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SUSCEPTIBILITY OF *ANOPHELES FARAUTI* TO INFECTION WITH DIFFERENT SPECIES OF *PLASMODIUM*

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ABSTRACT. A colony of *Anopheles farauti,* originally from the island of New Britain in Papua New Guinea, was tested for its receptivity to infection with different species of *Plasmodium* in comparison with *An. freeborni* and *An. stephensi.* This colony adapted well to feeding on monkeys and was infected with New World and Old World strains of *P. vivax* and *P. falciparum, P. ovale, P. cynomolgi,* and *P. brasilianum.*

KEY WORDS *Anopheles farauti, Anopheles freeborni, Anopheles stephensi,* mosquitoes, *Plasmodium*

INTRODUCTION

Different species and strains of anopheline mosquitoes are established in the Division of Parasitic Diseases' insectaries in Chamblee, GA, for transmission studies with malaria in nonhuman primates. Necessary to these studies are vectors capable of transmitting the wide variety of parasites that are continuously being adapted to primates for these studies. These studies have revealed that some species and strains of parasites are more readily transmitted by particular species of *Anopheles* mosquitoes. Some species of mosquitoes vary markedly in their susceptibility to infection with exotic strains of parasites. Under controlled laboratory conditions, particularly with the nonhuman primate malaria parasites, different species of *Anopheles* may be refractory to infection with 1 species of *Plasmodium* and be a predictable vector of another. In general, co-indigenous parasites and vectors have been more suitable combinations for transmission studies (Collins et al. 1975, 1976, 1977, 1986; Sullivan et al. 2001). This is particularly true for the vectors and malaria parasites of the New World (Li et al. 2001). The investigation of chloroquine-resistant strains of *Plasmodium vivax* from the western Pacific region has led to the colonization and examination of vectors from this region. One of the vectors of interest is *Anopheles farauti.* Previously, a laboratory-adapted strain of *An. farauti* from Irian Jaya was investigated (Collins et al. 2002). Unfortunately, this strain was lost. Subsequently, a colony of *An. farauti,* originally established in Australia in 1965 from field material collected from Rabaul, the capital of East New Britain Province (New Britain Island), Papua New Guinea, was provided to the Centers for Disease Control (CDC) by Robert Cooper as adult mosquitoes. This colony has been maintained continuously in the Division of Parasitic Diseases (DPD) insectaries since 2001. *Anopheles freeborni* and *An. stephensi* are the standards for comparison because they are most frequently used for many different experimental transmission studies at CDC and in other malaria research laboratories. Reported here are the results of comparative infectivity studies between these 3 species of *Anopheles* and different species of human and monkey malaria parasites.

MATERIALS AND METHODS

Mosquitoes: The CDC/DPD insectaries are maintained at 25°C and 70% relative humidity. Although this *An. farauti* colony has proven to be hardy, the 1st and 2nd instars are relatively fragile and will not tolerate overfeeding, excessive agitation, or overcrowding. Adult mosquitoes are housed on 1-gallon paper ice-cream-carton cages with mesh tops. Seven days after emergence, adult mosquitoes are allowed to feed on an anesthetized rabbit; 3 days after the blood meal, 8-oz cups containing 50 ml of distilled water are introduced into the cages. Egg laying occurs overnight; eggs are recovered, washed with 2% bleach solution (to control *Nosema* that is indigenous to the insectary), and collected on filter paper via vacuum filtration. Eggs are then washed into 9-in. × 12-in. enamelware pans with 500 ml distilled water and allowed to hatch. Larvae are fed on days 0, 1, 2, and 3 with active instant yeast (Saf-Instant; Safmex SA de CV, Toluca, Mexico). On day 2, pellets of fish food (Koi Floating Blend; Aquaticare, Victor, NY) are also added. As larvae grow, pans are split to avoid overcrowding and a powdered food consisting of a 1 : 1 : 1 : 1 mixture of dried Brewer's yeast (Sigma Chemical Co., St. Louis, MO), lactalbumin (Sigma Chemical Co.), milled New World Monkey Chow (Nutrition International, Brentwood, MO), and fish food that has been passed through a 40-mesh screen, is sprinkled on the surface once daily. Larval pans are usually split every other day. Pupation usually occurs over 4 days; pupae are harvested by the ice-water method.

