

FIGURE 1  
 CUMULATIVE PROPORTION OF SUBJECTS WITH P. FALCIPARUM PARASITEMIAS  
 BY STUDY GROUP

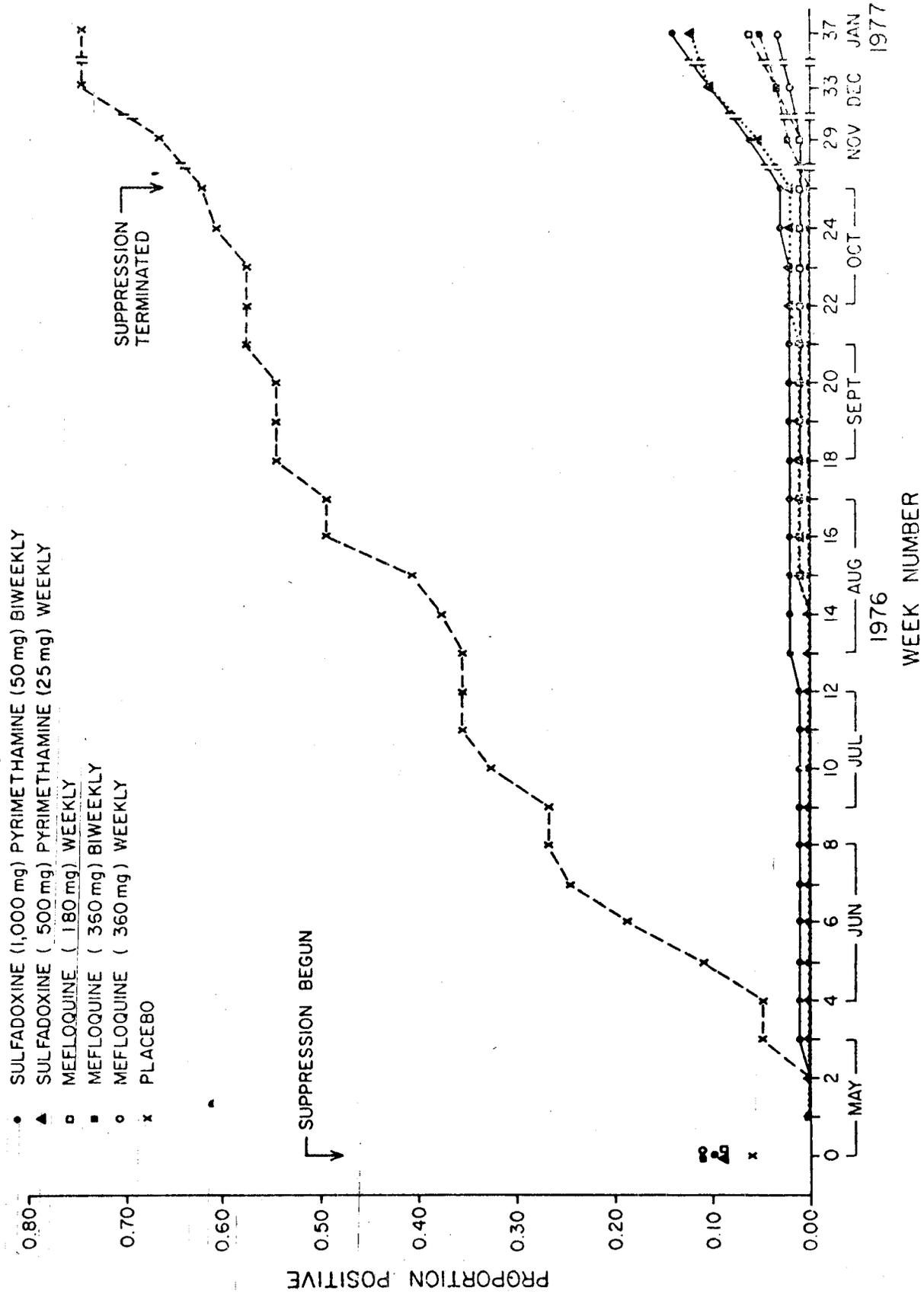
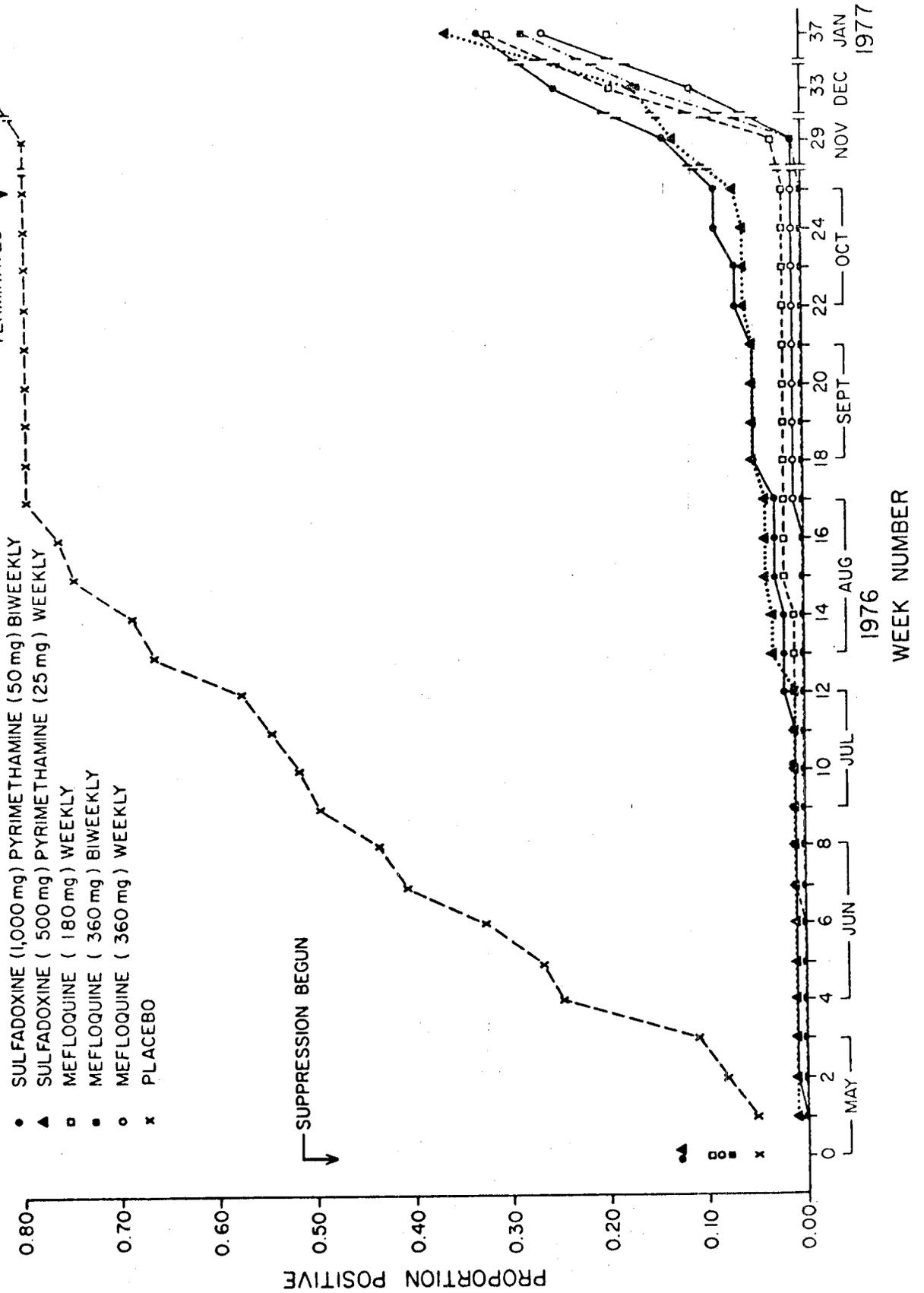


