

COMPARATIVE SUSCEPTIBILITY OF KNOWN AND SUSPECTED
SPECIES/STRAINS OF *Anopheles* TO
Plasmodium PARASITES

Principal Investigators : Bruce A. Harrison, LTC, MSC
Richard G. Andre, MAJ, MSC
Terry A. Klein, CPT, MSC

Associate Investigators : Suwattana Vongpradist
Franklin Wilson, SSG
Inkam Inlao
Vichit Punkitchar

Assistant Investigators : Nongnuj Maneechai
Ruan Thaopha
Sanit Nakngen

OBJECTIVES :

1. To determine and compare the susceptibility of colonized primary and suspected secondary vectors of malaria to human and simian parasites.
2. To observe the development of malaria parasites in anopheline species with varying degrees of susceptibility to malaria parasites.
3. To observe the feeding behavior of colonized primary and suspected secondary vectors of malaria under laboratory conditions.

BACKGROUND : During the last few years human malaria case rates in Thailand have increased drastically, and shifts have occurred in the prevalence of *Plasmodium* species (1, 2). A number of hypotheses have been proposed that possibly can explain this increase and/or the shifts in the *Plasmodium* species ratios, including : (A) changes in the behavior of the vector populations, e.g., a shift from zoophily to anthropophily; (B) development of physiologic or behavioristic resistance in the vector populations (3, 4); (C) development of drug resistance by the *Plasmodium* parasites (5, 6); (D) use of prophylactic and treatment regimens of drugs (Fansidar) that may actually enhance gametocyte production (7, 8); (E) misdirection of control efforts due to the misinterpretation of the species/strains of anophelines that have been serving as vectors in Thailand (9); and (F) misdirection of control efforts due to changes or unsuspected differences in the basic susceptibility levels of the Thai *Anopheles* vectors and suspected vectors to the malarial parasites. These last 2 points are of primary importance for understanding the epidemiology of malaria in Thailand. As noted elsewhere in this annual report (10, 11) the primary vectors previously called *An. balabacensis* and *An. maculatus*, now are known to be complexes of several sibling species which are poorly understood. Furthermore, the suspected vector previously called *An. philippinensis* now is

known to consist of at least 2, and probably 3, species of which *An. nivipes* apparently is the most abundant. This taxonomic confusion about the taxa we presume are serving as vectors, causes further complications, because we can no longer be certain which of the sibling species involved in these complexes are actually susceptible to malarial parasite infections. This is particularly critical since studies have demonstrated that malaria susceptibility is under genetic control (12) and that potential vector species and even different populations of a known vector can vary greatly in their susceptibility to the infection of malarial parasites (13).

During the past 3 years a large number of known vector and suspected vector species/strains have been colonized at AFRIMS (11) in preparation for vector susceptibility studies. The present study was designed to determine the susceptibility of these species/strains of *Anopheles* to *Plasmodium cynomolgi* (a *P. vivax*-like parasite), *P. falciparum* and *P. vivax*. Once the comparative susceptibilities of these species/strains of *Anopheles* have been determined, emphasis can be switched toward determining the basic behavioral patterns and control methods for those species determined to be the most likely vector candidates in nature.

MATERIALS AND METHODS : Colony mosquitoes will be tested for their susceptibility to develop infections of *Plasmodium cynomolgi* Mayer (B strain), *P. falciparum* (Welch) and *P. vivax* (Grassi and Feletti) parasites. The studies with *P. cynomolgi* will be conducted at AFRIMS, Bangkok, while the studies of the human malaria parasites will be conducted in Kanchanaburi Province and at AFRIMS, Bangkok. *Anopheles dirus* Peyton and Harrison, a very susceptible and proven malaria vector (14, 15, 16) will be used as a control, while *An. balabacensis* Baisas (Perlis form), *An. Fraser's Hill* form, *An. maculatus* Theobald (Kuala Lumpur, Nakhon Nayok and Huai Kuum strains), *An. minimus* Theobald (Tap Quang Strain), *An. nivipes* (Theobald) (Korat-Khonburi and Phrae strains), *An. philippinensis* Ludlow (Rayong strain) and *An. takasagoensis* Morishita will be tested for susceptibility. All of the test species/strains are suspected or known vectors of malaria parasites in Thailand and/or other countries.

