INTEGRATION OF CONTROL MEASURES FOR MALARIA VECTORS IN ENDEMIC AREAS OF THAILAND

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Abstract. Various vector control measures were applied in different endemic areas in two provinces, Saraburi and Chanthaburi, with comparison among different control measures. Application of IGR (insect growth regurator, pyriproxyfen) was introduced at Wat Tam Pra Pothisat, Tab-Kwang District, Saraburi Province. Some integration measures were performed at villages 6 and 8, Patavee, Makham District, Chanthaburi Province. In Tab-Kwang District with low malaria endemicity at the study site predators were not able to be released due to rapid velocity of running water. IGR could effectively control malaria compared to the basin released predators.

Another endemic area villages 6 and 8, Patavee, Makham, Chanthaburi Province was chosen. Highly endemic multidrug resistant malaria has been prevalent for many years in this area. Integration of Kanda's trapping system, application of IGR, use of both residual spraying and impregnated bed-net methods with etofenprox successfully interrupted malaria infection. The application of these methods as an integrated control system could be adjusted to environmental conditions. The results of this study suggest rapid effective vector control.

INTRODUCTION

Despite good malaria control in Thailand, there is still a high endemicity in many areas of the country. Endemicity varies from one site to another. These areas should be identified and ranked according to their endemicity. The control measures can then be adjusted for each area.

In Thailand, high endemicity is found in several provinces on the western and eastern borders, and in the southern area of Thailand (Malaria Division, 1985).

Development of insecticide resistance to compounds such as organophosphates has been observed. Their toxicity to non-target organisms further discourages the use of these insecticides. However, a new synthetic insecticide, etofenprox (Trebon, Mitsui Toatsu Chemicals Inc) has been shown to be highly active against anopheline mosquitos (Yoshimoto *et al,* 1988). It has mild acute toxicity against mammalias as well as fish and less impact on natural enemies (predators); no phytotoxicity was noted. It has not been shown to be cross-resistant to either carbamates or organophosphorous insecticides.

There are many other methods for vector control, including light traps sound traps for mating, promoting instinctive activities relating to reproduction such as swarming, mating, laying eggs and taking blood meals for ovarial development (Kanda *et al,* 1988, 1990). These are indispensable activities for their survival. The traps utilize their own physical, biochemical and eco-physiological stimulants, their activities do not interfere with others methods.

Pyriproxyfen (IGR) is a larvicide; it also interferes with the development of immature stages, resulting in prevention of both pupation and adult emergence (Jacob and Schoof, 1972). Larvivorous predators, such as the guppy (*Poecillia reticulata*), are also useful as agents against the vectors. These control methods could be integrated and used as effective mosquito control measures.

Normally the anopheline vectors of malaria appear at Thai breeding sites *eg* valley streams where they lay eggs, hatch, pass larval and pupal stages, then emerge to adults and mate. Integration of Kanda's sound trap, attractant for mating (emission of male's wing-beat which has been stimulated by dry ice) and attractance for biting (using organo-

chemical) (Kanda *et al,* 1990) has been shown to trap a number of anopheline species (Leemingsawat, 1989; Kanda *et al,* 1990). In addition, if IGR could be applied to the running stream (breeding site of the larval vector), it could further arrest the number of adult vectors (Kerdpibule, 1989).

On the other hand, the aim of control should also be focused on the biting sites where the houses or huts are situated. The vectors will bite and rest in-or out-side the house after taking their blood meals. Residual spray and/or bed-nets impregnated with an insecticide such as permethrin or etofenprox should be applied simultaneously with other control measures.

The present study aims to demonstrate a new approach to malaria vector control, manipulating natural phenomena in insects while avoiding the harmful effects and environmental pollution of using chemical insecticides.