Approximately 200 pupae are placed into 8-oz

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paper cups containing 100 ml distilled water and placed in 1-gallon paper ice-cream-carton cages for emergence. Adults are fed 10% sugar solution daily on a cotton pad. Adult mosquitoes, aged 3–6 days, are starved (denied sugar solution) the night before being used for feeding studies. Mosquitoes feed readily on monkeys, but very poorly through membranes. Feeding rates are increased in the dark.

Anopheles freeborni, F-1 strain originally from California, have been maintained continuously since 1944. Mosquitoes were laboratory-reared and maintained at the CDC/DPD insectaries in Chamblee, GA. *Anopheles stephensi,* originally from the area of Delhi, India, have been maintained for >10 years in the CDC/DPD insectaries. Mosquito infection was obtained by allowing the caged anophelines to feed directly on the tranquilized monkey. Comparative feedings with *P. malariae* and *P. ovale* on blood from chimpanzees were via parafilm membranes using a warm-water circulator (Rutledge et al. 1964).

Animals: *Aotus nancymai* and *A. vociferans* monkeys were wild-caught animals imported from Peru. *Saimiri boliviensis* and *Macaca mulatta* were laboratory-born animals. On arrival at the facility, all animals were quarantined for a 2-month conditioning period, weighed, and tested for tuberculosis. Parasitologic and serologic examination indicated that the animals were free of infection with malaria parasites before primary inoculation. All monkeys were splenectomized before or after exposure to infection. All surgeries were performed in an AAALAC (Association for the Assessment and Accreditation of Laboratory Animal Care, International, Inc.) -approved surgical suite appropriate for aseptic surgery. Protocols were reviewed and approved by the Centers for Disease Control and Prevention Institutional Animal Care and Use Committee, in accordance with procedures described in the U.S. Public Health Policy, 1986. Chimpanzees (*Pan troglodytes*) were housed and maintained at the Yerkes Regional Primate Research Center, Emory University, Atlanta, GA.

Monkeys were housed singly or doubly to avoid injuries caused by fighting with cage mates. Space recommendations for laboratory animals were followed as set forth in the *Guide for the Care and Use of Laboratory animals,* National Institutes of Health. All animals were fed a diet that has been proven to provide adequate nutrition and calories in captive monkeys used in malaria-related research. Feed was free of contaminants and freshly prepared. Daily observations of the animals' behavior, appetite, stool, and condition were recorded. An attending veterinarian treated all animals as medical conditions arose. Blood-stage parasitemia was monitored by the daily examination of thick- and thin-blood films by the method of Earle and Perez (1932). Infections in New World monkeys were terminated by treatment with chloroquine (30 mg base over 3 days) or mefloquine (20 mg base); in *M. mulatta,* infections with *P. cynomolgi* were treated with chloroquine (300 mg base over 3 days). All drugs were administered by oral intubation. Infections in chimpanzees were treated with chloroquine (1,500 mg base over 3 days) by intramuscular injection.

Parasites: Infections with *P. vivax* from the New World (Salvador I from El Salvador, Brazil I/CDC, Honduras I, and NICA from Nicaragua) and from the Old World India VII strain were induced in New World monkeys (*Aotus* or *Saimiri*). Three strains of *P. falciparum* from the New World (Santa Lucia from El Salvador, Haitian III, and Peru 01-134) and Ghana III from the Old World were also induced in New World monkeys. Infections with 6 different strains of *P. cynomolgi* from Asia (Berok, Gombak, Cambodian, Smithsonian, Langur, and *P. cynomolgi ceylonensis*) were induced in *M. mulatta* monkeys. Infections with the Peruvian I strain of *P. brasilianum* were induced in New World monkeys.

Infections with *P. malariae* (Uganda I) and *P. ovale* (Nigeria I) were induced in splenectomized chimpanzees (*Pan troglodytes)* via the intravenous inoculation of parasitized erythrocytes. Heparinized blood from these animals were fed to mosquitoes through parafilm membranes.

