Field trials of different traps for malaria vectors and epidemiological investigations at a foot-hill basin in Kanchanaburi, Thailand*

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(Received: July 27, 1988)

Key words: malaria endemicity, vector prevalence, response, wing-sound, breeding site.

Abstract: Malaria endemicity and the vector prevalence were investigated by using biting collections and trapping systems at a foot-hill malaria endemic area in Kanchanaburi, Thailand. A breeding basin, a stream-bed in a village Ban Tha Lam Yai, was selected as the study area. The buffalo bait caught the highest number of species (16 species) and individuals of anopheline mosquitoes; the number of individuals was 4 times more *Anopheles minimus* and 3 times more *An. maculatus* than that with the human bait. High parous rates of the two vectors throughout the year, 50% in September and 90% in May for *An. minimus*, 43% in October and 74% in March for *An. maculatus*, seemed to be participating in high infection rates of them and the stability of malaria endemicity in the village. The vectors were efficiently attracted to sound traps when those were combined with a hamster and dry ice. The number of vectors trapped was 3 times more than that with the human bait, but nearly a half of that with the buffalo bait. Sound traps segregated *An. minimus* into two groups according to the attractiveness at different sound frequency: one was 500–600 Hz and another was 800–1,000 Hz. *An. maculatus* was, however, attracted with a similar pattern to every frequency range from 350 to 800 Hz.

INTRODUCTION

Among anopheline mosquitoes, *Anopheles dirus*, *An. maculatus* and *An. minimus* have been known as principal vectors of human

malaria in Thailand. For decades, malaria control in Thailand has been carried out by using insecticide residual spray. This method has raised insecticide resistance in the vectors, and health hazards including environmental pollution, toxic side-effects and a high inci-

* This study was financially supported partially by grants-in-Aid (No. 61041079, No. 62043075 and No. 01044130) for Overseas Scientific Research from the Ministry of Education, Science and Culture of Japan and partially by a collaborative Research Project grant from PRO/WHO to the Collaborating Centre for Research and

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dence of destruction of non-target organisms have also resulted. Efforts have been made to develop other control methods, such as physical and biological methods (Ismail, 1983; Malaria Division, 1985). In malaria vector investigation a survey on population dynamics, actual transmission data on the malaria vectors and the human bait method are still being used, but there has been no improvement in results. Because the operation of this method poses major hazards for malaria workers, less hazardous methods have been sought. Recently a trap using artificial wingbeat sound has been proved to be an effective technique for trapping males of *Culicine* and *Mansonia* (Ikeshoji, 1985; Ikeshoji *et al.*, 1985; Kanda *et al.*, 1987, 1988). Furthermore, the success of sound trapping of females of these species was also reported (Kanda *et al.*, 1987; Leemingsawat *et al.*, 1988), the efficacy of this sound trap system has been demonstrated with genus *Mansonia*, vector of filariasis by Kanda *et al.* (1988), and with *Culex tritaeniorhynchus*, vector of Japanese encephalitis (Thongrungrat, unpublished). The present study was designed to select a malaria endemic area as the study site to reveal the epidemiological background, such as the parasite rate in the community, vectorial population dynamics and habitat. The physio-ecological characteristics of these vectors were utilized to apply the attraction to a sound trap system to their surveys and control. Thus, the present paper reports that use of the sound trapping collection method demonstrated its efficacy in monitoring population dynamics in comparison with bait traps and in proving the possibilities for reduction of the mosquito density of malaria vectors, *An. minimus* and *An. maculatus* in the study site.

MATERIALS AND METHODS

The selected study site is the stream-bed of a foot-hill basin (Fig. 1) in a village with a population of 1,773. The village, Ban Tha Lum Yai, 2 km distant from Ratanakosin Reservation Park, is a known endemic area of human malaria in Kanchanaburi (Malaria Division, 1986-1987). Major crops in the village are maize and sugar-cane, and cattle are raised for farm work. Houses are scat-

tered along a creek which runs through the middle of the village. The study was carried out from February 1987 to January 1988.