MATERIALS AND METHODS

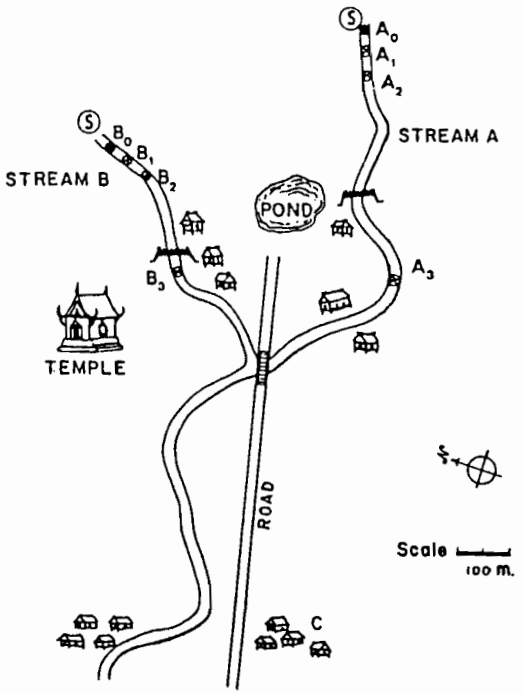
Study sites

Two endemic areas in Thailand were selected, one was an indigenous malaria area in Saraburi (Fig 1) and another was a multi-drug resistant falciparum malaria area in Chanthaburi Province (Fig 2). The former had low endemicity and the latter had high endemicity, with severe malaria.

Study site in Saraburi: Three basins were selected at Tab Kwang district, Saraburi Province. One was located at Wat Tham Pra Pothisat, an area with a fast running stream. No predator fish were detected in this area. The community consisted of about 200 people in 35 houses situated along the lower stream near the temple. IGR was applied in this stream for vector control.

The other two similar sized communities were selected around the study site. One, a hamlet with 200 inhabitants served as control. In another community with slow running water (500 inhabitants) guppy fish were introduced.

Study site in Chanthaburi (Fig 2): Two villages (6 and 8) were selected in Patavee, Makham District, Chanthaburi Province. Multi-drug resistant falciparum malaria has been documented in this area along the Thai-Cambodian border. Village 6



● application point
⊗ check point
A_1, A_2 and A_3 = 50, 120 and 530 m from A_0
B_1, B_2 and B_3 = 50, 20 and 270 m from B_0
C = station for mosquito collection
Ⓢ = spring

Fig 1–Experimental streams for S-31183 application (Wat Tham Pra Pothisat, Tab Kwang, Saraburi). (After Kerdpibule, 1989).

was divided into five communities (Fig 2). One community which applied integrated control (St C) consisted with 103 houses and huts (296 inhabitants). The other 4 communities surrounding St C (C1, C2, C3, and C4) had 49, 50, 58, 91 houses and huts and 154, 150, 141, 265 population, respectively, as shown in Fig 2 and Table 9.

At another study site, Sampang in village 8, IGR with running streams of two basins without any other control measure the vector density was monitored. The efficacy of using IGR alone in the high endemic area was assessed.

METHODS

1. Mosquito trapping: Kanda's trapping system (Kanda *et al,* 1990) was set to emit the sound of a

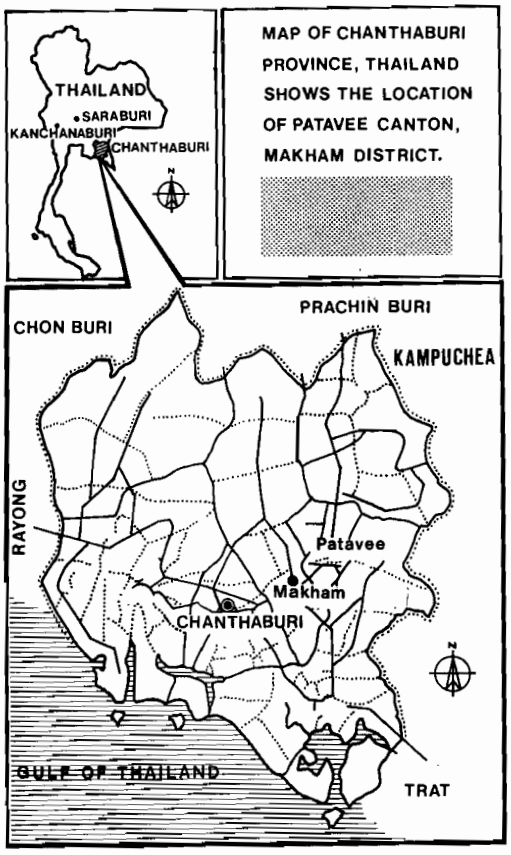


Fig 2–Map of Chanthaburi Province, Thailand shows the location of Patavee Canton, Makam District.