The studies will conform to the design depicted in Figure 1. Fifty, 3-4 days old female mosquitoes from the Control Group (*An. dirus*) and one or more of the Test Groups will be fed simultaneously on either a rhesus monkey infected with *P. cynomolgi*, or on a human patient infected with *P. falciparum* or *P. vivax* (see below "criteria for feeding on humans"). Blood-fed adults from each of the groups are removed, placed in a screened specimen cup and provided a 5% multi-vitamin solution as a food source. Mosquitoes not having taken a blood meal are discarded.

One half of the blood-fed mosquitoes from both the Control and Test Groups are dissected on day 7 and midguts are examined for the presence of oocysts and black spores of Ross. The mean oocyst size, range and frequency are also determined. When midguts are heavily infected, i.e., more than 100 oocysts, estimates of >100, >200, etc. are used.

The remaining mosquitoes from the Control and Test Groups are dissected on day 14 and the midguts and salivary glands are examined. The midguts are examined for the presence of mature and burst oocysts and black spores of Ross. The salivary glands are examined for their general condition, i.e., ruptured, discolored, etc., and the sporozoite rate will be determined, i.e., 1-10 sporozoites (+1), 11-100 sporozoites (+2), 101-1,000 sporozoites (+3), and >1,000 sporozoites (+4).

The monkey malaria studies will utilize rhesus monkeys that have been admitted to the WRAIR drug testing program. Mosquitoes are fed on the monkeys during the second peak of parasitemia, when there are good ratios of male and female gametocytes.

Criteria for feeding on humans

Persons to be considered for the AFRIMS study will consist of walk-in patients reporting to the zone malaria office in Tha Muang District, Kanchanaburi Province. They will have been identified by the malaria center personnel to have mild to moderate malaria parasite infections, based on thick/thin blood smear preparations. Patients will be screened and must meet the following criteria before being admitted to the study. The patient must: (A) have uncomplicated disease of mild to moderate severity; (B) have no other serious medical problems; (C) be a male; (D) be 20 years or older; (E) have asexual parasite levels between 1,000-100,000/mm³; (F) have gametocytes present on the blood smears; (G) volunteer for the study after a thorough explanation of the study and reading the fact sheet; and (H) sign a consent form.

RESULTS : During this period the studies testing the susceptibility of mosquito species/strains to the simian parasite, *Plasmodium cynomolgi* were concluded. During a previous report (17) only 4 species/strains, i.e., *An. balabacensis* (Perlis form), *An. maculatus* (Huai Kuum strain), *An. philippinensis* (Rayong strain) and *An. takasagoensis*, had been compared with the susceptibility of the control, *An. dirus*. During this period, the susceptibilities of the following species/strains were compared with that of *An. dirus*: *An. maculatus* (IMR - Kuala Lumpur strain), and *An. maculatus* (Thailand - Nakhon Nayok strain). In summing up these studies, *An. balabacensis* (Perlis form), *An. takasagoensis*, *An. maculatus* (IMR - Kuala Lumpur strain), and the *An. maculatus* (Thailand - Nakhon Nayok strain) demonstrated a high degree of susceptibility to *P. cynomolgi* (B strain) when compared to *An. dirus*, while *An. maculatus* (Huai Kuum strain), and *An. philippinensis* (Rayong strain) demonstrated a low susceptibility to this parasite. Black spores of Ross were detected in some of these species, particularly those with the lowest susceptibility levels.