FIGURE 2  
 CUMULATIVE PROPORTION OF SUBJECTS WITH P. VIVAX PARASITEMIAS  
 BY STUDY GROUP



## The Effect of Some Antimalarials on Gametocytogenesis and Sporogony in Human Malaria in Thailand

Investigators :

Edward B. Doberstyn, LTC, MC  
Richard G. Andre, MAJ, MSC  
Chaiphorn Teerakiartkamjorn, M.D.  
Phung Phintuyothin, MG, MC, RTA, (Ret.)  
Suphat Noeypatimanondh

Associate Investigators :

Chalit Thumraksa, M.D.  
Visit Benjapongse, M.D.  
Surin Pinijpong, M.D.

**OBJECTIVE :** To determine the effect of several therapeutic regimens on the sexual and sporogonic cycles of natural infections with both *P. falciparum* and *P. vivax*.

**BACKGROUND :** From data collected at this institution and elsewhere, it appears that sulfonamides in general and the sulfadoxine-pyrimethamine combination (Fansidar, Roche) in particular, stimulate gametocyte production in falciparum malaria. Recently it has been suggested that the two and three tablet therapeutic doses have different effects on the sexual forms of the parasite, that two tablets stimulate while three tablets depress falciparum gametocyte production (1). Vivax malaria, which is currently enjoying a dramatic increase in incidence in Thailand, has not been studied in regard to gametocyte response to Fansidar. Since Fansidar has been used in increasing amounts for the therapy of vivax and falciparum infections, it is necessary to know whether vivax gametocyte production responds to Fansidar in a similar fashion to that of falciparum. Although Fansidar is not recommended for vivax infections, it is used widely as self-treatment and as presumptive treatment where species diagnosis cannot be performed.

The new antimalarial, mefloquine hydrochloride, which has been seen to be a highly effective schizonticide administered as a single oral dose, has not been studied in terms of its effect on gametocytes. It is therefore not known whether gametocytocidal therapy must be added to the single-dose schizonticidal regimen.

This study has been designed to test Fansidar and mefloquine in terms of stimulation or suppression of gametocyte production in both falciparum and vivax parasitemias, and to compare these results with those associated with standard therapy - quinine in the case of falciparum and chloroquine in the case of vivax. Additionally, the effect of primaquine as a gametocytocide will be re-evaluated.

---

(1) Suphat Sucharit, Personal communication.

*Anopheles balabacensis* is the primary vector of malaria in Thailand, while *Anopheles maculatus* is the major vector in Malaysia. This study will also compare the vector efficiency of the two species for vivax and falciparum infections, as well as the effect of these parasites on the longevity of the mosquitoes.

**METHODS :** The study is currently underway at Phrabuddhabat Hospital, Saraburi Province, Central Thailand. Patients are admitted to the male medical ward from the hospital out-patient department or from clinics of the National Malaria Eradication Project, both in Phrabuddhabat and in Pak Chong (Nakorn Rajasima Province). Admission criteria for study subjects are :

1. Males at least 18 years of age.
2. Willingness to volunteer for hospitalization and follow-up. The intended procedure is carefully explained to the patient, and he is asked to sign a statement of understanding and agreement.
3. Uncomplicated disease of mild to moderate severity.
4. Asexual parasite count between 1,000 and 100,000/cu.mm.
5. Presence of gametocytes on the initial thick film.

The patients are randomly assigned to one of the following regimens. Each regimen will be administered to twenty patients.

*P. falciparum*

1. Mefloquine HCl, single dose, 1,500 mg., p.o. (If a patient weighs less than 50 kg., the dose will be 30 mg/kg.)
2. Fansidar, single dose
  - a. 2 tablets (50 mg. pyrimethamine, 1.0 gm. Sulfadoxine)
  - b. 3 tablets (75 mg. pyrimethamine, 1.5 gm. Sulfadoxine)
3. Quinine, i.v., or p.o., 650 mg. q. 8 hours for seven days.
4. Quinine, 650 mg. q. 8 hours for seven days, plus primaquine 15 mg/day x 5 days.

*P. vivax*

1. Mefloquine HCl, single dose 1,500 mg., p.o. (or less, as above)
2. Fansidar, single dose
  - a. 2 tablets
  - b. 3 tablets

3. Chloroquine 1,500 mg., total dose p.o.
4. Chloroquine 1,500 mg., total dose p.o. + primaquine 15 mg/day x 5 days.

The drugs are administered by one of the study physicians.

Upon completion of the 28 day follow-up period, vivax patients are given a course of primaquine 15 mg/day for 14 days. Recrudescences of *P. falciparum*, if they occur, are retreated with quinine-Fansidar on an individualized basis. Relapses of *P. vivax* are treated with chloroquine-primaquine.

Sixty laboratory-reared *Anopheles balabacensis* and 60 *Anopheles maculatus* (IMR strain) are fed on patients on days 0, 1, 7, 14 and 21. Ten engorged mosquitoes of both species are withheld for determination of longevity. Mosquitoes are also fed on uninfected volunteers for simultaneous control. The mosquitoes are reared and maintained in the AFRIMS Phrabuddhabat insectary. Mosquitoes are dissected 7 and 14 days post-feeding. Guts and glands are examined for oocysts and sporozoites and oocyst indices are determined.

Direct parasite counts, using a modification of the Earle-Perez technique, are performed before admission to the study, twice daily while in the hospital, and at every follow-up visit. Serum sulfonamide, quinine, and mefloquine levels are determined on days of mosquito feeding.

After discharge, patients are taken home in an AFRIMS vehicle and a map to their home is drawn. They are asked to return for follow-up visits on days 7, 14, 21 and 28. If they do not keep their appointments, patients are visited at home and are brought back to Pak Chong Headquarters of NMEP or to the Phrabuddhabat Hospital.

