al. (1990b). Except in the *Anopheles dirus* complex, no attempt was made to identify sibling species (e.g., for *Anopheles culicifacies* [Subbarao et al. 1988] and *An. maculatus* [Green et al. 1992]). After identification the head–thoraces of mosquitoes were separated and dried. At the laboratory, these specimens were stored at −80°C until the ELISA tests could be done 2–4 months later. Specimens were put in labeled 1.5-ml microcentrifuge tubes and ground in 50-μl BB NP-40 (5 μl NP-40/ml BB). The pestles were rinsed in PBS-Tw twice between grindings. The results were read visually and at 414 nm 30 or 60 min after adding substrate. Specimens were considered positive if their optical density values were greater than the mean value plus 3 to standard deviations of the negative controls (suspensions of triturated 3-day-old laboratory-bred *An. dirus* mosquitoes). The positive threshold was 0.063 for *P. falciparum* and 0.111 for *P. vivax*. Positive controls consisted of various serial dilutions of the provided circumsporozoite antigens.

Parasitology and spleen examinations: Thick blood films were taken from all persons present in the village during each survey. All blood films were stained with Giemsa and examined in the field and again checked in the laboratory by viewing 50 microscopic fields under 1,000× oil immersion. The same person checked all negative blood films by examining 100 microscopic fields. Only cases that were severe or complicated, or those with high parasite counts, were given antimalarial drugs (chloroquine or sulphadoxine-pyremethamine or mefloquine) according to standard regimens. All children between 2 and 9 years of age were examined for spleen enlargement using Hackett's index (Bruce-Chwatt 1985).

Meteorological data: Data were obtained from the meteorological station near Oktwin township and from the measurements carried out at the study village. The highest rainfall was 411 mm during the month of June 1993. The relative humidity was >80% in June 1993 and was lowest during the hot-dry period (<60% in February–April 1993). The lowest temperature recorded was 13.5°C in January 1993 and the highest was 37.6°C in April.

RESULTS

The prevalence of *P. falciparum* was >80% in all seasons; *P. vivax* was 6.1–12.3%; mixed infections ranged from 0 to 6.1%. Total parasite density index (PDI) was highest in postmonsoon (3.23) and lowest in the dry-hot season (1.85). The age-specific parasite and cumulative gametocyte rates for *P. falciparum* and *P. vivax* combined are shown in Table 1. The infant prevalence was highest in the cool-dry season (4/6 = 66.7%) as was the gametocyte rate (1.7%), both of which were lowest during April. Of the 177 cases detected during the study, 51 were visitors to the village.

Table 2. Total number of *Anopheles* caught in Tha-bye-wa village, Oktwin township, 1992–93.

Anopheles	Number caught				
	Human				
	Bednet	Indoor	Outdoor	Cattle	Total
dirus	93	23	31	71	218
minimus group	20	26	21	52	119
culicifacies group	127	38	60	712	937
maculatus group	10	35	36	210	291
annularis	1	0	2	0	3
philippinensis	0	0	1	0	1
aconitus	4	3	7	10	24
vagus	48	9	11	69	137
stephensi	71	39	78	103	291
varuna	7	3	6	22	38
kochi	11	3	4	16	34
tesselatus	6	4	7	6	23
Total	398	183	264	1,271	2,116

A total of 2,116 *Anopheles* representing 12 species were collected (Table 2); seasonal man-biting rates (per night and per year) are presented in Table 3. Using polytene chromosome analysis (Baimai et al. 1988b), the *An. dirus* were found to be species D (Myat-Myat-Thu, unpublished data); Baimai et al. (1988b) had earlier found exclusively *An. dirus* D at Tha-bye-wa village. The human-to-cattle biting ratio of *An. dirus* was 1.3:1 (n = 162) compared with 1:2.5 (n = 73) for *Anopheles minimus* Theobald (χ^2 = 15.15, $P < 0.0001$). Difference in the indoors to outdoors feeding patterns was not significant (χ^2 = 1.63, $P > 0.05$).