modified wing-beat with a frequency of 750 Hz around the breeding sites of vectors. The breeding

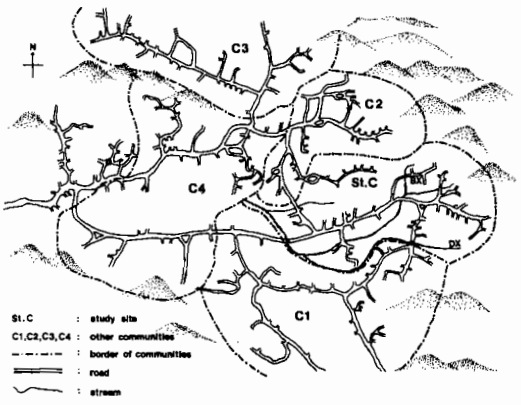


Fig 3- Map of study site in Village 6, Patavee Canton.

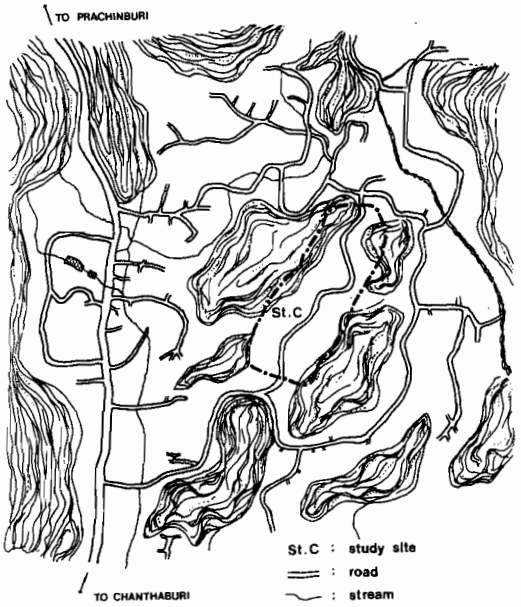


Fig 4–Map of Village 8, Patavee, Makham, Chanthaburi indicating the study site.

sites were identified at running streams around the residences of malaria patients in the communities where the malaria foci were found. The traps thus attracted vectors by emission of the simulated wing-beat, stimulation by dry ice and biting attraction by an organo-chemical, 2-keto-butyric acid. These mosquito stimulants and attractants worked by displaying synergistic function. Adjusting to the size of breeding sites the traps were variously set by altering their number. The trapping was utilized for monitoring the population of mosquito vectors and for reduction and elimination the vector population for control.

2. Mosquito collection adopting human bait double net method using dry ice at biting site: A pair of larger and smaller nets was set in the next room or under the eaves in each patient's residence building in order to know how large a vector population was to be monitored or controlled. A man lay in a smaller net which was closed inside a larger net with one side open to let vectors fly between the nets. Two blocks of 500 g of dry ice were put at both sites. The mosquito collection was performed from 1800 to 0600 hours the following morning; the mosquitos were identified and dissected.

3. Cattle bait trapping: At the residence area where cattle were available, an animal was used as a live mosquito bait in a mosquito net during one night (from 1800 hours in the evening to 0600 hours the following morning). The collected mosquitos were identified and dissected.

4. Insect growth regulator (IGR), pyriproxyfen application: Pyriproxyfen IGR was applied to the objective stream, at a concentration of 5 ppb (Kerdpibule, 1989). The technique adopted here was that which Kerdpibule reported in 1989. IGR was only applied to fast running water streams; it dried out during the dry season as the predator is not able to reside in such a stream at that time.

The applications of the IGR were made at the site indicated by marks Ao and Bo in Fig 1 in Saraburi, and by AX,BX and DX in Fig 2 in Chanthaburi, respectively.

