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# EXPERIMENTAL INFECTION OF *ANOPHELES FARAUTI* WITH DIFFERENT SPECIES OF *PLASMODIUM*

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ABSTRACT: Studies were conducted to determine the susceptibility of *Anopheles farauti* to different species and strains of *Plasmodium*. Mosquitoes were infected by feeding on animals or cultures infected with different strains of *P. vivax, P. falciparum, P. ovale, P. coatneyi, P. gonderi, P. simiovale, P. knowlesi,* and *P. brasilianum*. Infections of *P. vivax* and *P. coatneyi* were transmitted via sporozoites from *An. farauti* to monkeys. Comparative infection studies indicated that *An. farauti* was less susceptible to infection than *An. stephensi, An. gambiae, An. freeborni,* and *An. dirus* with the Salvador I strain of *P. vivax,* but more susceptible than *An. stephensi* and *An. gambiae* to infection with the coindigenous Indonesian XIX strain.

Periodically, new species and strains of anopheline mosquitoes are established in the laboratory to determine their susceptibility to infection with different species and strains of malarial parasites. The results of these studies allow us to select suitable parasite vector/parasite combinations for transmission studies and to determine relationships between experimental vectors and homologous and heterologous geographic isolates.

Previously, such relationships with *An. pseudopunctipennis* (Warren et al., 1980), *An. culicifacies* (Collins, Warren et al., 1986), *An. albitarsis* (Collins et al., 1985), and *An. gambiae* (Collins and Roberts, 1991) have been reported using our standard laboratory hosts *An. freeborni*, *An. dirus*, and *An. stephensi*. Several years ago, a colony of *An. farauti*, originating in Irian Jaya was established from eggs provided to us by the Naval Medical Research Unit in Jakarta, Indonesia. The range of distribution of *An. farauti* includes the Moluccas and extends eastward through New Guinea, the Admiralty Islands, the Bismarck Archipelago, the Solomon Islands, and the New Hebrides where it is a major vector of malaria. Here, the relative susceptibility of this mosquito is reported for several different species of human and nonhuman primate–infecting species of *Plasmodium*.

## MATERIALS AND METHODS

### Mosquitoes

Five species of *Anopheles* were used in the comparative infectivity studies. *Anopheles freeborni* (F-1 strain) was originally isolated in California in 1944 and has been maintained continuously since then. *Anopheles dirus* was obtained from the Walter Reed Army Institute of Research in 1964 and have been maintained in our insectary since then. *Anopheles gambiae* (G-3 strain, originally from The Gambia) was obtained from the London School of Hygiene and Tropical Medicine, and *Anopheles stephensi*, originally from New Delhi India, was obtained from the Naval Medical Research Institute. Eggs of *An. farauti*, originally from Irian Jaya, were obtained through the efforts of Michael J. Bangs, Naval Medical Research Unit, Jakarta, Indonesia. All mosquito colonies were maintained in the Centers for Disease Control (CDC) insectary in Chamblee, Georgia.

### Parasites

The Salvador I (Campbell et al., 1983), Indonesia XIX (Collins et al., 2000), and Thai 561 (Collins et al., 1992) strains of *P. vivax* and the Santa Lucia strain of *P. falciparum* (Collins et al., 1977) were maintained by serial passage in nonhuman primates (*Aotus, Saimiri,* and chimpanzees) or stored frozen over $N_2$. Three recently isolated strains of *P. falciparum* (Nigeria VI, Nigeria VII, and Malawi I) had not been adapted to grow in monkeys. For these parasites, all gametocytes were produced in vitro. The Nigerian I strain of *P. ovale* (Collins et al., 1987) has only been adapted to develop in chimpanzees.

*Plasmodium coatneyi, P. simiovale, P. gonderi*, and *P. knowlesi* (H strain) were maintained by serial passage in *Macaca mulatta* monkeys or stored frozen (Coatney et al., 1971). *Plasmodium brasilianum* (Peru III strain; Collins et al., 1990) was grown in New World monkeys (*Aotus* and *Saimiri*).

