

## Ecology of Malaria Vectors

Principal Investigators: Douglas J. Gould, Ph.D.  
R. N. Wilkinson, CPT, MSC

**OBJECTIVE:** To investigate the bionomics and population dynamics of known and potential vectors of human malaria in Southeast Asia and their relationship to the dissemination of chloroquine resistant strains of *P. falciparum*.

**DESCRIPTION:** Specific factors being studied in the process of defining actual and potential vector species in Thailand include the following: incidence of malarial oocysts and sporozoites in wild anopheline populations, susceptibility of colonized strains of *Anopheles* to infection with *P. falciparum*, patterns of biting activity of vector species, ovipositional habits of anopheline mosquitoes and the viability of their eggs under various environmental conditions.

### PROGRESS:

1) *Malaria field studies in Prachinburi province.* Entomological field studies were continued in Prachinburi Province in support of a longitudinal study of the epidemiology of malaria in the area. Results of studies described in the previous Annual Report (Segal, 1971)<sup>1</sup> indicated that the risk of malaria was greater in the forest than in the village setting. *Anopheles balabacensis* was shown to be the predominant malaria vector in the area. Between April and November, 1972, anopheline collections from human—bait and vegetation were made simultaneously on nine nights in forest and village locations to compare vector densities in the respective areas. Except for one night, these collections were made between dusk and dawn. In these collections, a total of 403 *A. balabacensis* were collected, biting humans and resting on vegetation in the vicinity of collectors in the forest, as opposed to only 30 in the village site. The number collected biting per man per night is shown in Table 1 for both locations. In addition, 421 *A. balabacensis* were collected biting or resting in the forest on seven separate nights, while in the village only 55 *A. balabacensis* were collected on ten nights. The breakdown of the number of these mosquitoes collected biting per man per night is shown in Table 2. The only *A. balabacensis* found infected with either oocysts or sporozoites were collected in the forest during the dry season in January and February 1973. In January four of 92 collected biting contained either oocysts and/or sporozoites while one additional *A. balabacensis* collected resting also was found to contain oocysts. Two of 47 *A. balabacensis* collected biting on one night in February contained oocysts. Blood films were obtained from five people living in a nearby hut and trophozoites of *P. falciparum* were demonstrated in the blood from four of these individuals. One slide had *falciparum* gametocytes as well as asexual stages.

Efforts were made during this period to determine how *A. balabacensis* populations survive the extended dry season. Previous studies had shown that eggs were not important in over—dry season survival, and larvae were not found at the time either in Ban Bu Phram valley or at the edge of the forest. During the dry season covered by this report, *A. balabacensis* larvae were found in rock pools alongside partially dry stream beds on the steep slopes of surrounding hills and in shallow pits dug at stream margins by villagers as a source of water in the forest. Identical pools were dug out by SMRL personnel and *A. balabacensis* larvae were collected from these new pools within 10 days. Thus, it appears that during the dry season the life cycle of *A. balabacensis* is maintained in the forest and that breeding extends into the central portion of the valley during the rainy season as breeding sites become available there.

2) *Susceptibility of A. balabacensis and A. minimus to infection with P. falciparum prior to and after standard chloroquine therapy.* The suggestion has been made that the wide—spread use of chloroquine may have contributed to the rise of chloroquine resistant strains of *P. falciparum* (Langer, et al., 1966).<sup>2</sup> Romkaran & Peters (1968)<sup>3</sup> have shown that the infectivity of a chloroquine—resistant strain of *P. berghei* for *A.*

*stephensi* is enhanced after treatment with chloroquine. No differences were observed in the infectivity of drug sensitive strains after treatment with chloroquine. A study was undertaken to determine if chloroquine enhanced the infectivity of *P. falciparum* for *A. balabacensis* and *A. minimus*. Laboratory reared strains of these two species were fed simultaneously on 29 human subjects with *P. falciparum* infections in which gametocyte densities were at least 25 per cmm. Mosquitoes were fed on these subjects immediately prior to administration of 1500 mg chloroquine base (day 0) and on days 2 and 7 when treatment was completed. All patients were admitted to the district hospital in Phra Phutthabat for the first 7 days of study. During this period blood films were taken daily and examined for parasites. After the subjects were discharged from the hospital, follow-up blood films were taken on days 14, 21 and 28 to determine the status of chloroquine resistance, using WHO criteria. Mosquitoes which fed on these subjects were dissected 8 to 10 days later and the oocysts in their guts counted. The proportions of mosquitoes with oocysts (per cent positive) and the mean number of oocysts per infected mosquito (oocyst index) were used to compare the susceptibility of the two mosquito species to untreated and treated falciparum malaria.