There are several significant findings from these studies. (A) All members of the Leucosphyrus Complex tested to date, i.e., *An. balabacensis* (Perlis form), *An. dirus* and *An. takasagoensis*, are highly susceptible to *P. cynomolgi* (B strain). Apparently, the distribution of this complex in Asia is very closely correlated with the distribution of simian malaria (18). (B) One of the 3 strains of *An. maculatus* demonstrated a much lower susceptibility to this parasite than the other 2 strains. Although the Thailand (Huai Kuum) strain

possesses distinct Karyotype heterochromatin differences from the other 2 strains, all 3 strains are considered conspecific based on polytene chromosome and cross mating studies (11). Thus, the differences detected here demonstrate the importance of intraspecific strain variation in the susceptibility to malaria parasites. (C) *An. philippinensis* demonstrated a low susceptibility to this parasite. Previous studies in Malaya (19, 20) found that *Anopheles philippinensis* sensu lato, was a moderate to good laboratory host for *P. cynomolgi* (B strain). These differences, however, cannot be resolved because of the uncertainty of what species actually was involved in the *An. philippinensis* of those authors. Since their work, *An. nivipes*, a sibling species of *An. philippinensis*, was found common and widely distributed in Malaya. During our studies, *An. nivipes* was not tested because of inadequate numbers due to colonization problems.

Beside testing the susceptibilities of these species/strains to *P. cynomolgi*, these efforts were designed to develop and evaluate the methods and techniques that are necessary for conducting human malaria susceptibility studies. The *P. cynomolgi* studies were terminated in preparation for the beginning of *P. falciparum* and *P. vivax* studies.

A manuscript (21) regarding the *P. cynomolgi* susceptibility studies is in preparation.

Preliminary aspects of the human malaria susceptibility studies were initiated early this FY with the submission of a protocol for clearance by respective Health officials and Human Use Committees. This protocol was approved by the Thailand Ministry of Health and the Human Use Committee, Office of the Surgeon General, U.S. Army, in May 1981. Subsequently, arrangements for a field study site have proceeded rapidly. With the kind assistance of personnel of the Thailand Malaria Division, the malaria zone office, Tha Muang District, Kanchanaburi Province, Malaria Region 5, was chosen as the field study site. This zone office screens a large number of potential *P. falciparum* and *P. vivax* patients each week. The human malaria susceptibility studies were actually initiated in Tha Muang, Kanchanaburi, on 5 October 1981, and patients meeting the criteria for the study have been volunteering on a regular basis since that time.

These studies are continuing.