### Animals

*Aotus vociferans, Aotus nancymai, Aotus lemurinus griseimembra,* and *Saimiri boliviensis* animals were infected with the different strains of *P. vivax* and *P. falciparum* in the CDC animal facilities, Chamblee, Georgia. Chimpanzees (*Pan troglodytes*) were infected and maintained at the Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia. All animals were splenectomized either before or during infection to increase the probability of producing gametocytes or infection.

Infections in the animals were monitored by the examination of Giemsa-stained thick and thin blood films made by the method of Earle and Perez (1932). During periods when infection was thought likely, mosquitoes were fed on the tranquilized animal or via membrane feeding on heparinized blood (Collins, McClure et al., 1986). Gametocytes from in vitro culture were diluted in heparinized human blood and offered to mosquitoes through Parafilm membranes (Campbell et al., 1980).

After 7–10 days of extrinsic incubation at 25 C, mosquitoes were dissected and examined for the presence of oocysts. Because not all species of mosquito were fed each day, oocyst counts were compared only when either *An. farauti* or its paired feeding species were positive. Salivary glands were dissected and examined beginning 12 days after feeding.

For transmission, mosquitoes were fed directly on the tranquilized recipient monkey, or alternatively, the salivary glands were dissected into 20% fetal bovine serum in phosphate-buffered saline (pH 7.2), and the released sporozoites were injected into the femoral vein of the recipient animal (Collins et al., 1988). Ten or 12 days after sporozoite injection, daily blood films were made to determine the prepatent period.

## RESULTS

Mosquitoes (6,302) were dissected and examined; 1,896 (30.1%) were positive. Infection was 31.9% (495 of 1,552 examined) for *An. stephensi*, 24.8% (266 of 1,074) for *An. gambiae*, 41.5% (416 of 1,002) for *An. freeborni*, 47.2% (376 of 796) for *An. dirus*, and 17.7% (333 of 1,878) for *An. farauti*. Comparisons were only made when at least 1 of the paired lots was positive.

Sixty-three paired comparative feedings between *An. farauti* and the other anopheline mosquitoes (*An. stephensi, An. free-*

