

## Longitudinal Malaria Studies in Rural Northeast Thailand

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**INTRODUCTION:** A prospective longitudinal study of the transmission of Plasmodium falciparum malaria was begun at Ban Bu Phram and Ban Tablan, Tambon Tungpo, Prachinburi Province. Initially, attention was to be focused on the patterns of parasitemias and clinical disease associated with chloroquine-sensitive and chloroquine-resistant strains of the falciparum parasite. All parasitemias in Bu Phram were to be treated with chloroquine in an effort to determine whether intensive chloroquine pressure could be shown to measurably increase the proportion of chloroquine-resistant infections. Most falciparum infections were found to be resistant to chloroquine treatment from the outset (see below). The objectives of the study were therefore restated as follows:

### OBJECTIVES:

- (1) To study the variables associated with the acquisition, duration, and density of P. falciparum parasitemias.
- (2) To measure the prevalence of chloroquine-resistant falciparum infections and evaluate the usefulness of chloroquine as a control measure.
- (3) To assess the feasibility of successfully performing a prophylactic drug trial.

**DESCRIPTION:** Two Northeast Thai villages, Ban Bu Phram and Ban Tablan, Prachinburi Province, were selected for study. They are located in a heavily-forested mountainous region approximately 100 kilometers south of Nakorn Ratchasima (Korat) on Route 304. On the basis of hospital reports, chloroquine-resistant falciparum malaria and vivax malaria were considered to be endemic. The villages were mapped and censused in mid-April 1971. There were 380 persons resident in Bu Phram and 605 in Tablan at that time. A random sample of households was drawn and included 252 persons in both villages. Both village samples ("Family Group") were representative of the entire village populations and comparable to one another.

Investigators visited each family sixteen times, at approximately two-week intervals, between late April 1971 and early January, 1972. During each visit they inquired about a history of fever and/or headache and collected capillary blood specimens for quantitative parasite counts and, on one occasion, for glucose-6-phosphate dehydrogenase (G-6-PD) determination and hemoglobin electrophoresis. In Bu Phram, all Family Group subjects with falciparum or vivax parasitemia, symptomatic or asymptomatic, were treated initially with chloroquine (Comer et al, 1968). In Tablan, only those with symptomatic parasitemias were treated.

Sickcall was held weekly and non-Family Group persons ("Sickcall Group") with complaints related or unrelated to malaria were seen and treated. All parasitemias found in members of this group were treated with chloroquine.

Parasite densities were determined by relating the parasite—leukocyte ratio on the blood film to the total leukocyte count. The methemoglobin elution test (Gall et al, 1965) was used to identify G—6—PD deficient, heterozygous, and normal persons. Hemoglobin electrophoresis, carried out on cellulose acetate strips, was used for family pedigree analysis.

The data accumulated permitted study of the association of falciparum and vivax infection rates, average number of parasitic and gametocytic episodes per infected subject, parasitemia—associated morbidity, and parasite densities with variables under consideration. A history of fever preceding or fever coincident with documented parasitemia was recorded as evidence of symptomatic infection since other symptoms, complications, and mortality from malaria were not observed. Data from the study villages were pooled for this report, since chloroquine treatment of asymptomatic parasitemias (Ban Bu Phram) was without demonstrable effect (see below).

PROGRESS: Variables Associated with Infection: The associations of P. falciparum and P. vivax infections with the variables of age and sex, time and place of parasite acquisition, and G—6—PD deficiency were studied. Forty—six percent of subjects studied were parasitemic with P. falciparum and 23 percent with P. vivax during the study period (Table 1). Parasitemia rates were similar in all subjects except for those under one year of age. Males were infected in greater proportion than females (Table 2).

Peak transmission occurred during May, June, and July, with lower levels occurring during the remainder of the study period. In the ten week period from mid—May through July, 61 percent of the "new" falciparum infections and 47 percent of the "new" vivax infections were found. The largest collections of Anopheles balabacensis were also made during this period and four mosquitoes were found infected (Gould and Wilkinson, 1972).

Twenty—nine percent of Family Group subjects resided in shelters in fields away from the village for some part of the study period (Table 2). These subjects were at greater risk of acquiring infection than subjects denying any overnight absences from the village. Whether subjects presumably infected in the field were infected early in the season and introduced infection into the village is under study.

