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

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OBJECTIVES :

1. To determine and compare the susceptibility of colonized primary and suspected secondary vectors of malaria to human and simian *Plasmodium* 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 : In Thailand, the number of human malaria cases has increased appreciably over the last few years, and has been accompanied by a major shift in the prevalence of the *Plasmodium* species (1). Overall the percentage of *P. vivax* infections increased from 17.02% in 1971 to over 40% in the last 4 years of the 1970's (1). Although finalized data for 1980 are not currently available, data for the first months of 1980 (2), show a significant reversal in this trend and a very serious increase in *P. falciparum*. A number of factors have been proposed that can possibly help explain the increase and shifts in the ratio of the *Plasmodium* infections, including : (A) changes in the behavior of the vector populations, e.g., a shift from zoophilic to anthropophilic; (B) the development of physiologic or behavioristic resistance in the vector populations (3, 4); (C) the development of drug resistance in the *Plasmodium* parasites (5, 6); (D) the use of prophylactic and treatment regimens of drugs (Fansidar) that may actually enhance gametocyte production (7); and (E) the determination of basic susceptibility levels of the anopheline vectors or suspected vectors to the malarial parasites in Thailand. The last point is of primary importance since these basic levels have not been determined for a number of the test species, and will provide a more accurate perspective of the epidemiology of human malaria in Thailand.

In recent years it has been demonstrated that potential vector species and vector populations vary greatly in their susceptibility to the infection of malarial parasites (8). Earlier Ward (9) had already demonstrated that the susceptibility of *Aedes aegypti* to *Plasmodium gallinaceum* is genetic. Investigations into the systematics of suspected and incriminated vector species also pose new questions. The Leucosphyrus Complex of *Anopheles*, for instance, is apparently an assemblage of similar nearly indistinguishable separate species. Although *An. balabacensis* has previously been considered a primary vector of

malaria in Thailand it is now known that this species does not occur in Thailand. What was originally thought to be *An. balabacensis* in Thailand is now known to be at least 3-4 separate species or forms (10), of which the most common, *An. dirus*, has been described as a new species. The major question now is, "Is the primary vector of malaria in Thailand, *An. dirus* or one of the forms, or are they all equally important as vectors of malaria parasites?" Other species such as *An. maculatus* and *An. philippinensis*, although incriminated as vectors of malaria (1), have not been fully investigated to provide insight into their species status (10) or their relation to susceptibility to *Plasmodium* spp.

The present study has been designed to provide insight into the susceptibility of some of these suspected and incriminated vectors of *Plasmodium* spp. in Thailand.

METHODS : 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. *Anopheles dirus* Peyton and Harrison, a highly susceptible and proven malaria vector (11, 12, 13) will be used as a control, while *An. balabacensis* Baisas (Perlis Form), *An. maculatus* Theobald (Kuala Lumpur, Nakhon Nayok and Huai Kuum Strains), *An. minimus* (Theobald) (Nakhon Ratchasima and Chiang Mai 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 to be vectors of malaria parasites in other countries and several are suspected vectors in Thailand.

Fifty adult, 3-4 day old female mosquitoes from the Control Group (*An. dirus*) and one or more of the test groups are 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). Blood-fed adults from each of the groups are removed and placed in a screened specimen cup and provided a 5 percent multi-vitamin solution as a food source. Unfed mosquitoes are discarded.

One half of the blood-fed mosquitoes from both the Control and Test Groups are dissected on day 7 and midguts examined for the presence of oocysts and black spores of Ross. The mean oocyst size, range and frequency are also determined. However, 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 both 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 determined, i.e., 1-10 sporozoites, +1; 11-100 sporozoites, +2; 101-1,000 sporozoites, +3 and > 1,000 sporozoites, +4.

Criteria for Feeding.

Human malaria studies are performed on patients that have been admitted to a hospital for treatment. The patients are screened and must meet the following criteria before being admitted to the study. The patient must : a) be 18 years

of age or older, b) have uncomplicated disease of mild to moderate severity, c) have an asexual parasite count between 1,000-100,000/cu.mm., d) have both male and female gametocytes in the blood film and ê) volunteer for the study after a thorough explanation of the study is presented to him.

Monkey malaria studies are performed on rhesus monkeys that have been admitted to the WRAIR drug testing program. Mosquitoes are fed on the second peak of parasitemia when both male and female gametocytes are present.