RESULTS : To date 68 patients with *P. vivax* infection have been admitted to the study and 33 patients with *P. falciparum*.

#### Therapeutic Results (Table 1)

*P. vivax* : The mefloquine, chloroquine, and chloroquine-primaquine regimens were alike in their effect on fever clearance and asexual parasite clearance. In the two groups treated with Fansidar, unacceptable therapeutic results have been obtained. The two tablet-dose of Fansidar eliminated initial asexual parasitemia in only six of ten patients studied. Fever clearance and parasite clearance in the remainder of the patients was very sluggish (Table 2). Three tablets of Fansidar was successful in clearing parasitemia in all of eight patients studied, however, the parasite clearance time was prolonged at 90 hours.

*P. falciparum* : Since only 33 patients infected with *P. falciparum* have been studied to date, there are too few patients in each group to compare therapeutic efficacy.

Table 1. Results of Therapy

*P. vivax*

Treatment	Number of Patients	Mean Initial Asexual Count	Mean Fever Clearance Time (hours)	Mean Asexual Parasite Clearance Time (hours)
Mefloquine	15	5,324	36	46
Fansidar (2)	10	8,435	69	73*
Fansidar (3)	8	7,283	47	90
Chloroquine	18	5,832	40	52
Chloroquine-Primaquine	17	7,064	48	42

*P. falciparum*

Treatment	Number of Patients	Mean Initial Asexual Count	Mean Fever Clearance Time (hours)	Mean Asexual Parasite Clearance Time (hours)
Mefloquine	5	23,732	48	84
Fansidar (2)	7	11,332	87	73
Fansidar (3)	4	13,050	68	72
Quinine	9	15,351	51	68
Quinine-Primaquine	8	14,192	57	80

\* Four patients in this group had not cleared asexual parasitemia by Day 7. This value is the mean of those which did clear before Day 7.

Table 2. Results of *P. vivax* Therapy Using Two Tablets of Fansidar

Patient No.	Initial Asexual Count	Mean Fever Clearance Time (hours)	Mean Asexual Parasite Clearance Time (hours)	Comment
11-0348	34,390	42	53	
11-0353	6,300	80	No clearance by Day 7*	
11-0355	4,452	30	64	
11-0366	13,800	80	No clearance by Day 7*	
11-0367	8,310	No Fever	52	
11-0376	3,080	72	No clearance by Day 7*	
11-0388	5,796	120	102	Relapse Day 21*
11-0399	1,160	28	20	
11-0400	1,700	96	140	Relapse Day 28*
11-0401	5,360	72	No clearance by Day 7*	

\* Patients were retreated with chloroquine-primaquine

#### Gametocyte Counts (Table 3)

*P. vivax* : Initial gametocyte counts in the five groups were comparable and their rates of disappearance paralleled those of the asexual parasites. It is interesting to note, however, that gametocytes persisted in the two-tablet Fansidar group for a longer period on the average than in any other group. This reflects the fact that Fansidar was not successful in eliminating initial parasitemia in four of the ten patients studied. In addition, the mean gametocyte count of the eight patients treated with three tablets of Fansidar seemed to be higher than in any other group. Whether this represents stimulation by the drug is not yet clear.

*P. falciparum* : It is noteworthy that once again falciparum patients treated with Fansidar showed much higher peak gametocytemias than patients in the other treatment groups. It is too early to comment on other features of this portion of the study.

Table 3. Mean Gametocyte Counts on Days of Mosquito Feeding

Drug	Day				
	0	1	7	14	21
<i>P. vivax</i>					
Mefloquine	186	93	0	0	0
Fansidar (2)	368	90	100	5	0
Fansidar (3)	431	250	0	0	0
Chloroquine	230	55	0	0	0
Chloroquine-Primaquine	128	26	0	0	0
<i>P. falciparum</i>					
Mefloquine	163	160	220	30	15
Fansidar (2)	3	60	350	794	88
Fansidar (3)	3	10	1,580	1,490	620
Quinine	29	50	60	10	—*
Quinine-Primaquine	68	260	—*	—*	—*

\* No feeds - no gametocytes or lost to follow-up.

### Mosquito Feeding

*P. vivax*: Fifty-four per cent of patients studied to date have been infectious to vector mosquitoes before treatment (Table 4). It appears that overall *Anopheles balabacensis* is more likely to be infected with *P. vivax* than *Anopheles maculatus*. Mefloquine and Fansidar-treated patients are apparently equally infectious to mosquitoes on Day 1 following therapy. The chloroquine and the chloroquine-primaquine treated groups were much less likely to infect mosquitoes of either species on the day following therapy.