RESULTS

Seventy paired feedings were made between *An. farauti* and the other 2 species of mosquito. Mosquitoes (2,781) were dissected and examined; infection was 26.6% (235 of 884 examined) for *An. farauti,* 50.6% (496 of 981) for *An. freeborni,* and 47.9% (439 of 916) for *An. stephensi.* Comparisons between 2 species were considered only when at least 1 of the 2 species of mosquito being compared was infected. In all but 1 instance, *An. freeborni* had a higher mean percentage infection and higher number of oocysts per positive gut than did the *An. farauti* (Fig. 1). *Plasmodium ovale* was the exception. Examination of the data for New World versus the India VII strain of *P. vivax* revealed that the differences were more pronounced between *An. farauti* and the other 2 mosquito species, suggesting a better relationship between the India VII strain of parasite and this vector (Figs. 1 and 2).

Anopheles farauti was not able to be infected with *P. malariae* from Africa, and the *P. malariae* infection rate and oocysts per positive gut levels were relatively low for both *An. freeborni* and *An. stephensi.* Further feedings may result in some level of infection for *An. farauti. Plasmodium brasilianum* is considered by many to be identical to or markedly similar to *P. malariae* (Coatney et al. 1971). Feedings on *P. brasilianum* resulted in a high level of infection and high mean numbers of oocysts per positive gut in *An. farauti.* Thus, one would expect that further feedings on *P. malariae* may result in higher levels of infection for *An. far-*