The 72 percent of subjects who gave no history of overnight absence were presumably infected in the village proper. The night—time patterns of activity of 22 Family Groups were observed (Table 4) and compared with vector mosquito collections (Gould and Wilkinson, 1972). Seventy—four percent of An. balabacensis collected were caught after 2300 hours, when all subjects were inside the house, asleep, and those who had mosquito nets were using them. Infection rates in those subjects using nets (infants and females) were lower than rates in non—users. Two cycles of house—spraying with residual DDT were completed by National Malaria Eradication Program (NMEP) personnel during the study period.

The prevalence of the gene for G—6—PD deficiency was 16.1% in male Family Group subjects. Thirteen males and two females were found to be enzyme—deficient. Fifteen (female) heterozygotes were identified by a combination of the methemoglobin elution test and/or family pedigree. Enzyme—deficient and heterozygous Family Group subjects were not infected with P. falciparum in lesser proportion and were not parasitemic less often than comparable enzyme—normal relatives or the remainder of the Family Group (Table 5) (Segal et al, 1972).

Usefulness of Chloroquine in Treatment of P. falciparum: The usefulness of chloroquine treatment of both asymptomatic and symptomatic falciparum parasitemias was studied. (All vivax parasitemias were sensitive to chloroquine). Prior use of chloroquine in the villages was assessed by determining what proportion of villagers suspected of being parasitemic might have (ideally) been treated by the NMEP house visitor. (The house visitor dispensed chloroquine based on a history of fever). Data accumulated (Table VI) suggested that he might have treated six percent of falciparum parasitemias, twelve percent of vivax parasitemias, and few non—parasitemic persons. On three occasions, self—treatment with chloroquine (for fever) was observed by the investigators.

In Bu Phram, all parasitemias found in Family Group subjects were treated; in Tablan, only symptomatic parasitemias were treated. Parasitemic subjects in Bu Phram were treated approximately three times as often (Table 7). Nevertheless, the average number of episodes of parasitemia experienced was similar to that of Tablan. The average number of episodes of gametocytemia and parasite densities experienced were also similar.

Subjects from either the Family Group or Sickcall Group under twenty years of age and with falciparum parasitemias higher than 1000 asexual parasites per cubic millimeter of blood were treated with chloroquine to determine the proportion of chloroquine-resistant P. falciparum strains. Thirty-eight such subjects were found, treated, and followed for 28 days (Table VIII). Only five of the 38 (0.13) were cured of their infection. The majority of strain responses were of the R2 pattern. Subjects cured of their infections were somewhat older (median age 11.0 years) than those with resistant infections (median age 6.0). Subjects with either sensitive or resistant infections were equally distributed by sex and village.

Subjects not responding to chloroquine and having continuing symptoms (R3 pattern) were treated with quinine; all were cured. Subjects from among those described above, if not symptomatic, were retreated with chloroquine if they had received no other treatment and if their parasite density was still greater than 1000 per cubic millimeter on day 28 of follow-up. Of five such subjects, three demonstrated falciparum infections "more sensitive" to chloroquine on retreatment (Table IX).

Sickcall was held weekly to identify parasitemic persons outside the Family Group. The majority of persons presented with complaints seemingly unrelated to parasitemia. Parasitemia was suspected and capillary blood for smear taken from 303 (0.18) of the 1963 persons seen. One hundred fourteen (0.38) of these 303 persons were infected with P. falciparum and 33 (0.11) with P. vivax. All parasitemic persons found at sickcall were treated with chloroquine.

Feasibility of Prophylactic Antimalarial Drug Trials: The prospects for successful completion of a prophylactic antimalarial drug trial were assessed using census data, participation rate data, and infection rate data obtained during the study period. In a trial restricted to persons ten years of age and older, 937 persons (0.73 of the population) would be eligible for study. The minimum number of subjects required was calculated, based on the following assumptions: 1) parasitemia rates similar to those observed during the previous study, 2) A two-fold reduction of falciparum parasitemia by the drug under study, and 3) participation rates higher than the lowest rate attained during the previous study (55 percent). A minimum of 103 subjects, in each of the study groups (drug and placebo) would be required. Thus, such a drug trial is feasible.