5. Predator application: Guppy fish were released into a slow running stream in the middle of the community of 500 population. The velocity of the stream was slow enough for the predator to reside. The release of guppy fish was done only once as the number of guppies was enough all seasons without any additional supply.

RESULTS

1. Vector control in Saraburi

The study commenced in December 1989, by comparing three communities in Wat Tham Pra Pothisat, Tab Kwang District, Saraburi Province. The vector control performed by the government was residual DDT spraying.

IGR was applied in the 2 streams (A and B, Fig 1), which run around Wat Tham Pra Pothisat. Surveillance was performed by monitoring the vector population and recording malaria cases in the malaria clinics in the respective study communities until February 15, 1993. The first application of the IGR was on December 9, 1989. Mosquito collection was performed by both human bait and cattle bait methods. Identification and age determination of mosquitos collected were performed. The IGR was applied at intervals (Table 1). The application of IGR was not regular due to variation of seasonal duration, the amount of rainfall and duration of the dry season. Application of IGR was exclusively applied into running water against the *An. dirus, An. maculatus* and *An. minimus.* The concentration was always kept at 5 ppb by adapting to the amount of water running in the stream as described by Kerdpibule (1989).

The vectors and the malaria cases in the IGR applied basin, the non-IGR applied basin and the basin with predator are shown in Table 1. The efficacy of predator and IGR was evaluated using the number of mosquito and nulli-parity to indicate the vector density. It was shown in this study that the predator alone was able to effectively control the breeding of the vector based on the numbers of collected mosquitos. No malaria case was found in the community where the predator was released: in this basin the stream was slowly running and guppy fish were able to survive all year round. On the other hand, the number of mosquitos was also reduced after the application of IGR for 8 weeks. The occurrence of malaria cases was not seen later than 8 weeks after the first application of IGR in the community, whereas a few cases were consistently been found in the non-IGR application community (Table 1-2).

2. Vector control in Chanthaburi

Efficacy test of Pyriproxyfen in a high endemic community, Sampang, Village 8, Patavee: The experiment was performed to evaluate the efficacy of IGR in basins of the community Sampang. After the application of the IGR, Kanda's sound trapping system was set-up at the breeding site and human bait collection with a double-net was performed at the biting site. The collection was performed up to 22 weeks after the application of IGR. Collected mosquitos were identified and examined for their parity.

The results of human bait collection at biting site and sound trapping at breeding sites are shown in Tables 3 and 4, respectively. The number of mosquito vectors was drastically reduced after the application of IGR at the breeding site (Table 2 and 3); all 3 species of vectors *(ie An. dirus, An. maculatus* and *An. minimus*) were detected at the biting site (Table 3). However quite a number of parous, highly infectious mosquitos survived until 11 to 15 weeks (Table 3). Monitoring of active case detection showed no significant alteration of monthly malaria cases in the study community

Table 1

Number of malaria case and adult vectors in the comparison between two larval control methods at running streams in foot-hill area, Saraburi, since December 1989 up to February 1991.