Blood survey. Malariometric active case detection was performed by examining blood smear of malaria cases or of people with malaria-like symptoms, to find out the malaria incidence in the village.

Mosquito collection. 1) Human and animal baits: The buffalo bait trap was performed using the same buffalo bait trap for two consecutive nights at intervals of approximately 30 days, to know the seasonal prevalence, to compare with other trapping methods, and to know population dynamics as suggested from their physiological age at the stream-bed, the main breeding site of the malaria vectors in the village. A part of the female anopheline vectors collected by the buffalo bait were dissected for evaluation of the parous rates in relation to mosquito longevity. Living baits of both human and animals (buffalo, dog, hamster and chicken) were used to know the host preference of anopheline mosquitoes and also to compare the efficacy of mosquito collection among other trapping methods. In bait collection with a buffalo, a mosquito net trap described by Kanda (1979) (Fig. 2) was used. The other animal bait trappings were carried out by adopting the same ones described by Sucharit and Supavej (1987). In these human bait and animal bait trappings, two of each animal were fixed side by side at about 50 m intervals at the stream-bed for 2 consecutive nights at the end of January 1988. Outdoor human bait trapping was performed on two men at the stream-bed, 50 m apart, by biting catch collection during September 1987 to January 1988.

2) Sound traps: Two types of modified sound traps (cage and cylinder traps) designed by Kanda *et al.* (1988) (Figs. 3 and 4) were used to compare their attractiveness. One with various frequencies of wingbeat sound was also used. These sound traps combined with a hamster and dry ice (Kanda *et al.*, 1988) were set at fixed sites in the stream-bed (Fig. 1) with the sound traps alternately emitting different wingbeat frequencies in their respective operation. All the sound traps were set from 6:00 p.m. to 6:00 a.m. for two consecutive nights. They

Fig. 1 Study site, a basin in Ban Tha Lam Yai, Kanchanaburi Province.
 Fig. 2 Buffalo bait trapping, a buffalo covered with a mosquito net as animal bait.
 Fig. 3 Setting a sound trap connecting cylinder and cage (see text).
 Fig. 4 Counting and identification of mosquitoes trapped (identification is tentative).

were at a height of 0.5 m above the ground in the shade of a bush or low tropical tree at 30 m intervals along the stream-bed (Figs. 1 and 3). Sound was emitted under the conditions described by Kanda *et al.* (1988). A soundless control trap was also set similarly without a soundspeaker.

Comparison was made of the efficacy of the four different systems of traps with two each of buffalo bait and human bait at the study site in the same night in March and August, 1987. Response of female mosquitoes to the two types of trapping systems (cage type and cylinder one) emitting various frequencies of sound with dry ice and hamster was tested by using 2 traps of each for two

consecutive nights in August 1987 (Table 7). In view of the higher trapping efficacy among various sound traps, four cage-type sound trap systems, respectively emitting 500, 600, 700, 800 and 1,000 Hz of wing sound frequencies, were tested for the response of *An. maculatus* and *An. minimus* at the same site for two consecutive nights around each month-end from March 1987 to January 1988.

3) Mosquito counting and identification: All mosquitoes collected were counted, identified with hand lens (Fig. 4) and recorded on the following day. Identification of *An. maculatus* group was made according to the key of Rattanarithikul and Green (1986).

RESULTS

1. Blood survey

The results of active case detection are shown in Table 1. The average monthly positive rates were 29% in 1986 and 24% in 1987, the highest were 40% in August 1986 and 41% in December 1987. The data indicate high parasite rate during these 2 years with no significant decrease. This fact suggested epidemiological stability and high endemicity of malaria.

