

## Studies on Malaria Vectors

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**OBJECTIVE:** To investigate the bionomics and population dynamics of known and potential vectors of human malaria in Southeast Asia, their relationship to the dissemination of chloroquine-resistant strains of *P. falciparum*, and to apply the information acquired to the development of effective methods of control for these vectors.

**DESCRIPTION:** Specific factors being studied in the process of defining actual and potential vector species in Thailand include the following: incidence of malarial parasites in wild anopheline populations, susceptibility of colonized strains of *Anopheles* to infection with *P. falciparum*, patterns of biting activity of suspect anopheline species, and ovipositional habits of proven vector species.

### PROGRESS:

1) *Malaria field studies in Prachinburi Province:* Entomological field studies were continued in the Bu Phram valley of Kabinburi District. Major emphasis was on a continuation of the survey for dry season breeding sites of *A. balabacensis*. As reported in previous years, ovipositional sites of this malaria vector have been located during the dry season along stream beds on the forested slopes of the hills surrounding Bu Phram valley. Water trapped in rock holes from the previous rainy season or fed by persistent springs on the slopes provide sites for low density breeding of *A. balabacensis* during the dry season. Because of the time and effort required to locate these sites in the difficult terrain of this area, application of larval control measures based upon ground surveys alone would be impractical. The possibility of applying infrared aerial photography as a survey technique for locating dry-season breeding sites was explored during this reporting period. Aerial photographs of jungle areas taken with infrared film can delineate small areas of water beneath the forest canopy because of differences in the infrared reflectivity of the adjacent vegetation. Through the cooperation of the 432 Technical Reconnaissance Wing, 7/13 Air Force, USAF, aerial photographic surveys of the entire Bu Phram valley and adjacent hills were flown during January and March 1974, using Kodak 2424 infrared aerographic film. A third survey of this area is planned for after the onset of the monsoon rains. Processing and interpretation of these aerial surveys is being carried out by the School of Public Health, University of Texas, through contract with the National Aviation and Space Agency, Houston.

(2) *Susceptibility of A. balabacensis and A. minimus to infection with P. falciparum prior to and after standard chloroquine therapy:* Results of studies given in the last Progress Report (1) indicated that when *A. balabacensis* and *A. minimus* were fed on patients with chloroquine-resistant *falciparum* malaria prior to and 2 and 7 days after chloroquine therapy there was no increase in the proportion of mosquitoes of either species infected after the initiation of therapy; however, the oocyst indices (mean number of oocysts per infected mosquito) for *A. balabacensis* were 1.7 and 1.8 times greater, respectively, on days 2 and 7, than on day 0 (Table 1). A similar increase in oocyst indices was not observed for *A. minimus*. Since gametocyte densities in the donor patients on days 0, 2 and 7 were not significantly different, it is difficult to explain the observed increases in oocyst indices on that basis. These observations do, however, agree with observations on the enhancement of infectivity of *P. berghei* for *A. stephensi* fed on mice infected with chloroquine-resistant strains of the parasite after the mice had been treated with 1

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or 10 mg/kg body weight of chloroquine (2). The apparent enhancement of the infectivity of chloroquine-resistant strains of *P. falciparum* for *A. balabacensis* (but not *A. minimus*) lends support to the hypothesis that this mosquito species has been a contributing factor to the rapid increase of chloroquine-resistant strains of *P. falciparum* in Southeast Asia.

Table 1. Median and Ranges of Oocyst Indices for Mosquitoes which Fed on Infective Patients on Days 0, 2 & 7

| Day | Number<br>Inf. Pts. | Median (Range) Oocyst Indices |                   |
|-----|---------------------|-------------------------------|-------------------|
|     |                     | <i>A. balabacensis</i>        | <i>A. minimus</i> |
| 0   | 13                  | 3.3 (0-120.2)                 | 2.8 (0-24.9)      |
| 2   | 11                  | 5.7 (0-29.2)                  | 2.4 (1.0-9.5)     |
| 7   | 6                   | 6.1 (2.1-46.7)                | 3.1 (1.0-32.9)    |