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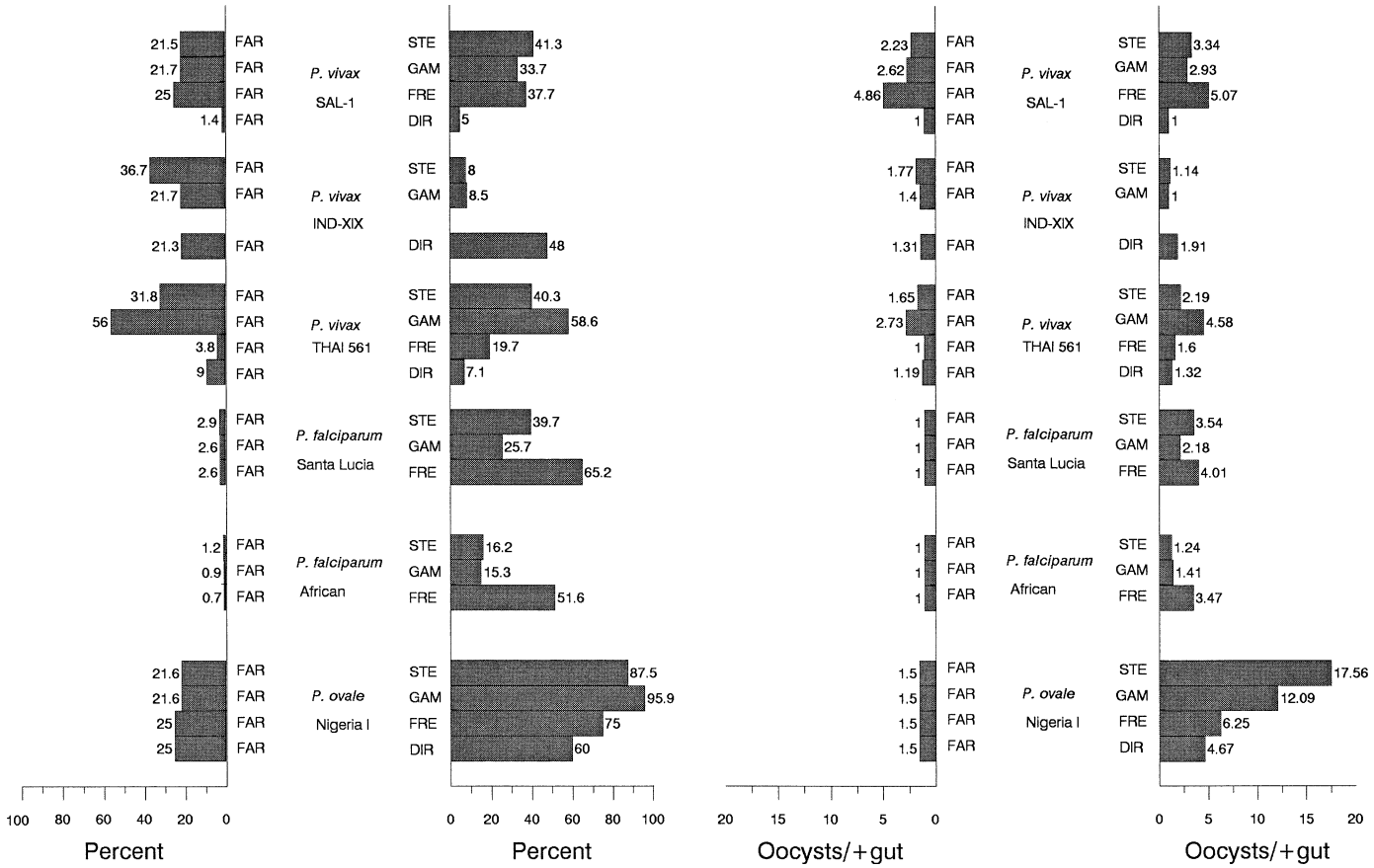


FIGURE 1. Comparative percent infection and mean number of oocysts per positive gut between *Anopheles farauti* (FAR) and 4 other anopheline mosquitoes (*An. stephensi*, STE; *An. gambiae*, GAM; *An. freeborni*, FRE; *An. dirus*, DIR) for *Plasmodium vivax* (Salvador I, Indonesia XIX, and Thai 561 strains), *P. falciparum* (Santa Lucia strain from El Salvador and 3 African strains: Nigeria VI, Nigeria VII, and Malawi I), and *P. ovale* (Nigeria I strain).

*borni*, *An. gambiae*, and *An. dirus*) were made with the 3 strains of *P. vivax* (Fig. 1).

Fifty-three paired comparative feedings were made on animals or culture-produced gametocytes of *P. falciparum* (Fig. 1). In all instances, the *An. farauti* were markedly less frequently infected than *An. stephensi*, *An. gambiae*, and *An. freeborni*. Only 6 paired feedings were made on blood from a chimpanzee infected with *P. ovale*.

Five different species of nonhuman primate–infecting species of *Plasmodium* were used for comparative feedings (Fig. 2). Thirteen paired comparative feedings were made on rhesus monkeys infected with *P. coatneyi*, 12 paired feedings with *P. gonderi*, 11 paired feedings with *P. simiovale*, and 15 paired feedings with *P. knowlesi*. Nine paired feedings were made on New World monkeys infected with *P. brasilianum*.

There were 7 attempts to transmit infection using sporozoites produced in *An. farauti*; 5 of these were successful. Fifteen mosquitoes infected by feeding on a chimpanzee infected with the Salvador I strain of *P. vivax* were fed on a splenectomized *Saimiri boliviensis* monkey (SI-145) following 13 days of extrinsic incubation. The prepatent period was 22 days.