2. Mosquito surveys

Monthly prevalence of anopheline mosquitoes was surveyed by buffalo bait trap-pings from February 1987 to January 1988 (Table 2), and 16 species of *Anopheles* were collected. Among these species the principal malaria vectors, *An. maculatus* and *An. minimus*, were highly abundant, especially from August to November. The parous rates of *An. maculatus* and *An. minimus* collected from a buffalo bait trap are shown in Table 3. The rates were high throughout the period, ranging 50 to 90% in *An. minimus* and 43 to 74% in *An. maculatus*. Slightly lower rates were observed in September, October

and November when the mosquito density was higher (Table 2). In addition to buffalo bait traps in the village, the relative importance of all suspected blood donors: human, dog, chicken and hamster, were used for bait traps by simultaneous catch at the stream-bed. The results of simultaneous bait traps are shown in Table 4. *An. minimus* fed on all the baits tested, and *An. maculatus* bit 4 kinds of baits except for chicken. But other species did not bite any bait except buffalo. This result indicates that buffalo may be the main blood donor to the vectors; human, a partial donor and dog, to a lesser extent. Similarly, only *An. maculatus* and *An. minimus* were attracted to human bait according to data obtained from September 1987 to January 1988 (Table 5). Thus, it is strongly suggested these two species are the principal vectors in the village. Among 3 forms of *An. maculatus*, A, B and G, form A was most abundant.

Comparison of effectiveness of various collection methods is shown in Table 6. A buffalo bait trap and a sound trap combined with dry ice and a hamster were highly effective out of 9 kinds of traps tested. Response to various frequencies of wingbeat sound and type of trap systems, (cage type

Table 1 Number of malaria parasite positive cases among the community at Ban Tha Lum Yai, Kanchanaburi, in 1986 and 1987.

Month	1986					1987				
	No. exam.	No. posit.	P.f.	P.v.	Posit. rate	No. exam.	No. posit.	P.f.	P.v.	Posit. rate
Jan.	279	80	39	41	0.29	187	51	18	33	0.27
Feb.	196	41	26	15	0.21	160	38	5	33	0.24
Mar.	179	34	21	13	0.19	112	30	9	21	0.27
Apr.	169	26	18	8	0.15	145	45	16	29	0.31
May	282	85	28	57	0.30	252	47	24	23	0.19
Jun.	333	84	43	41	0.25	530	112	60	52	0.21
Jul.	455	158	95	63	0.35	591	115	74	41	0.19
Aug.	490	198	140	58	0.40	428	97	45	52	0.23
Sept.	437	165	109	56	0.38	591	115	74	41	0.19
Oct.	366	121	76	45	0.33	381	57	22	35	0.15
Nov.	236	78	38	40	0.33	556	148	84	64	0.27
Dec.	193	61	26	35	0.32	195	80	38	42	0.41
Mean	301.2	94.3	54.9	39.3	0.29	344.0	77.9	39.1	38.8	0.24

P.f., *Plasmodium falciparum*; P.v., *Plasmodium vivax*.

Table 2 Number of *Anopheles* collected with buffalo bait traps in Ban Tha Lum Yai, Sri-Sawat, Kanchanaburi, from Feb. 1987 to Jan. 1988.

<i>Anopheles</i>	Feb.	Mar.	May	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
<i>aconitus</i>	1						5	3	3
<i>barbirostris</i>	7	32	6	1	1		3	2	21
<i>culicifacies</i>		7	14	78	154				1
<i>dirus</i>					1	1			
<i>kochi</i>		10			11	42	221		7
<i>karwani</i>					1				
<i>maculatus</i>									
A	9	161	134	134	249	269	303	42	130
B	4	96	42	53	98	122	116	12	6
G			7	23	15	26	25	1	50
I					6				
H					2	6	47	22	
(Total)	13	257	183	210	370	423	491	77	186
<i>minimus</i>	7	69	51	129	115	50	180	101	65
<i>nivipes</i>		30	20	9	63	89	23	12	4
<i>peditaeniatus</i>	58	91			2	90		19	99
<i>philippinensis</i>			25		55		2	3	
<i>ramsayi</i>		28	76	9	31	7	158		21
<i>sinensis</i>	17								
<i>tesselatus</i>				1		3	44	2	1
<i>vagus</i>	2	59	8	31	479	309	258	12	
<i>varuna</i>	2	4					3	1	1

Identification of *An. maculatus*: after Rattanaarithikul and Green (1986).

Table 3 Parity of two species of anopheline vectors collected from a buffalo bait trap at Ban Tha Lum Yai, during March 1987 to January 1988.