Larv contr	Non treated			Predator			Pyriproxyfen		
Bait	HB	CB	%NP	HB	CB	%NP	HB	CB	%NP
An. minimus									
Appl (19 Dec 1989)									
Pre-appl	6	27	51.8	2	0	-	3	196	62.0
2 (w) aft	4	14	50.0	0	0	-	4	69	11.1
4	1	11	54.5	1	0	-	12	79	7.7
8	0	0	-	0	0	-	0	25	12.0
Pre-appl	0	2	-	0	0	-	2	15	47.1
Oct	0	5	60.0	0	0	-	3	45	54.5
Nov (flooded)	-	-		-			-	-	
Dec	6	103	60.0	4	0	25.0	22	428	52.0
Appl (20 Dec 1990)									
2 (w) in 1991	3	17	55.0	8	0	62.5	14	323	7.5
4	3	18	44.4	3		33.3	12	183	16.7
8	0		42.9	0	0	-	15	65	46.3
HB human bait, CB cattle bait, %NP, nulliparity									
An. maculatus									
Appl (19 Dec 1989)									
Pre-appl	0	0	-	0	0	-	2	19	47.3
2	0	3	33.3	0	0	-	0	5	20.0
4	0	1	-	0	0	-	0	5	20.0
8	0	1	-	0	0	-	0	0	-
Pre-appl									
Sep 1990	2	8	37.5	2	0	-	4	21	42.9
Oct 1990	2	19	52.4	0	0	-	1	22	54.5
Nov (flooded)									
Dec	0	14	42.9	0	0	-	12	532	55.6
Appl (20 Dec 1990)									
2	0	8	50.0	0	0	-	6	45	9.6
4	0	0	-	0	0	-	0	14	14.3
8	0	0	-	0	0	-	4	7	45.6
No. of malaria cases									
Before appl	7/200			0/500			6/200		
4 (w)	7/200			0/500			2/200		
8	7/200			0/500			1/200		
12 (w)	7/200			0/500			0/200		
12 (months)	8/200			0/500			0/200		
18 (m) 1991	10/200			0/500			0/200		
24 (m) 1992	6/200			0/500			0/200		
36 1993	2/200			0/500			0/200		

HB human bait, CB : cattle bait, %NP : nulliparity

Table 2

Number of mosquitos collected by human bait double net method at the entrance of a house (biting site) in the endemic area of multi-drug resistant malaria after application of pyriproxyfen in Village 8.

Weeks	Before	After 1	5	10	14	22
Anopheles						
aconitus		2				
barbirostris	6	36	6	13	15	0
dirus	3	2	0	0	2	3
maculatus	3	4	0	0	1	4
minimus	0	2	0	0	0	0
nivipes	13	41	5	3	2	12
nigerrimus	2	12	0	1	0	0
pallidus	0	0	4	0	0	0
karwari	3	11	0	1	0	0
ramsayi	0	0	0	0	0	7
sinensis	4	0	0	0	0	0
peditaeniatus	0	0	0	0	0	2
Culex	46	113	54	31	252	95
Aedes	0	33	3	100	55	31
Mansonia	3	9	25	27	8	4
Coquillettidia	0	7	4	2	0	1

compared to 4 in the vicinity (Table 5).

Integrated control measure at village 6, Patavee: This area has been established as having a high endemicity of multi-drug resistant falciparum malaria, thus urgent mosquito control was required. Kanda's trapping system was applied at the breeding site of village 6, Patavee, Makham District, Chanthaburi Province (Table 6). Thirty two speakers

Table 3

Number of anopheline vectors collected by human bait in double-net following IGR (Pyriproxyfen) application at a biting site in the endemic area of multi drug resistant falciparum malaria, Village 8, Patavee, Makham, Chanthaburi.

IGR appl (week)	*An. dirus* Total	P	N	*An. maculatus* Total	P	N	*An. minimus* Total	P	N
Before									
4	8	5	3	10	4	6	2	2	0
0	27	8	19	33	12	21	50	15	35
After									
4	13	11	2	56	51	5	29	26	3
11	4	3	1	32	28	4	15	12	3
15	3	2	1	17	10	7	2	2	0

P : parous, N : nulliparous

Table 4

Number of mosquitos trapped by Kanda's sound trap system after application of pyriproxyfen into streams breeding site of the anopheline vectors of falciparum malaria at a community, Sampang in Village 8, Patavee, Makham, Chanthaburi Province.

IGR appl	Anopheline vectors				Other Anophel	*Cx.*	*Ae.*	*Mn.*
	dirus	mae	mini	Total				
Weeks after								
0	2	1	2	5	14	79	9	0
5	0	0	0	0	17	58	7	0
10	0	0	0	0	5	87	0	6
15	0	0	0	0	3	31	0	1

were set at the breeding site of vectors. The malaria vectors were *An. dirus, An. minimus* and *An. maculatus* which breed in running streams of the village. The village was divided into 5 communities as shown in Fig 2.