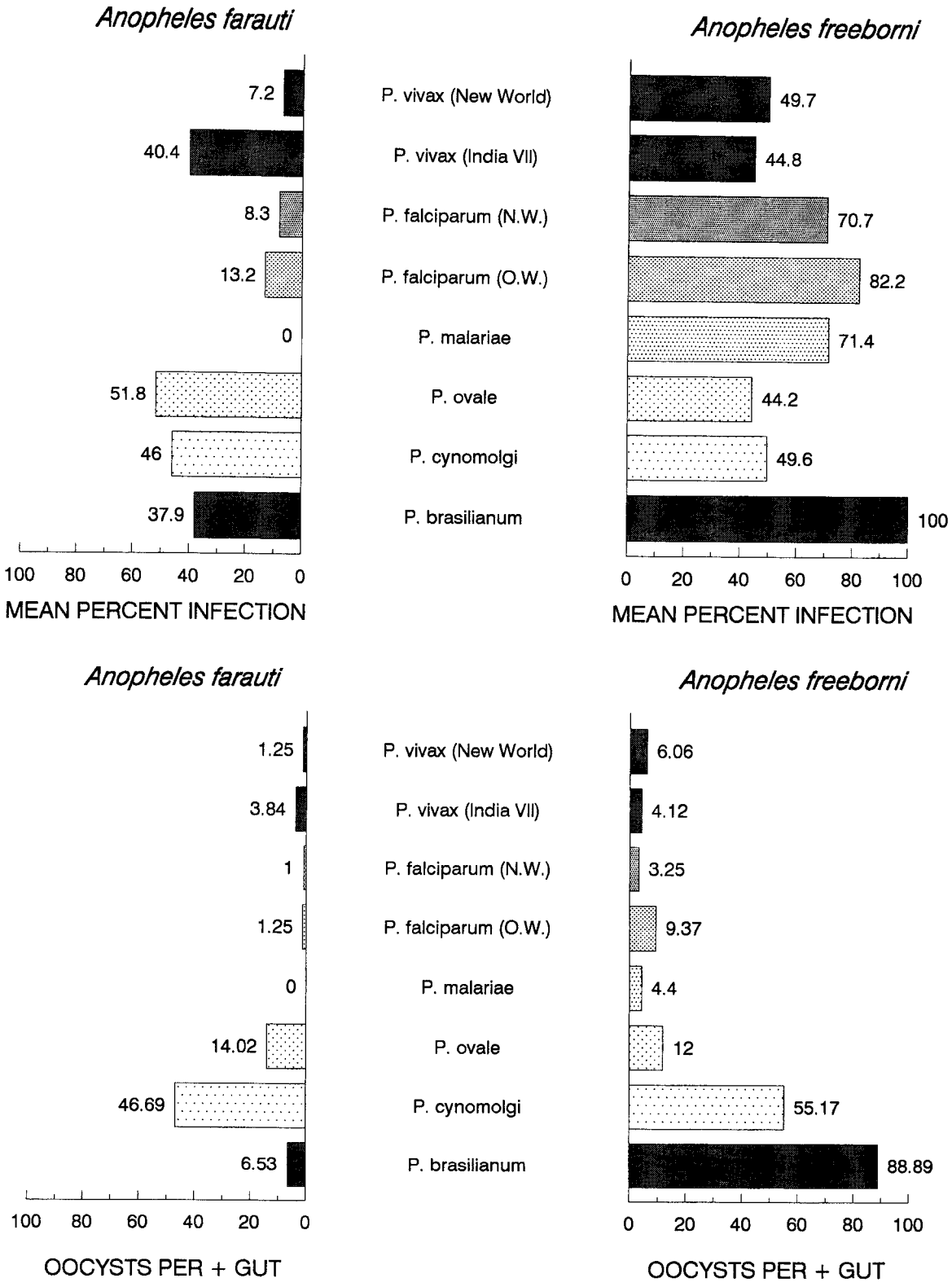

Fig. 1. Comparative mean percent infection and mean number of oocysts per positive gut for *Anopheles farauti* and *An. freeborni* mosquitoes following feeding on nonhuman primates infected with different species of *Plasmodium*.

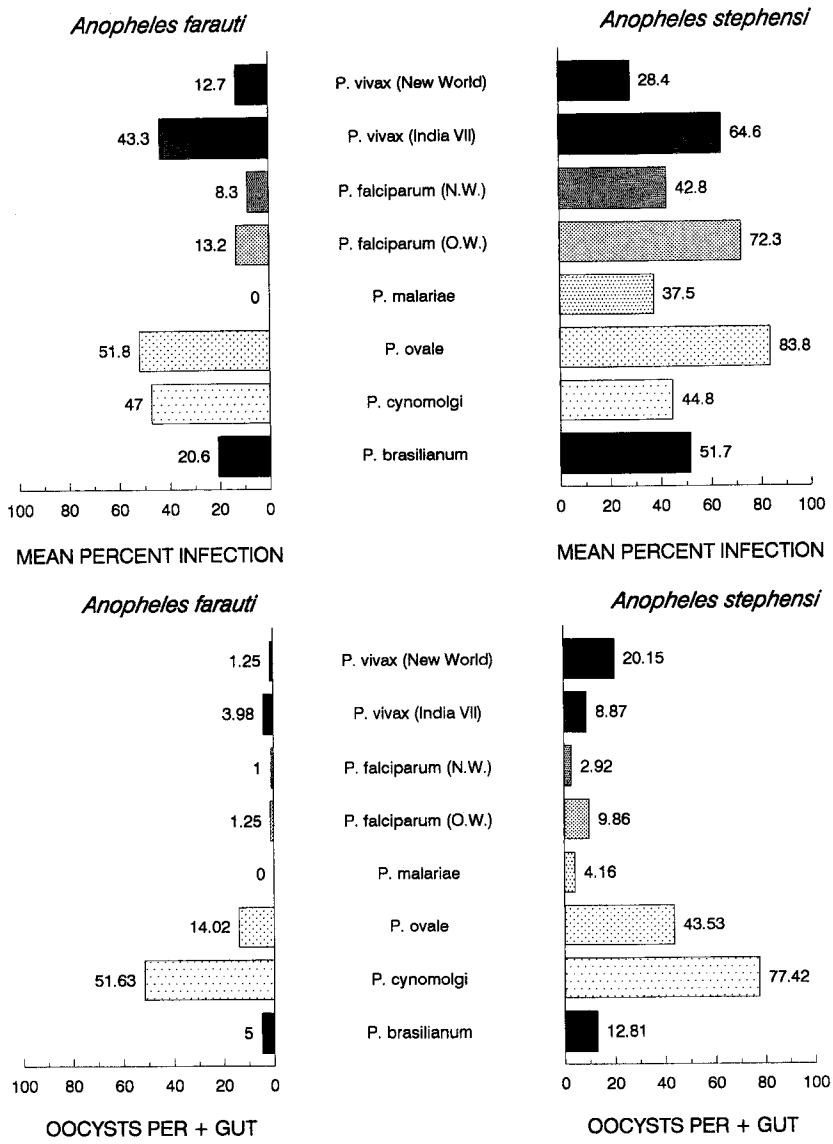

Fig. 2. Comparative mean percent infection and mean number of oocysts per positive gut for *Anopheles farauti* and *An. stephensi* mosquitoes following feeding on nonhuman primates infected with different species of *Plasmodium.*

auti. In comparison with *An. freeborni, An. farauti* was more susceptible to an African *P. ovale* parasite induced in chimpanzees (Figs. 1 and 2).