	Mar.	May	Sept.	Oct.	Nov.	Dec.	Jan.
<i>Anopheles minimus</i>							
No. exam.	69	51	115	60	347	101	39
Nulliparous	8	5	58	23	112	19	7
Parous 1	48	35	55	4	221	82	31
Parous 2	13	11	2	33	14	0	1
Parous rate	0.88	0.90	0.50	0.62	0.68	0.81	0.82
<i>Anopheles maculatus</i>							
No. exam.	257	—	364	423	490	29	61
Nulliparous	68	—	191	241	196	12	18
Parous 1	145	—	153	171	269	16	41
Parous 2	44	—	20	11	25	1	2
Parous rate	0.74	—	0.48	0.43	0.60	0.59	0.70

Parity was examined following the method of Detinova (1962).

Table 4 Number of *Anopheles* collected with human and domestic animal bait traps, in January 1988.

<i>Anopheles</i>	Bait of traps	Human	Buffalo	Dog	Hamster	Chicken
	Number of trap	2	2	2	2	2
<i>aconitus</i>			1			
<i>barbirostris</i>			2			
<i>kochi</i>			10			
<i>maculatus</i>		6	24	1	2	
<i>minimus</i>		29	78	1	11	2
<i>peditaeniatus</i>			45			
<i>ramsayi</i>			1			

Table 5 Number of *Anopheles* collected with a human bait trap (two men two consecutive nights) at Ban Tha Lum Yai, Kanchanaburi, during September 1987-January 1988.

Species	Sept.	Oct.	Nov.	Dec.	Jan.
<i>An. maculatus</i>					
A	22	46	17	1	6
B	4	5	6	1	3
G	8	1	5	0	5
(Total)	34	52	28	2	14
<i>An. minimus</i>	45	26	39	36	24

Identification of *An. maculatus*: after Rattanaarithikul and Green (1986).

Table 6 Number of mosquitoes responding to various systems of traps in March and August 1987.

Type of systems	D	H	S	D+H	D+S	S+H	D+H+S	Buffalo	Human
No. of trap	4	4	4	4	4	4	4	2	2
March*	0	0	0	5	6	3	64	107	18
August*	3	1	2	17	9	5	118	339	41

* This experiment was performed for a relatively lower population in March and a higher population in August. D, dry ice; H, hamster; S, cage-type trap emitting 530 Hz of wingbeat sound.

or cylinder type) was tested on females of two species, *An. maculatus* and *An. minimus*. Table 7 shows that the sound trap of the cage type was more effective to attract female *An. minimus* and *An. maculatus*. The results proved that females of the two species were attracted more to the cage type emitting some preferable range of frequency of sound.

The sound trap of the cage type combined with dry ice and a hamster caught a number of female mosquitoes from March 1987 to January 1988 (Tables 8 and 9). *An. minimus* was abundantly collected by the trap

emitting sound frequencies between 500 and 600 Hz and between 800 to 1,000 Hz. At 700 Hz they were trapped in lower numbers; this tendency was also indicated in Table 7. *An. maculatus* was trapped in a wider range of frequencies, though the catch was lower in number.

DISCUSSION

In order to develop and improve the technical aspect of field survey and researches on malaria, and to utilize background infor-

Table 7 Number of female mosquitoes collected with two types of trapping systems emitting various sound frequencies.

Frequency (Hz)	<i>An. minimus</i>			<i>An. maculatus</i>		
	Cylinder	Cage	Total	Cylinder	Cage	Total
350	0	12	12	0	0	0
400	6	7	13	0	2	2
500	7	19	26	0	3	3
600	3	16	19	0	2	2
700	3	4	7	0	3	3
800	6	29	35	0	2	2
1,000	4	16	20	0	3	3
Soundless*	3	2	5	0	0	0

* The trap combined with a hamster and dry ice without sound emitting.

Table 8 Number of female *An. minimus* collected with a sound cage trap emitting various sound frequencies and combined with a hamster and dry ice, from March 1987 to January 1988.