An integrated control measure was performed at St C (Fig 2). The adult vector trapping was commenced at the same time with the application of the IGR 5 times during a 7 day period. The 3 species of anopheline mosquitos were efficiently trapped by these systems at the breeding sites as shown in Table 6 and Fig 2. Using these traps, *An. dirus* and *An. minimus* disappeared but some *An. maculatus* was still detected. However, continuation of IGR application at a concentration of 5 ppb in the running stream at an interval of 3 months successfully kept the vector density at a low level either at the breeding site or the biting site (Table 6, 7). In addition to these control measures residual spray and impregnated bed-net were applied at an interval of 6 months.

Using Kanda's trapping system at the breeding site (Table 6) and human bait double net mosquito collection (Table 7) at the biting site, it was found that adult vectors appeared 22 weeks after the application of the IGR at various sites shown in Fig 2. The malaria cases however were strikingly decreased in the study community (Table 8).

DISCUSSION

In general, the mosquito vectors can be estimated at breeding sites from their bionomical activities. The 3 species of anopheline vectors lay eggs in the stream, larval and pupal growth occurs, then hatching and emergence of adults. The newly emerged adults rest for a while and mate. They then spend their early lives around the breeding site. In their adult stage, they leave the breeding site for the biting site where blood meals are available. After a blood meal, the reproductive organs develop (during resting), the gravid females then return to the breeding site for laying eggs.

Table 5

Number of multi-drug resistant malaria cases in Village 8, Patavee from October 1992 to January 1994 after application of pyriproxyfen into streams of basins, reside area.

Communities	Houses	Huts	Population	Mal case	1993	1994		
				1992-1993	Oct	Nov	Dec	Jan
Study site	79	2	217	45 (19)*	3 (2)*	5 (1)*	8 (8)*	2 (2)*
4 Commun	200	19	582	22 (16)*	2 (0)*	5 (3)*	9 (9)*	3 (3)*

( )* Indigenous cases.

Table 6

Number of mosquitos trapped by Kanda's sound trap system following the integrated control measure at a breeding site of the anopheline vectors of falciparum malaria in Village 6, Patavee.

IGR & Etofen applied		Anopheline vectors				Other anophel	*Cx.*	*Ae.*	*Coq*
		dirus	mac	mini	Total				
Day	0	23	16	10	39	0	136	8	0
	1	11	11	7	29	3	149	11	4
	3	19	13	3	35	0	211	21	2
	5	7	8	1	16	4	109	4	1
	7	1	5	0	6	1	157	3	3
Cumul No.		61	53	21	135	8	762	47	10
Week	2	0	3	0	3	4	177	2	1
	5	0	1	0	1	1	111	2	3
	10	0	0	0	0	1	246	1	1
	14*	0	0	0	0	1	187	6	2
	22	0	2	0	2	4	71	4	1
	30**	2	6	0	8	0	49	3	0
	46	0	0	0	0	2	83	2	3
	54**	2	4	3	9	28	118	5	0
	58	0	0	0	0	3	107	5	1
	63	0	0	0	0	35	97	3	0
	68*+	0	0	0	0	39	2	0	7

*+Integrated control, *IGR application

Although there are various conditions (such as characteristics of vector, seasonal rainfall, topography, etc) that could affect the bionomics of the vectors, integrated control measures can be modified accordingly. In this study we have used Kanda's sound trapping system for adult vectors simultaneously with the application of IGR to the stream to eliminate the larval and pupal vectors, as well as residual spray and impregnated bed-nets (etophenprox, a new insecticide which is effective against target organisms less toxic and free from chlorine and organo-phosphorous compounds - Nakatani *et al,* 1982; Udagawa 1988; Yoshimoto *et al,* 1989; Prasittisuk *et al,* 1992) application at the biting site, to protect against biting by adult vectors. The evaluation of this approach was by monitoring the vector population through the sound trap and double mosquito net collection.

The results of integrated control measures in Saraburi Province (low endemic area) suggested that Kanda's sound trapping system at the breeding site is not necessary if residual spray at the biting site has already been implemented as larvae could be controlled effectively by predators or by IGR application at the breeding site (Table 1). The application of Kanda's sound trapping system (Kanda *et al,* 1990) should be reserved for high endemicity areas as it is rather expensive and requires a great deal of manpower; although it is environmentally safe, its utility is limited.