Plasmodium cynomolgi was highly infectious to all three species of mosquito, with the oocysts per positive gut ratio for *An. farauti* versus *An. freeborni* (Fig. 1) being 46.69 : 55.17 and for *An. farauti* versus *An. stephensi* (Fig. 2) being 51.63 : 77.42.

In none of the feedings was *An. farauti* readily infected with *P. falciparum.* It was shown to be susceptible to both New World and Old World strains, but the infection rate and oocysts per positive gut were extremely low. Further studies with additional strains may be needed to select a strain of parasite adapted to develop well in this vector.

DISCUSSION

This strain of *An. farauti* from New Britain, Papua New Guinea, was shown to be susceptible to infection with different species and strains of *Plasmodium* from widely different geographic regions. Cooper (1994) had reported the infection of this strain of *An. farauti* with the chloroquine-resistant AMRU-1 strain of *P. vivax* from Papua New Guinea. In comparison with *An. freeborni* and *An. stephensi, An. farauti* appeared to be more receptive

to strains of *P. vivax* from the Old World than from the New World. In the previous report (Collins et al. 2002) with *An. farauti* from Irian Jaya, the ratio of infection between *An. farauti, An. freeborni,* and *An. stephensi,* when fed on animals infected with the Salvador I strain of *P. vivax,* was 25.0 : 37.7 and 21.5 : 41.3. The ratio of infection for the Salvador I strain with the New Britain strain was 2.0 : 74.6 and 16.0 : 54.0. When results from feedings on the 4 New World strains of *P. vivax* were combined, the ratios were 7.2 : 49.7 and 12.7 : 28.4. Both strains of *An. farauti* were markedly less susceptible to infection than were the other 2 species of *Anopheles.* The Irian Jaya strain was shown to be more susceptible to infection with a chloroquine-resistant strain of *P. vivax* from Indonesia (Indonesia XIX) than was *An. stephensi*; however, no comparisons were made using *An. freeborni.* With the New Britain strain, the Old World *P. vivax* comparison was with the India VII strain. Here, the ratios of infection between *An. farauti, An. freeborni,* and *An. stephensi* were 40.4 : 44.8 and 43.3 : 64.6. It was apparent that the New Britain strain of *An. farauti* were readily susceptible to infection with this Old World strain of *P. vivax,* even though its origin was outside the range of distribution for *An. farauti.*

Infection of *An. farauti* was not obtained with *P. malariae,* whereas high-density infection was obtained with *P. brasilianum.* This was surprising in that these 2 parasites are very similar and are considered by some to be identical species. The high levels of susceptibility of all 3 species to *P. cynomolgi* is not surprising in that many species of *Anopheles* mosquitoes are susceptible to this parasite. Yet none of these mosquitoes are found where the parasite is found. *Anopheles farauti* occurs in New Britain, Papua New Guinea, which is east of the Wallace's Line, which delineates the boundaries where nonhuman primates are found (and therefore their malarial parasites). *Anopheles freeborni* is from California, where there are no monkeys nor monkey malaria parasites. *Anopheles stephensi* occurs in northern India in the presence of abundant nonhuman primates. However, monkey malaria parasites have not been reported from northern India. It has been postulated that their absence is due to the absence of mosquitoes belonging to the *An. leucosphyrus* group of forest-dwelling vectors needed to maintain transmission. Thus, even though *M. mulatta* and *An. stephensi* coexist in northern India, *P. cynomolgi* is not transmitted in this setting.

These initial studies with this strain of *An. farauti* from New Britain support its use for a number of laboratory-based investigations. It is susceptible to infection with different strains of New and Old World *P. vivax* and *P. falciparum* as well as *P. cynomolgi, P. ovale,* and *P. brasilianum.* It appears to be hardy and relatively long lived. In comparison, *An. freeborni* is relatively short lived; in most instances, *An. farauti* has survived longer than *An. stephensi* under our laboratory conditions. It feeds poorly through parafilm membranes, but feeds readily on rabbits and monkeys.

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