More than 53% of *An. dirus* Peyton and Harrison landing on humans were caught during the second quarter of the night (2100–2400 h), whereas *An. minimus* bit about equally in both the first and second quarters (47.1 and 42.6%) (Table 4). The difference in the biting period of *An. dirus* and *An. minimus* was significant (χ^2 = 4.68, P = 0.03).

Based on ELISA results (Table 5), *An. dirus* and *An. minimus* were the principal vectors. With the exception of one *An. dirus* caught biting a cow, all positives were caught indoors during October and January attempting to feed on human bait. The cow-biting *An. dirus* was positive for both *P. falciparum* and *P. vivax*, the remaining *An. dirus* were positive for only *P. falciparum*, and both *An. minimus* were positive for only *P. vivax*. The entomological inoculation rate (EIR), calculated from human landing

Table 3. Man-biting rates (MBR) of *Anopheles* in Tha-bye-wa village, Oktwin township, 1992–93.

Anopheles	MBR (per night)				MBR (annual)[1]
	Oct.	Jan.	Apr.	Jul.	
dirus	2	0.10	0.07	2.25	401
minimus	0	0.80	0.60	0.40	164
culicifacies	0	0.08	5.30	0.03	494
maculatus	0.22	1.70	0.10	0	184
annularis	0.05	0	0.02	0	6
philippinensis	0.02	0	0	0	2
aconitus	0	0.35	0	0	32
vagus	0.08	0.05	0.77	1	173
stephensi	0	0.37	4.80	0	472
varuna	0	0.07	0.19	0.17	39
kochi	0.50	0	0	0	46
tesselatus	0	0	0	0.48	44

[1] Mean MBR (per night) × 365.

Table 4. Human landing rhythms of confirmed and suspected malaria vectors in Tha-bye-wa village, Oktwin township.

Time (h)	Percent total caught			
	Anopheles dirus (n = 147)	*Anopheles minimus* (n = 68)	*Anopheles culicifacies* (n = 224)	*Anopheles maculatus* (n = 81)
1800–2100	29.9	47.1	36.6	49.4
2100–2400	53.7	42.6	35.7	50.6
2400–0300	16.4	10.3	13.4	0.0
0300–0600	0.0	0.0	14.3	0.0

Table 5. Numbers of *Anopheles dirus* and *Anopheles minimus* captured on humans and number sporozoite-positive by ELISA.

Survey date	Number tested (sporozoite-positive)		
	An. dirus	*An. minimus*	Sum
Oct. 1992	40 (3)	0	40 (3)
Jan. 1993	7 (1)	32 (2)	39 (3)
Apr. 1993	2 (0)	23 (0)	25 (0)
Jul. 1993	50 (0)	12 (0)	62 (0)
Total	99 (4)	67 (2)	166 (6)

catches, for 160 man-nights was 0.0345 or 13.7 infective bites/year.

In larval surveys, *An. dirus* larvae were collected from small rock pools under dense shade 1.5 km from the southwestern edge of the village in both the postmonsoon and cool-dry seasons. During the hot-dry season we could not find the breeding sites. During the monsoon season *An. dirus* immatures were also found in bamboo stumps in the deep forest. *Anopheles minimus* was found in small jungle streams during January, April, and July, whereas *An. maculatus* was found during the October, January, and April surveys in these same sites. During the hot-dry season, the breeding sites of *An. culicifacies* Giles were found (together with *Anopheles stephensi* Liston) in small pools in the beds of the village creeks and in unused, hand-dug water pits in the partially dried-out creek beds within and around the village.