Ten mosquitoes infected on another chimpanzee with this strain of *P. vivax* were fed on *S. boliviensis* monkey SI-1707 after 13 days of extrinsic incubation. The prepatent period was 23 days.

Sporozoites of this strain of *P. vivax*, dissected from the salivary glands of *An. farauti* mosquitoes after 13 and 14 days, were injected intravenously into *S. boliviensis* monkeys. Monkey SI-1957 was injected with 20,000 sporozoites. The prepatent period was determined to be 33 days, although continuous high-density parasitemia did not develop until day 54. Monkey SI-760 was injected with 75,000 sporozoites. The animal was splenectomized 7 days after injection. The prepatent period was 16 days. One *S. boliviensis* monkey was injected with 3,000 sporozoites and another was fed upon by 12 positive mosquitoes infected with the Salvador I strain of *P. vivax*; no infections were detected during 60 days of observation.

Rhesus monkey R-8904 was infected via the bites 3 *An. farauti* infected with *P. coatneyi*. The extrinsic incubation period was 12 days; the prepatent period was 13 days.

## DISCUSSION

Different strains of anopheline mosquitoes vary in their ability to be infected by malarial parasites. In addition, infectivity to mosquitoes is often linked to the geographic origin of the parasite. These observations suggest that, particularly during periods of geographic isolation, the parasite–vector relationship is reinforced such that heterologous parasites might have difficulty in being introduced into these areas. Therefore, one