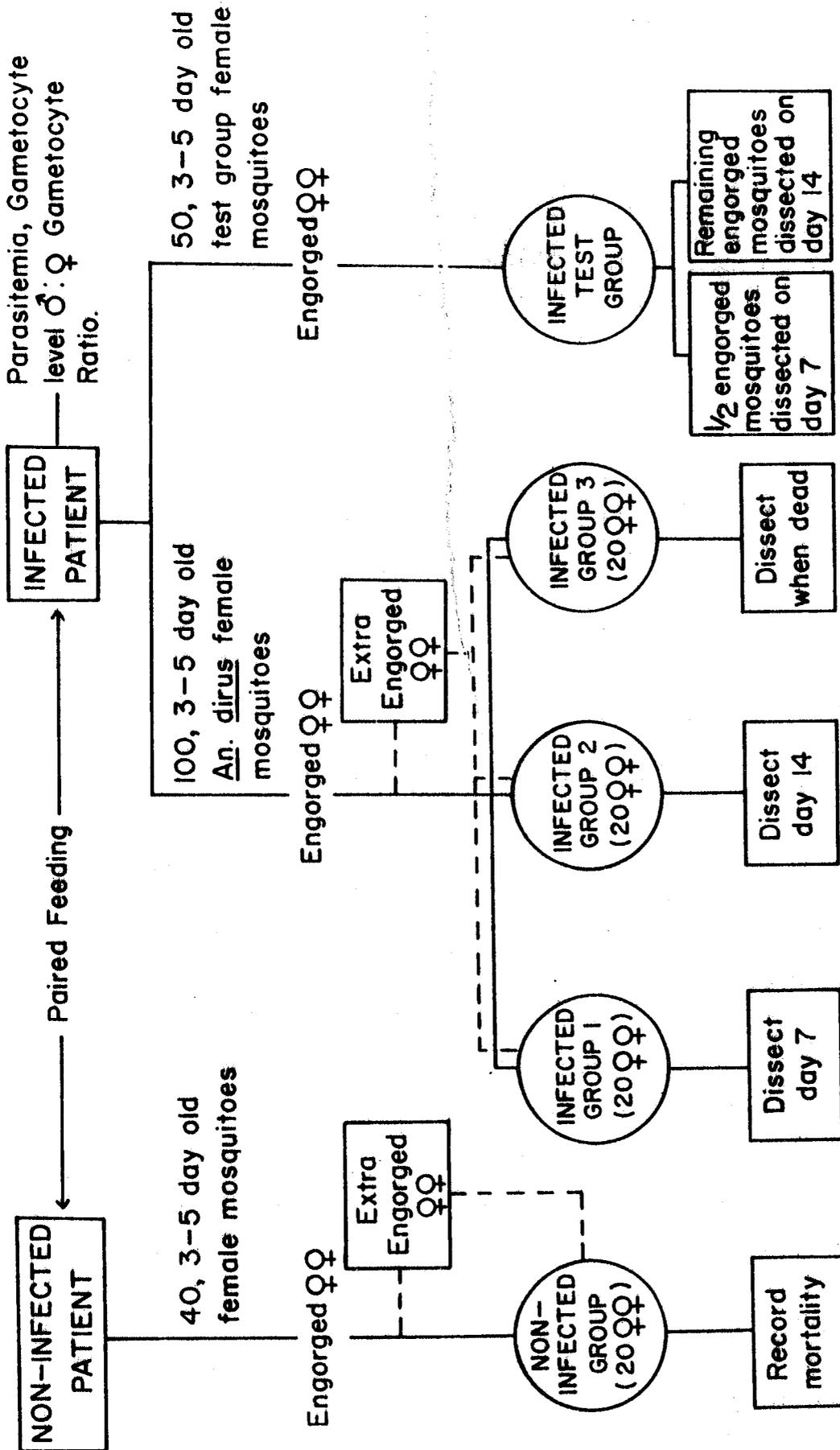


Figure 1. Diagram illustrating the sequence of events for each replicate.

REFERENCES :