Seventeen of the subjects were infectious to at least one mosquito species on either the pre- or one of the post-treatment feedings. Two of the subjects were not infectious on the pre-treatment feed but one of these was infective on the second day and the other was infective on day 7. A twenty-eight day follow-up was completed on 13 of the 17 subjects. Ten of these exhibited an RI response and 3 were RII. Three subjects could not be followed after discharge and an additional subject was switched to quinine and tetracycline therapy prior to discharge. The results of the dissection of the groups of mosquitoes which fed on these subjects are shown in Table 3. There was a decrease in the median percent infected for both mosquito species after chloroquine treatment. These results suggest that chloroquine treatment did not enhance the infectivity of chloroquine resistant strains of *P. falciparum*; however, no RIII level resistant strains were studied.

In this study, there was little difference in the susceptibility of the two mosquito species to infection with falciparum gametocytes, although previous studies indicated that *A. balabacensis* were more susceptible than *A. minimus*. Techniques used were the same in both studies; however, in the present investigation higher proportions of *A. minimus* fed and more were dissected than previously. In addition, the larval diet in the *A. minimus* colony was changed between studies. This change resulted in greater longevity in the *A. minimus* colony.

3) Irritability of *A. balabacensis* to DDT. Tests to determine the irritability of *A. balabacensis* to DDT have been initiated using the procedures recommended by the WHO. Initial results are inconclusive and further studies are planned.

#### REFERENCES:

1. Segal, H.E.: Longitudinal malaria studies in rural Northeast Thailand. Ann. Prog. Rep. SEATO Med. Res. Lab. 1971-72.
2. Langer, B.W., Rutledge, L.C. and Gould, D.J.: Chloroquine: Retention by *Anopheles stephensi* after ingestion in a blood meal. Mosq. News 28 (3): 455-58, 1968.
3. Ramkaran, A.E. and Peters, W.: Infectivity of chloroquine resistant *Plasmodium berghei* to *Anopheles stephensi* enhanced by chloroquine. Nature 223 (5206): 635-36, 1969.

Table 1. *A. balabacensis* collected biting man on simultaneous nights in the forest and village settings

Month	No. nights	Forest		Village	
		No. Coll.	No./man night	No. Coll.	No./man night
Apr	1	100	16.6	3	0.6
May	4	60	5.0	7	0.3
Jun	2	30	3.0	1	0.1
Aug*	1	4	2.0	1	0.5
Oct	1	7	3.5	2	0.5
Nov	1	8	4.0	5	1.3
Totals	10	209	6.15	19	0.48

\* Collection between 1800–2400 hrs.

Table 2. *A. balabacensis* collected biting man on different nights in the forest and village settings

Month	No. nights		Forest		Village	
	Forest	Village	No. Coll.	No./man night	No. Coll.	No./man night
Apr	—	2	—	—	4	0.4
Jun	3	5	90	5.6	18	0.9
Jul	—	2	—	—	6	1.4
Aug*	1	1	26	6.5	15	3.8
Jan*	1	—	92	30.6	—	—
Feb*	2	1	81	5.8	0	0
Totals	7	10	289	7.8	43	1.2

\* Collections between 1800–2400 hrs

Table 3. Results of dissections of *A. balabacensis* and *A. minimus* which were infected when fed simultaneously on subjects either prior to or on day 2 or 7 after starting chloroquine treatment.

Species	Day	Percent Positive		Oocyst Index	
		Median	Range	Median	Range
<i>A. balabacensis</i>	0*	48	0-100	4.1	1.0-120.2
	2**	40	0-100	8.3	1.0-29.2
	7***	24	0-100	5.8	2.1-46.7
<i>A. minimus</i>	0*	49	0-100	2.6	1.0-25.0
	2**	38	0-100	3.3	1.0-9.5
	7***	26	0-93	3.5	2.5-32.9

\* Day 0-29 subjects  
 \*\* Day 2-23 subjects  
 \*\*\* Day 7-19 subjects