Frequency (Hz)	Mar.	May	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
500	6*(1.5)	8 (1.0)	10 (1.3)	67 (8.4)	24 (3.0)	43 (5.4)	13 (1.6)	24 (3.0)
600	8*(2.0)	0	9 (1.1)	116 (14.5)	53 (6.6)	36 (4.5)	19 (2.4)	77 (9.6)
700	0	1 (0.1)	4 (0.5)	38 (4.8)	57 (7.1)	28 (3.5)	5 (0.6)	0
800	14*(3.5)	0	18 (2.2)	213 (26.6)	449 (56.1)	217 (27.1)	37 (4.6)	50 (6.3)
1,000	11*(2.8)	5*(1.2)	6 (0.8)	207*(51.8)	228*(57.0)	182*(45.5)	20*(5.0)	50 (6.3)
Control	6 (0.8)	1 (0.1)	0	18 (2.3)	102 (12.8)	69 (8.6)	16 (2.0)	0

Average number of mosquitoes per trap was shown in the parenthesis. Four (* marked) or eight traps were used in the tests and soundless trap with a hamster and dry ice in a cage was used as control.

Table 9 Number of female *An. maculatus* collected with sound cage trap emitting various sound frequencies and combined with a hamster and dry ice, from May 1987 to January 1988.

Frequency (Hz)	May	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
500	0	3 (0.4)	6 (0.8)	1 (0.1)	10 (1.3)	4 (0.5)	10 (1.3)
600	0	2 (0.3)	20 (2.5)	6 (0.8)	28 (3.5)	5 (0.6)	11 (1.4)
700	1*(0.3)	4 (0.5)	14 (1.8)	2 (0.3)	10 (1.3)	3 (0.4)	9 (1.1)
800	1*(0.3)	8 (1.0)	12 (1.5)	47 (5.9)	52 (6.5)	8 (1.0)	31 (3.9)
1,000	0	5 (0.6)	12*(3.0)	38*(9.5)	47*(11.8)	2*(0.5)	14 (1.8)
Control	0	0	0	12 (1.5)	19 (2.4)	0	0

Average number of mosquitoes per trap was shown in the parenthesis. Four (* marked) or eight traps were used in the tests and soundless trap with a hamster and dry ice in a cage was used as control.

mation on future control project, malario-metric investigation and vector surveys were first carried out. The malaria parasite rate in the study area remained high in 1986–1987 (Table 1). This result suggests that perennial stable malaria has been endemic in this

village. In addition, high to holoendemicity of malaria in this village according to data of the Malaria Division, Ministry of Public Health (Malaria Division, 1986–1987) supports the present results of high parasite rate. Despite the low density of the vector popula-

tions, their high parous rates might be thought to support the stability of malaria transmission in the area. The decrease of parous rate from September to November might be due to increase of mosquito density by newly emerged females, as monitored by the buffalo bait (Table 2) and also by sound trapping (Tables 8 and 9). The high-risk situation of malaria endemicity in this village underlines the urgency to implement malario-metric and vector surveys, and malaria control in this area.

The human bait method can effectively monitor the mosquito population, but its use in field operation is controversial because of the hazards to man. On the other hand, sound trapping emitting animal scent and evaporating CO₂ from dry ice produced parallel fluctuation of population density derived from results of both human and buffalo baits which were performed monthly and seasonally. Moreover, the number of mosquitoes collected by this trapping system was 3 to 4 times larger than that collected with human bait, though it was 1/3 to 1/4 of that collected with buffalo bait. These facts proved the effectiveness of this trapping system not only for monitoring the fluctuation of mosquito population but also in terms of protection of malaria workers from malaria infection. When this sound trap system is used, the blood meal preference of vectors which is epidemiologically required may not be analyzed. This problem can, however, be solved by other ways such as the precipitin test of the vectors collected.