1. Pinichpongse, S. 1980. Current status of malaria in Thailand, 1980. Presentation at 1st Natl. Mal. Conf., 17-19 Nov., Had Yai, Thailand.
2. Status Report. 1981. Malaria Division, Dept. Comm. Dis. Cont., Ministry Public Health, Bangkok (June), 41 p.
3. Schoof, H.F. 1970. Physiological resistance and development of resistance in field populations. Misc. Publ. Entomol. Soc. Am. 7: 45-52.
4. Elliot, R. 1972. The influence of vector behavior on malaria transmission. Am. J. Trop. Med. Hyg. 21: 755-763.
5. Burke, A.T.C., S. Puhomcharoen, F.C. Cadigan, D.J. Gould and K. Pinswasdi. 1966. Prevalence of malaria exhibiting reduced sensitivity to chloroquine in southern Thailand. Trans R. Soc. Trop. Med. Hyg. 60: 225-230.
6. Dixon, K.E., R.G. Williams, U. Pitaktong, T. Pongsupat and S. Aruwatana. 1980. Recent chemotherapeutic malaria studies at AFRIMS. Presentation at 1st Natl. Mal. Conf., 17-19 Nov., Had Yai, Thailand.
7. Andre, R.G. and E.B. Doberstyn. 1978. A preliminary report on the evaluation of the sporonticidal effect of pyrimethamine-sulfadoxone, quinine and quinine-pyrimethamine against *Plasmodium falciparum*. pp. 64-66. in Tan, Dora S.K. (ed). 1978. Current concepts in the diagnosis and treatment of parasitic and other tropical diseases in South East Asia. Proc. 18th SEAMEO-TROPMED Semin., Rajiv Printers, Petaling Jaya, Selangor, Malaysia. 205 p.
8. Andre, R.G., E.B. Doberstyn, C. Teerakiartkamjorn, P. Phintuyothin and S. Noeypatimanondh. 1978. Sporonticidal effect of antimalarials in *P. vivax* malaria. AFRIMS Ann. Prog. Rep., October 1977-September 1978, pp. 139-141.
9. Harrison, B.A., E.L. Peyton, V. Baimai and T.A. Klein. 1980. Changing species concepts and distributions for known and potential vectors of human malaria parasites in Thailand. Presentation at 1st Natl. Mal. Conf., 17-19 Nov., Had Yai, Thailand.
10. Harrison, B.A., T.A. Klein, E.L. Peyton and R. Rattanarithikul. 1982. Mosquito survey and taxonomic studies. AFRIMS Annual Progress Report, October 1980 - September 1981, pp.
11. Baimai, V., C.A. Green, B.A. Harrison and T.A. Klein. 1982. Mosquito cytogenetic, electrophoretic and cross mating studies. AFRIMS Annual Progress Report, October 1980-September 1981, pp.
12. Ward, R.A. 1965. Some effects of the mosquito host on malarial parasite. Proc. XII Int. Cong. Entomol., London. p. 731.

13. Rutledge, L.C., D.E. Hayes and R.A. Ward. 1970. *Plasmodium cynomolgi*: Sources of variation in susceptibility of *Anopheles quadrimaculatus*, *A. balabacensis* and *A. stephensi*. *Exp. Parasitol.* 27: 53-59.
14. Peyton, E.L. and B.A. Harrison. 1979. *Anopheles (Cellia) dirus*, a new species of the Leucosphyrus Group from Thailand (Diptera: Culicidae). *Mosq. Syst.* 11: 40-52.
15. Scanlon, J.E. and U. Sandhinand. 1965. The distribution and biology of *Anopheles balabacensis* in Thailand (Diptera: Culicidae). *J. Med. Entomol.* 2: 61-69.
16. Wilkinson, R.N., D.J. Gould, P. Boonyakanist and H.E. Segal. 1978. Observations of *Anopheles balabacensis* (Diptera: Culicidae) in Thailand. *J. Med. Entomol.* 14: 666-671.
17. Harrison, B.A. and T.A. Klein. 1981. Comparative susceptibility of known and suspected species/strains of *Anopheles* to *Plasmodium* parasites. AFRIMS Annual Progress Report, October 1979 - September 1980, pp.
18. Coatney, G.R., W.E. Collins, M. Warren and P.G. Contacos. 1971. The Primate Malarias. U.S. Dept. H.E.W., U.S. Gov. Printing Office, Washington. 366 p.
19. Warren, M., D.E. Eyles, R.H. Wharton and C.K. Ow Yang. 1963. The susceptibility of malayan anophelines to *Plasmodium cynomolgi bastianellii*. *Ind. J. Malariol.* 17: 85-105.
20. Wharton, R.H., D.E. Eyles, M. Warren and W.H. Cheong. 1964. Studies to determine the vectors of monkey malaria in Malaya. *Ann. Trop. Med. Parasitol.* 58: 56-77.
21. Klein, T.A., B.A. Harrison, S. Vongpradist and I. Inlao. Comparative susceptibility of known or suspected vector species/strains of *Anopheles* to *Plasmodium cynomolgi* (B strain). (Manuscript in preparation).