**Table 1.**  
**Age-specific incidence rates for falciparum and vivax parasitemias among Family Group subjects.**

Age group	Population at risk	Falciparum parasitemia	Vivax parasitemia	Parasitemias combined
< 1	17	2 (0.12) *	1 (0.06)	3 (0.18)
1-4	35	14 (0.40)	10 (0.29)	24 (0.69)
5-9	37	20 (0.54)	11 (0.30)	31 (0.84)
10-19	50	30 (0.60)	14 (0.28)	44 (0.88)
20-29	45	22 (0.49)	11 (0.24)	33 (0.73)
30-39	33	11 (0.33)	5 (0.15)	16 (0.48)
> 40	35	18 (0.51)	6 (0.17)	24 (0.69)
All ages	252	117 (0.46)	58 (0.23)	175 (0.69)

\* Number (proportion) infected

**Table 2.**  
**Sex-specific incidence rates for falciparum and vivax parasitemias among Family Group subjects.**

Sex	Population at risk	Falciparum parasitemia	Vivax parasitemia	Parasitemias combined
Male	127	69 (0.54) *	33 (0.26)	102 (0.80)
Female	125	48 (0.38)	25 (0.20)	73 (0.58)
Both sexes	252	117 (0.46)	58 (0.23)	175 (0.69)