*P. falciparum*: It is too early to evaluate mosquito feeding data from this group of patients, however, it may be noted that two tablets of Fansidar gave rise to a number of positive mosquito feeds on Day 7, 14 and 21 following therapy.

Table 4. Summary of Mosquito Feeding Results

*P. vivax*

Therapy	Day of Feed	<i>A. balabacensis</i>		<i>A. maculatus</i>	
		Positive	Feed	Positive	Feed
Mefloquine	0	7/17	41%	4/17	24%
	1	4/14	29%	2/14	14%
	7	0/0	-	0/0	-
	14	0/0	-	0/0	-
	21	0/0	-	0/0	-
Fansidar (2)	0	2/10	20%	1/10	10%
	1	4/11	36%	5/10	50%
	7	1/3	33%	1/3	33%
	14	0/1	0%	0/1	0%
	21	0/0	-	0/0	-
Fansidar (3)	0	6/8	75%	4/8	50%
	1	3/6	50%	4/6	67%
	7	0/0	-	0/0	-
	14	0/0	-	0/0	-
	21	0/0	-	0/0	-
Chloroquine	0	7/17	41%	6/17	35%
	1	2/11	18%	0/11	0%
	7	0/0	-	0/0	-
	14	0/0	-	0/0	-
	21	0/0	-	0/0	-
Chloroquine-Primaquine	0	2/11	64%	9/11	82%
	1	0/10	0%	0/10	0%
	7	0/0	-	0/0	-
	14	0/0	-	0/0	-
	21	0/0	-	0/0	-

### Vector Engorgement and Survival

*P. vivax* : The percentage of mosquitoes taking a bloodmeal upon being exposed to infected patients was similar in all treatment categories (Table 5). In nearly every case larger numbers of *Anopheles balabacensis* fed than *Anopheles maculatus*. Likewise, larger number of *Anopheles balabacensis* survived to be dissected than *Anopheles maculatus*. These data suggest that *Anopheles balabacensis* may be a more effective vector of *vivax* malaria from Thailand.

Table 5. Vector Engorgement and Survival

*P. Vivax*

Therapy	Day	No. of Lots Fed	<i>Anopheles balabacensis</i>		<i>Anopheles maculatus</i>	
			% Engorgement	% Survival	% Engorgement	% Survival
Mefloquine	0	17	70	66	62	48
	1	15	75	75	74	53
	7	6	77	55	53	57
	14	3	68	65	59	62
	21	4	74	63	54	57
Fansidar (2)	0	10	78	73	72	67
	1	11	83	66	86	58
	7	3	84	71	72	64
	14	2	60	66	53	32
	21	0	-	-	-	-
Fansidar (3)	0	8	80	89	69	55
	1	6	67	61	83	51
	7	3	91	49	63	50
	14	2	71	71	53	43
	21	1	80	74	78	100
Chloroquine	0	17	82	85	71	53
	1	13	82	69	81	61
	7	3	60	67	63	55
	14	2	78	78	75	54
	21	2	83	69	75	50
Chloroquine-Primaquine	0	11	77	83	77	56
	1	10	85	72	84	53
	7	3	86	90	66	45
	14	2	44	90	58	48
	21	0	-	-	-	-

Mosquitoes were held separately for longevity analysis in order to compare the effect of the parasite on the vector. Mosquitoes fed on uninfected volunteers throughout the course of the study survived a mean length of 26.4 days (*A. balabacensis*) and 18.4 days (*A. maculatus*).

Mosquitoes from infected lots were compared with those fed at the same time on normal volunteers (simultaneous controls). *A. balabacensis* from infected and control lots showed little difference, but uninfected *A. maculatus* appear to

have significantly greater longevity than those from infected lots.

*P. falciparum* : It is too early to completely evaluate data from this group of patients. However, it seems that *Anopheles balabacensis* may again be a more efficient vector of *P. falciparum*, as seems to be the case with *P. vivax*.

Data is still being collected in this study, and it is anticipated that at least another six months will be required for completion.

Table 6. Longevity in *P. vivax*-infected and Uninfected Vector Mosquitoes

		<i>Anopheles balabacensis</i>		<i>Anopheles maculatus</i>	
		Infected	Uninfected	Infected	Uninfected
Mosquitoes fed before patient treatment	No. of lots	22	19	20	13
	Longevity (day)	Mean	23.5	16.9	21.0
	Range	1-48	1-51	2-34	2-34
Mosquitoes fed one day after patient treatment	No. of lots	9	8	11	10
	Longevity (day)	Mean	29	19.6	24.8
	Range	5-48	6-53	4-40	1-34