RESULTS : Monkey malaria susceptibility studies have been completed for *An. philippinensis*, *An. balabacensis* (Perlis form), *An. takasagoensis* and *An. maculatus* (Huai Kuum strain). The oocyst frequencies of *An. philippinensis*, *An. balabacensis* (Perlis Form), *An. takasagoensis* and *An. maculatus* (HK) were highly correlated with the oocyst frequency of *An. dirus*, i.e., r = 0.83, 0.85, 0.74 and 0.82 respectively. Furthermore, *An. balabacensis* (Perlis Form) and *An. takasagoensis* demonstrated a high degree of susceptibility to *P. cynomolgi* (B strain) when compared to *An. dirus*, while *An. maculatus* (HK) and *An. philippinensis* demonstrated a low susceptibility (Figure 1). *Anopheles philippinensis* had a high incidence of black spores of Ross, with 63% positive for oocysts also having spores. In addition, 25% of *An. philippinensis* that did not have oocysts also had black spores of Ross. *Anopheles maculatus* (HK) had a lower incidence of these spores than *An. philippinensis*, with 23.4% of oocyst infected specimens having spores and 8.3% of uninfected specimens having spores. *Anopheles takasagoensis* had a very low incidence of these spores, while none were observed in *An. dirus* and *An. balabacensis* (Perlis Form).

Preliminary data indicate that *An. maculatus* (NN and KL) appear to be intermediate between *An. balabacensis* (Perlis Form) and *An. maculatus* (HK). *Anopheles nivipes* (KB and CM) have not been tested due to insufficient colony specimens.

Human malaria studies are awaiting approval from the Royal Thai Surgeon General. These studies are continuing.

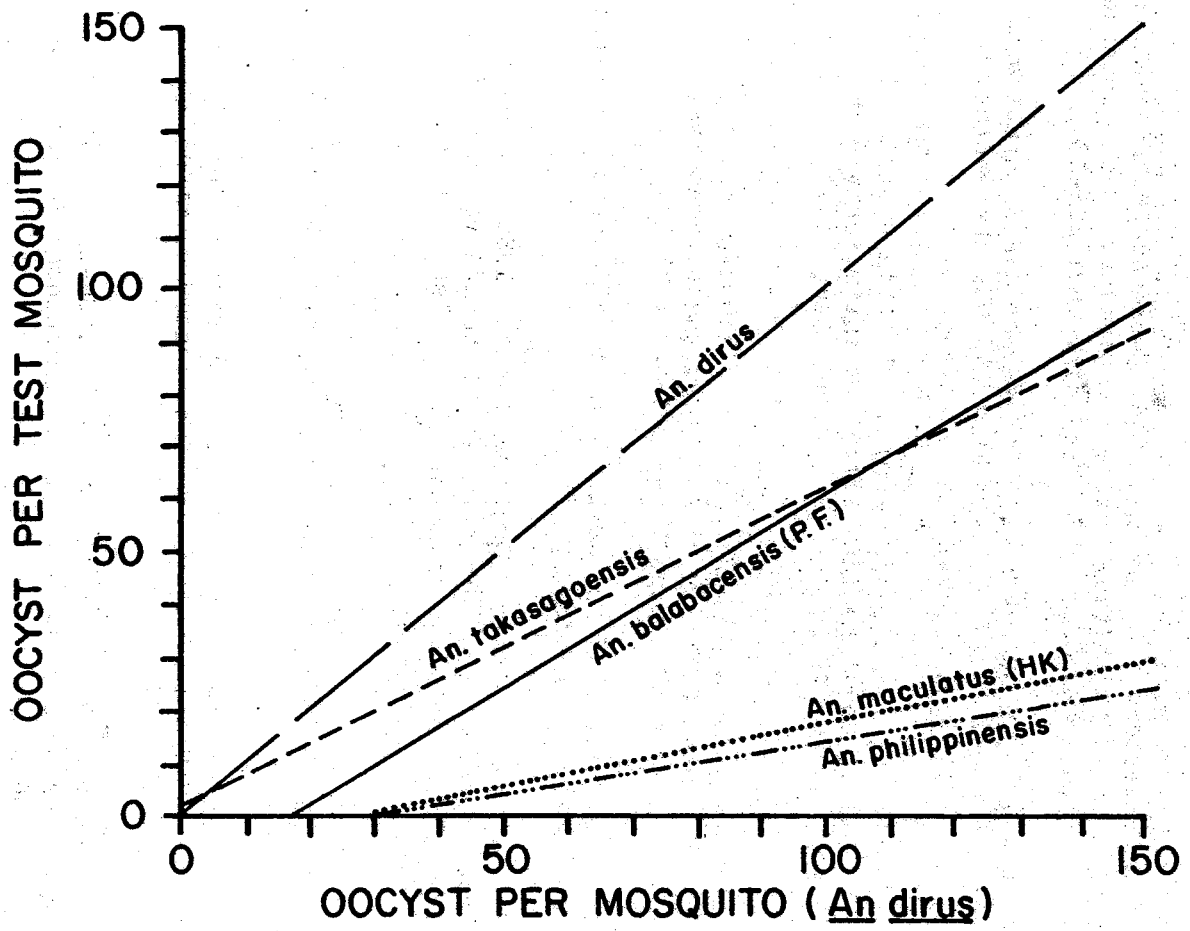

Figure I. Regression of mean number oocyst count in An. balabacensis (Perlis Form), An takasagoensis, An maculatus (Huai Kuum strain) and An philippinensis (Rayong strain) on the mean oocyst count of An. dirus (Bangkok colony strain).

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