It has been shown that in high endemicity areas in Chanthaburi Province, Kanda's sound trapping system should be employed in addition to the use of predator and/or IGR (Table 5, 6, 7, 8). Kanda's sound trapping system stimulates and attracts mosquitos physically and ecophysiologically. It stimulates mosquito activities, such as flying, swarming and mating by dry ice and by sound. When the mosquitos gather around the trap, they are trapped by tangle foot glue. These procedures do not destroy or interfere with the environment or ecosystem as mating, feeding, swarming, gathering and egg

Table 7

Number of mosquitos collected by human bait in double-net method following the integrated control measure at a biting site in the endemic area of multi-drug resistant falciparum malaria in Village 6, Patavee, Makham, Chanthaburi Province.

Applic	Anopheline vectors			Other anophelines				*Cx.*	*Ae.*	*Ar.*	*Mn.*	*Coq.*
	dirus	mac	mini	Total	barb	nivi	other					
Before	14	3	0	17	9	21	6	49	5	4	0	0
0*+	7	5	0	12	6	21	12	113	33	0	9	7
After												
1(w)	2	4	2	8	34	41	0	0	0	0	0	0
5	0	0	0	0	6	5	4	54	3	0	21	4
10	2	1	0	3	15	0	5	121	21	0	8	0
14*	1	0	0	1	0	1	0	0	0	0	0	0
22	0	0	0	0	1	0	1	0	0	0	0	0
30*	1	0	0	1	5	13	5	63	8	3	0	0
46*	0	0	0	0	2	1	7	0	0	0	0	0
54*+	2	4	3	9	4	9	6	0	0	0	0	0
58*	0	0	0	0	1	0	2	20	10	0	0	0
63	0	0	0	0	10	5	12	0	0	0	0	0
68*	0	0	0	0	31	0	8	5	2	0	7	0

*+Integrated control, *IGR application

Table 8

Number of malaria cases after integrated control on anopheline vectors in Village 6, Patavee, Makham, Chanthaburi (after active case detection).

Communities	Study site	Other 4 communities
No. houses and huts	103	246
No. population	296	710
No. malaria cases		
Oct '91 - Sept '92	45	127
Oct '92 - Sept '93	3 (2 Pv)	23
Oct '93 - Sept '94	3 (2 Pv)	39

laying are indispensable for their lives (Kanda, 1992).

In this study IGR alone worked well in a low endemicity area, it reduced the larval vectors in the stream (lower part of the applied site). The larvae were then washed away far from their usual habitat. The concentration of IGR did not affect any other organism or the environment as it was kept at 5 ppb in the stream (Kerdpibule, 1989).

The vectors for vivax malaria could not be controlled by IGR as it was not applied to stagnant water (breeding place for vectors of vivax malaria) in this study. Thus, it is not surprising to see vivax malaria in this study (Table 8).

These two integrated control methods are representative examples among various strategies. The present study showed how to eliminate malaria effectively and rapidly in highly endemic and hypoendemic falciparum malaria areas together with the already existing control measures used by the government. In order to derive rapid effective vector control with low cost, less manpower, resulting in fewer malaria cases, the strategy of combination of the control methods should be considered according to the situation.

Employment of predators would be very important for long term vector control as seen in this study at Tab Kwang District, Saraburi where guppy fish can be released into the community stream by the people in the community. The application of IGR in this study aimed at the control of breeding in

Table 9

Number of malaria cases found at communities around the one applied integrated control in Village 6, Makham, Chanthaburi from October 1991 to September 1992 (after case detection).

Community	No. houses and huts	Population	Malaria cases	Indigenous cases
Study site	103	296	45	33
C1	49	154	14	10
C2	50	150	63	46
C3	58	141	27	16
C4	91	265	23	7

Cn : Communities applied DDT or distributed impregnated bed-net

running streams, not stagnant streams. Thus, the vector of vivax malaria was not controlled; guppy fish could play a major role in this respect.

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