DISCUSSION

In Myanmar, *An. dirus* appears to be adapted to a wider variety of shady habitats than has been reported elsewhere. We have already reported on its occurrence in water wells within a coastal village where all shade comes from occasional fruit trees and scrub (Tun-Lin et al. 1986, 1988). Baimai et al. (1988b) have identified only *An. dirus* species D from materials originating in Kwan-ka-thaung (well-breeding *An. dirus*) and Oktwin (forest-breeding *An. dirus*), Myanmar; all specimens examined from Bangladesh have also been cytogenetically classified as species D (V. Baimai, unpublished data). In contrast, the predominant *An. dirus* vector in northern and eastern Thailand has been species A. The site we chose for this study differs from those in neighboring countries such as Bangladesh (Rosenberg and Maheswary 1982) and Thailand (Ismail et al. 1974, 1975; Rosenberg et al. 1990a, 1990b, 1990c) by being in an area that has received little or no insecticide treatment.

A variety of practical constraints limited our study at this remote area to only one period per quarter; therefore the interpretation of our findings is necessarily more suggestive than if we had been able to conduct monthly collections. Our results suggest that, similar to the findings in Thailand (Ismail et al. 1974), *An. dirus* (as *Anopheles balabacensis* Baisas) appeared to transmit malaria year-round in the deep forest but with peak transmission during the postmonsoon and cool-dry seasons. In our study during the cool-dry season, both *An. dirus* and *An. minimus* gave positive ELISA results, and this was supported by the high infant parasite, gametocyte, and crude parasite rates in this season. This finding was also reported in Thailand showing highly efficient dry-season transmission of malaria (Rosenberg et al. 1990b). Because the principal vectors gave positive ELISA results, and our collections were made in or near dwellings, we concluded that malaria was transmitted within the village.

Unlike most other studies, we found evidence that cattle may be deflecting parasite-positive *An. dirus* from humans. The human-to-cattle biting ratio of *An. dirus* was 1.3:1, and parasite-positive rates from *An. dirus* caught from cattle and humans were found to be 1% (1/98) and 4% (4/99), respectively. *Anopheles minimus* was found to bite cattle more frequently than *An. dirus* ($P < 0.01$). In Thailand, *An. minimus* was also found to be zoophilic (Ratanatham et al. 1988), and the animal-biting densities were high throughout the year. We did not determine parasite-positive rates of *An. minimus* caught from cattle; all *An. culicifacies* or *An. maculatus* were found negative.

During the postmonsoon season, 3 parasite-positive *An. dirus* from human bait and a single parasite-positive *An. dirus* from cattle bait were caught biting in the second quarter of the night. Most villagers were asleep by then, suggesting the potential importance of bednet use. In his studies in southern Thailand, Baimai et al. (1988a) found that out of 128 *An. dirus* D collected from humans, 60 mosquitoes (47%) were caught biting in the third quarter (2400–0300 h) of the night. Only 8 mosquitoes (6%) and 29 mosquitoes (23%) were caught in the first (1800–2100 h) and second (2100–2400 h) quarters of the night, respectively. According to Ratanatham et al. (1988) in studies carried out in upper central Thailand, however, *An. minimus* tended to be an early evening biter of humans in the cool-dry season and an early morning biter of humans in the wet season, thereby increasing the chance of human–vector contact. In this

unsprayed area, *An. dirus* bit almost equally indoors and outdoors; the houses were without doors or proper walls. *Anopheles minimus* was also found to bite indoors slightly more than outdoors contrary to the findings in the foothill area (Myo-Paing et al. 1988). The 2 parasite-positive *An. minimus* caught during the cool-dry season were also caught biting indoors.

During a preliminary study in July 1987, the man-biting rate of *An. dirus* was 4.6 (Myo-Paing et al. 1989), which is about twice the rate we obtained in July 1993. During a preliminary study in February 1989 (cool-dry season) at the same study site (Myo-Paing et al. 1990a), the crude parasite rate was 46.8%, and the infant parasite rate was 75% (3/4). This is much higher than our findings (31.3 and 66.7%, respectively) in January 1993 (cool-dry season), although the sample size is too small for significance. Although a number of factors could cause the apparent reduction in malaria cases, the opening of a small private pharmacy selling indigenous and antimalaria drugs as well as the effects of commercial logging may have had an impact. The establishment of more small pharmacies could curb malaria disease, even though transmission would be little affected, and so should be encouraged and given support under the supervision of the local health authorities.

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