Table 2. Summary of the Duration of the Immature Stages of Colonized *Anopheles minimus*

| Stage      | Day Present | Day 50 Percent<br>Attain a Given Stage | Percent<br>Mortality |
|------------|-------------|--|----------------------|
| 1st instar | 0-7         | —                                      | 1.9                  |
| 2nd "      | 2-11        | 3                                      | 0.7                  |
| 3rd "      | 5-18        | 7                                      | 0.1                  |
| 4th "      | 6-25        | 10                                     | 0.7                  |
| Pupae      | 8-26        | 13                                     | 5.8                  |

3) Determination of volume of blood meals ingested by *A. minimus* and *A. balabacensis*: Comparison of the infection rates occurring in colonized strains of *A. balabacensis* and *A. minimus* fed simultaneously on falciparum malaria patients indicated that a significantly higher proportion of *A. balabacensis* became infected and also that the mean number of oocysts developing in the gut of infected *A. balabacensis* was higher than in *A. minimus*. These differences could be related to the volume of blood ingested by each species or to differences in the ability of each to concentrate the cellular components of their blood meals.

The volumes of blood meals taken by laboratory-reared *A. balabacensis* and *A. minimus* were compared by feeding them simultaneously on rabbits whose RBC and serum had been labelled with Cr<sup>51</sup> and I<sup>125</sup>, respectively. In order to collect excreta discharged by the mosquitoes during engorgement, they were

individually housed in filter paper-lined vials whose nylon-screened mouths were placed against the shaved sides and back of the donor rabbit. Blood meal volumes and the composition of mosquito excreta were determined by comparing the radioactivity of the engorged mosquitoes and the filter papers containing their excreta with that of a sample of blood from the donor rabbit taken at the time of feeding.

Four separate feeding experiments were completed in which a total of 72 *A. balabacensis* were fed on rabbits with radioisotope-labelled blood. The mean volume of blood ingested by this species was 2.69  $\mu$ l (range 0.14–4.95  $\mu$ l). The excreted portion of the blood meal was measured for 8 *A. balabacensis* and averaged 0.12  $\mu$ l per individual; this excreted portion consisted almost entirely of serum globulins. The mean volume of blood ingested by 17 *A. minimus*, on the other hand, was 1.38  $\mu$ l. Excreta discharged during feeding, for 8 of these mosquitoes, was approximately the same as for *A. balabacensis*—0.11  $\mu$ l per mosquito. These initial experiments suggest that differences in rate of infection observed between the two anopheline species can at least partially be explained by these differences in blood-meal volumes.

4) *Bionomics of laboratory-reared Anopheles minimus*: A colony of *A. minimus* has been successfully maintained at SMRL since 1970. Observations on this colony made during the present reporting period are given below. The SMRL strain of *A. minimus* was established from the progeny of 32 engorged females collected feeding on buffaloes in Saraburi province and returned to the field insectary at Phra Phutthabat. The insectary was maintained at 24–28°C and 55–90% relative humidity. In our insectary, these mosquitoes laid a total of 1572 eggs (mean: 49/female), ninety per cent of which were fertile. Adults reared from the offspring of the wild-caught females were forced-mated and their progeny formed the basis of the present colony, which has been maintained by the same forced mating technique used with the *A. balabacensis* colony (3). After several generations were fed on human blood, adaptation to feeding on hamsters was accomplished. As with the colonized strain of *A. balabacensis*, female *A. minimus* are mated after they have taken a blood meal. Oviposition is on the surface of water in paper cups, in preference over moist filter paper or sand. An average of 90 eggs per female are deposited between 3 and 15 days following forced-mating. Eggs hatch two to three days after oviposition. The larvae were fed a mixture of two parts Bacto Liver Powder and one part each of ground laboratory guinea pig and rat-mouse food. First and second instar larvae were fed twice and third and fourth instars three to four times daily. The duration of the larval stages ranges from 8 to 26 days, with a mean of 3–4 days for each instar (Table 2). Fifty per cent of *A. minimus* larvae in this laboratory strain pupate by the 13 th day, and 96 per cent of larvae reaching the pupal stage complete development to adults. Average larval mortality for the past year has been approximately 3 per cent. The sex ratio of emerging adults during this period has been 44 per cent male and 56 per cent female. The average production from the colony was 3045 males and 3501 females per month. Mortality of emerging adults has been approximately 6 per cent (Table 2). Sixty-four per cent of pupation occurs between 1800 and 0600 hours, while the majority of adults (59%) emerge during the same period.

#### REFERENCES:

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3. Esah, S. and Scanlon, J.E.: Mosquito News 26:509, 1966.