\* Number (proportion) infected

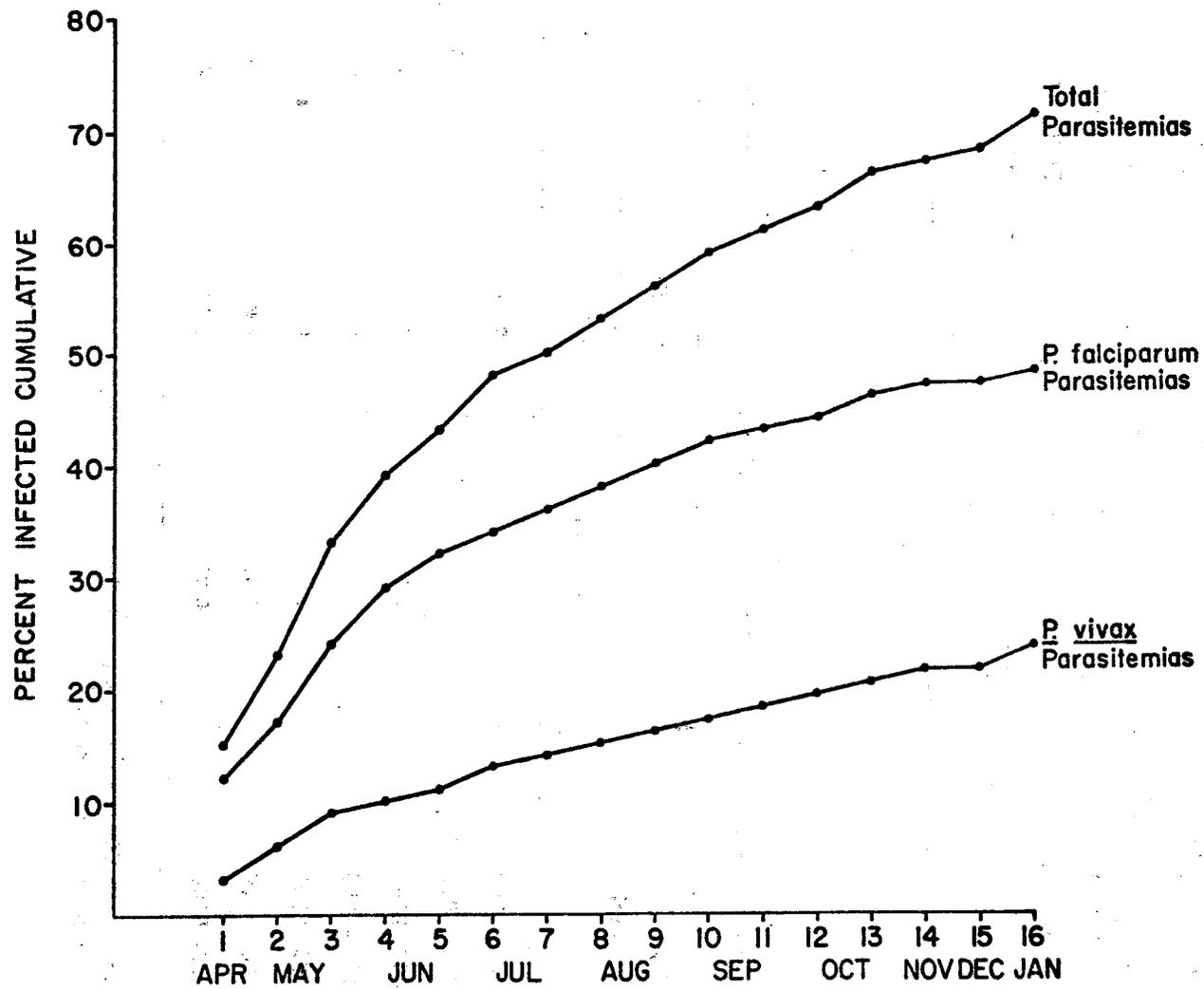


FIGURE 1. CUMULATIVE PERCENT FAMILY GROUP SUBJECTS INFECTED.

**Table 3.**  
**The relationship of a history of overnight absence from the village to falciparum and vivax parasitemias in Family Group subjects.**

Absence history	Population	No. (proportion) infected
Absent	58	42 (0.72)
Not absent	146	76 (0.52)
Total	204	118 (0.58)

**Table 4.**  
**Nighttime activities of 22 Family Groups observed during biting\* collections.**

Time period	Activity		
	Inside house	Asleep	Under mosq. net
1800-1900	0.67 <sup>+</sup>	0.05	0.01
1900-2000	0.88	0.28	0.14
2000-2100	0.95	0.75	0.48
2100-2200	0.96	0.92	0.57
2200-2300	0.97	0.93	0.60
2300-0500	1.00	1.00	0.55
0500-0600	0.98	0.72	0.64

\* These represent 50% of the Family Groups  
<sup>+</sup> proportion of persons observed.

Table 5.  
P. falciparum Infection rates and average number of parasitemic episodes  
in G-6--PD deficient, heterozygous, and normal Family Group members.

Population	Sex	No. persons	No. (proportion) infected	No. of parasitemic episodes	
				Total	Average +
Deficient	Male	13	9(0.69)	45	5.0
Deficient	Female	2	0	0	0
Heterozygous (test)	Female	9	2(0.22)	4	2.0
Heterozygous (all)*	Female	15	4(0.27)	10	2.5
Enzyme—normal relatives	Male	24	9(0.38)	31	3.4
	Female	20	4(0.20)	8	2.0
Remainder of Family Group	Male	73	39(0.53)	105	2.7
	Female	72	30(0.42)	129	4.3

\* This group includes individuals classified by pedigree as well as by the methoglobin elution test.  
+ Number of parasitemic episodes per infected person.

Table 6.  
Parasitemic and non-parasitemic-associated fevers among Family Group subjects

Smear	No. infected subjects	No. parasitemic episodes	No. febrile episodes	Proportion febrile episodes
<u>P. falciparum</u>	118	421	36	0.06
<u>P. vivax</u>	57	104	12	0.12
Negative	0	0	48	0.01*

\* Based on 3219 negative smears

Table 7.  
The effect of chloroquine treatment on the number of episodes of falciparum parasitemia.

Village	No. subjects infected, total	No. treatments		No. episodes of parasitemia	
		total	per infected subject	total	per infected subject
Bu Phram	62	172	2.8	229	3.7
Tablan	56	50	0.9	190	3.4

Table 8.  
Response to chloroquine treatment of selected Family and Sickcall Group subjects with falciparum parasitemias.

Response pattern	Number	Proportion
Sensitive	5	0.13
R1	6	0.16
R2	22	0.58
R3	5	0.13
Total	38	1.00

Table 9.  
Response to chloroquine retreatment of selected Family and Sickcall Group Subjects with falciparum parasitemias.

Patient (sex, age)	Initial response	Retreatment response
B 48 (F,8)	R1	R1
SB 34 (F,3)	R3	R2
B 12 (F,7)	R2	R2
SB 59 (F,6)	R3	S
SB 45 (F,6)	R3	R1