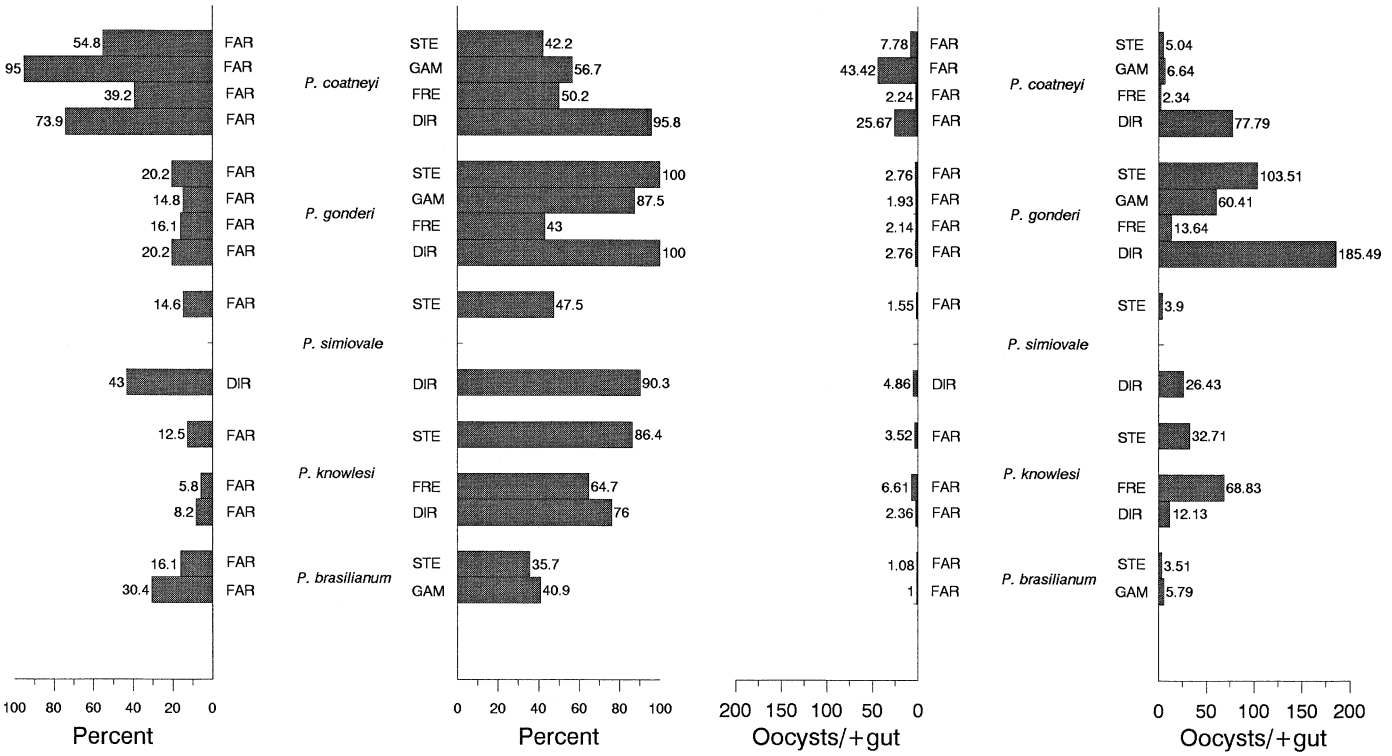


FIGURE 2. Comparative percent infection and mean number of oocysts per positive gut between *Anopheles farauti* (FAR) and 4 other anopheline mosquitoes (*An. stephensi*, STE; *An. gambiae*, GAM; *An. freeborni*, FRE; *An. dirus*, DIR) for *Plasmodium coatneyi*, *P. gonderi*, *P. simiovale*, *P. knowlesi*, and *P. brasilianum*.

would not be surprised when a parasite such as the Salvador I strain of *P. vivax* is comparatively less infective to *An. farauti* from Irian Jaya than the Indonesia XIX strain of the parasite from Irian Jaya. A similar close relationship was observed when strains of *P. vivax* from distant geographic areas were compared with Central American strains of the parasite for infectivity to the coindigenous *Anopheles albimanus* mosquito (Li et al., 2001).

Malaria is highly endemic in areas of Irian Jaya where *An. farauti* is a major vector. In the present studies, this mosquito was markedly susceptible to the chloroquine-resistant Indonesia XIX strain of *P. vivax*. Nonetheless, the mosquito was capable experimentally of transmitting the Salvador I strain of *P. vivax* to susceptible monkeys.

The results indicated that this mosquito was barely susceptible to infection with the Santa Lucia strain of *P. falciparum* or 3 strains of the parasite from Africa. Additional studies would be needed to determine if a coindigenous isolate of *P. falciparum* would be more infective in comparison with our 4 standard mosquito species.

All 5 species of monkey malaria parasites, *P. coatneyi*, *P. gonderi*, *P. simiovale*, *P. knowlesi*, and *P. brasilianum*, were infective to *An. farauti*. Comparatively, *P. coatneyi* appeared to be the most suitable combination for transmission studies. *Anopheles dirus* was more susceptible to infection with the Asian monkey malaria parasites than was *An. farauti*. Because *An. farauti* is a self-mating colony and *An. dirus* requires tedious force mating, there may be an advantage to using the self-mating species of mosquito.

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## *ERRATUM:*

Due to a printer's error at Allen Press, a key was compressed in the article by Braswell et al. (**88:** 28–35). On p. 35, the key should have been printed as follows:

### Key to *Taeniacanthodes* spp. adult females

1 Total number of spines and setae on terminal endopod segment of legs 3 and 4 = 2; first free thoracic segment narrower than second, providing a necklike appearance to the cephalothorax–thorax junction. . . . . *T. gracilis*

Total number of spines and setae on terminal endopod segment of legs 3 and 4 = 3; first free thoracic segment wider than second. . . . . 2

2 Leg 5 composed of 2 segments; total number of armature elements (spines and setae) on leg 4 exopod segment 3 = 6. . . . . *T. haakeri*

Leg 5 composed of 1 segment; total number of armature elements on leg 4 exopod segment 3 = 7. . . . . *T. dojirii*