An. minimus was highly attracted to sound traps with frequencies from 500 to 600 Hz and from 800 to 1,000 Hz. This phenomenon may be due to the difference of their mating behavior, and some genetic divergence may ethologically exist. The details of this ethological phenomenon will be published in a separate paper. The differences of wing-beat frequency within various colonies and members of a given species and among different taxa were proved by Kanda *et al.* (1986) and Ogawa and Kanda (1986). According to measurements of wing-beat frequency, members of the *An. minimus* group showed slightly different frequencies. In *An. minimus s.s.*, females showed 464.8 ± 7.6 Hz (mean $\pm$ S.E.), whereas males 713.1 ± 22.4 Hz. In *An. mini-*

mus pampanai type, female showed 473.0 ± 5.2 Hz, whereas males 709.5 ± 10.5 Hz. As far as the present result obtained from one of the anopheline species is concerned, this seems to show the genetic divergence relating to several other biological characteristics in the phylogeny of this species group (Kanda *et al.*, 1984; Sucharit *et al.*, 1986; Komalamisra, 1989). On the other hand, *An. maculatus* was trapped in a more limited range of sound frequency. This may suggest the species in this village consisted of either one group or many (Table 5). In the previous experiment (Kanda *et al.*, 1986), *An. maculatus* showed much intertaxonomic variability in their wingbeat sound frequencies.

The biting preference test of these two vectors and other anopheline species proved that the vectors fed preferably on buffalo and human baits, but not on dog or chicken (Tables 2, 4 and 5). Hamster was experimentally an efficient blood donor, although the efficiency was one-third of human bait. Buffalo was a good blood donor to all anopheline species in the area. Despite the fact that human was a tentative or partial donor compared to buffalo in the village, human malaria transmission has been stably maintained.

Possible utilization of this trapping system arises as one means of future control of the vectors. If buffalos were protected from the anopheline's biting, density of this species would be greatly reduced, though some ethological changes may occur, especially in their biting activity for a while.

ACKNOWLEDGEMENTS

Sincere gratitude is extended to Dr. Masamichi Fujii, President of St. Marianna University, for the promotion of the scientific researches project of the university for Dissertation Ph.D. and research support for this study in Thailand as advisor, Dr. Tozo Kanda for his supervision of this project. Thanks is also extended to Associate Professor Dr. Supat Sucharit, Head of Department of Medical Entomology, Faculty of Tropical Medicine and Mr. Kamhang Suratint, and the staffs of the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University and Department of Medical Zoology, St. Marianna University. I thank Dr. Toshiaki Ikeshoji, Professor, Faculty of Agriculture, the University of Tokyo, for his reading of the manuscript and suggested

revisions.

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摘 要

タイ国カンチャナブリ省山麓地帯溪谷でのマラリアの疫学的研究と種々トラップ法による媒介ハマダラカ捕獲の試み

タイ国カンチャナブリ省、山麓溪谷に沿う山村マラリア流行地タ・ラム・ヤイ村において、媒介蚊の消長と、マラリア原虫検出状況を検討し、また、ヒトや動物をおとりにした吸血採集法と羽音による音響捕獲法との有効性を比較検討した。吸血採集では、水牛をおとりにした場合に最も多くのハマダラカ類が採れ、16種に及んだ。ヒトを吸血したのはそのうちの2種で、*An. minimus* は水牛の場合のほぼ1/4、*An. maculatus* は1/3が採集され、その他の動物では同じくこれら2種が採れたが、数ははるかに少なかった。このことからヒトのマラリアの媒介蚊はこれら2種が主役をなし、おもに水牛を吸血して繁殖しているものと推測された。経産蚊率は両種とも高く *minimus* は9月の0.5が最低、5月の0.9が最高、*maculatus* は10月の0.43が最低、3月の0.74が最高という値を示し、マラリアの高度安定流行に関与しているとみられた。これら2種は音響トラップに著明に応答し、ドライアイスとハムスターを併用した場合に、捕獲が促進された。しかもヒト吸血の3倍を捕獲し年間の消長のモニタリングに、ヒトおとり吸血に代わり有用と思われる。なお、種群内に異なった振動数に応答するものがみられ、*minimus* 群では500~600 Hzに応答するものと、800~1,000 Hzに応答する2集団が別れて捕獲された。これに対し *maculatus* 群では、各振動数の音響に一樣にそれぞれ応答する雌ハマダラカを採集した。このことは遺伝的分化との関連を示唆するものと考えられた。