ANOPHELES GAMBIAE AS A HOST FOR GEOGRAPHIC ISOLATES OF *PLASMODIUM VIVAX*

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ABSTRACT. The G-3 strain of *Anopheles gambiae* was compared with 2 other strains of *An. gambiae* and *An. freeborni*, *An. stephensi*, and *An. dirus* for susceptibility to infection with 7 different geographic strains of *Plasmodium vivax*. Ratios of infection varied, indicating that certain strains of *P. vivax* were more infectious to the G-3 strain of *An. gambiae* than to other anopheline species/strains. Based on the comparative number of oocysts per mosquito, the relationships between the 3 strains of *An. gambiae* were closer than between the G-3 strain of *An. gambiae* and the 3 other species of *Anopheles*. *Anopheles gambiae* appears to be a very useful host for laboratory studies with *P. vivax* from different geographic origins.

INTRODUCTION

To study parasite-vector relationships and to select suitable vectors for laboratory transmission studies with different species of *Plasmodium*, we have adapted different strains and species of *Anopheles* mosquitoes to our laboratory. The different strains of *P. vivax* that have been adapted to develop in nonhuman primates come from different geographic areas, and efforts have been made to determine the relationships between coindigenous and noncoindigenous parasites and vectors.

Anopheles gambiae Giles is recognized as a major vector of *P. falciparum*, *P. malariae* and *P. ovale* throughout much of sub-Saharan Africa. However, most of the indigenous populations of this area are unable to support the development of *P. vivax* infection. This resistance to *P. vivax* infection by indigenous populations of sub-Saharan Africa is primarily due to the Duffy blood group negative genotype *FyFy* in which erythrocytes lack the Duffy blood group determinants Fy^a and Fy^b (Miller and Carter 1976, Welch et al. 1977). Since *An. gambiae* has little opportunity to be exposed naturally to infection with this parasite, we attempted to determine the susceptibility of several strains of *An. gambiae* to infection with 7 different strains of *P. vivax* being studied in nonhuman primates in our laboratory.

MATERIALS AND METHODS

Strains of Plasmodium vivax and infection of primates: Seven different strains of *P. vivax* were employed in the study (Table 1). Infections were induced in splenectomized *Aotus* (n = 67) and *Saimiri* (n = 32) monkeys and chimpanzees (n = 14). Animals were infected either by the intravenous inoculation of parasitized blood or by sporozoites, through either the bites of infected mosquitoes or the intravenous inoculation of sporozoites harvested from dissected mosquito salivary glands.

Mosquito strains: The G-3 strain of *An. gambiae*, Giles, originally from The Gambia, provided by G. Davidson of the London School of Hygiene and Tropical Medicine, has been maintained in our insectary since 1979. The KWA strain, originally from Tanzania, and the Zanzibar (ZAN) strain were acquired from field collections in 1984. *Anopheles freeborni* Aitken, (F-1 strain originally from Marysville, CA), *An. dirus* Peyton and Harrison, originally from Thailand, and *An. stephensi* Liston, originally from India were compared with the G-3 strain of *An. gambiae*. These species have been maintained in our insectary for over 10 years. Mosquitoes were reared at 25°C in deionized water and fed finely ground dog food daily. Pupae were harvested with ice water, and the adults were allowed to emerge in large screen-topped ice-cream-carton cages. Mosquitoes in breeding cages were allowed to feed on a tranquilized monkey or rabbit 3–5 times a week. Adults were fed 5% Karo™ solution daily. The *An. dirus* are maintained by force-mating. For feeding on monkeys infected with *P. vivax*, mosquitoes were transferred to small screen-topped ice-cream-carton cages. Mosquitoes fed on the tranquilized monkey directly through the mesh. In some instances, mosquitoes were infected by feeding through Parafilm™ membranes on heparinized blood from chimpanzees containing gametocytes. Infected mosquitoes were held in an incubator at 25°C and 60% relative humidity until they were dissected and examined for the presence of oocycts between 7 and 10 days after feeding. Salivary gland dissections were usually begun on day 12 and were made only to confirm the completion of the sporogonic cycle of the

different strains of *P. vivax* in the different species/strains of mosquitoes.

Comparative tests: For comparative feedings, the different mosquito species/strains were caged in ice-cream-carton cages and allowed to feed on the restrained tranquilized animals or through membranes on heparinized chimpanzee blood. Only lots of mosquitoes that were fed during the same time period were compared. On many occasions, none of the mosquitoes became infected. This study compares only those feedings in which one or both of the lots were infected. The G-3 strain of *An. gambiae* was considered the standard for our comparative infection studies. *Anopheles freeborni*, *An. dirus* and *An. stephensi*, as well as the KWA and ZAN strains of *An. gambiae* were compared with the G-3 strain of *An. gambiae* for percent infected and Gut Infection Index (GII). The GII for one lot of mosquitoes was the mean number of oocysts per gut × 100. Mean Infection Ratio is the ratio of the mean percent infection of each strain/species of *Anopheles* relative to the mean percent infection of *An. gambiae* G-3 strain. A ratio >1.00 indicates that the strain tested is more susceptible than the G-3 standard; a ratio <1.00 indicates that G-3 is more susceptible than the other strain. Mean Gut Infection Index Ratio indicates the intensity of oocyst infection and is the ratio of the geometric mean GII of each strain/species of mosquito relative to the geometric mean GII of the G-3 strain (× 100).

RESULTS

A total of 673 comparative feedings in which one or both of the species/strains were infected were selected. There were 168 paired feedings between G-3 and *An. freeborni*, 187 between G-3 and *An. stephensi*, 151 between G-3 and *An. dirus*, 89 between G-3 and the KWA strain of *An. gambiae*, and 78 between G-3 and the ZAN strain of *An. gambiae*. All of the mosquito species and strains supported the full cyclical development of parasites to sporozoites in the salivary glands. However, the presence and number of oocysts were the only criteria used for comparative analysis.

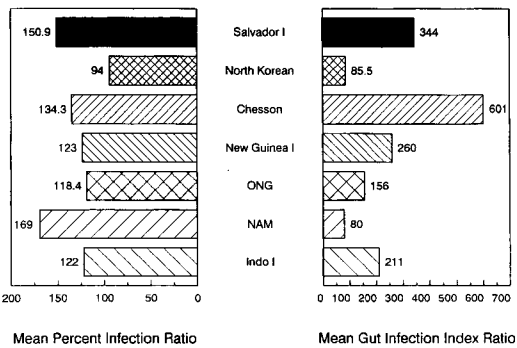

Fig. 1. Mean percent infection and Gut Infection Index ratios for 168 comparative feedings of *Anopheles gambiae* G-3 and *An. freeborni* on animals infected with 7 different isolates of *Plasmodium vivax*. Ratio = (*An. freeborni*/*An. gambiae* G3) × 100.

A comparison of the mean percentage infection ratio for the 168 paired feedings indicated that *An. freeborni* were more susceptible than *An. gambiae* G-3 to all the strains except the North Korean (Fig. 1). A comparison of the intensity of oocyst infection for each of the paired feedings indicated that *An. freeborni* were more heavily infected with the Salvador I, Chesson, New Guinea I, ONG and Indochina I

Table 1. Origin of 7 strains of *Plasmodium vivax* and number of monkeys and chimpanzees used in comparative susceptibility studies.

Strain of *P. vivax*	Primate hosts (no. used)	Geographic origin	Year	References
Salvador I	*Aotus* (20) *Saimiri* (30) Chimpanzees (8)	El Salvador	1969	Contacos et al. 1972, Collins et al. 1976
North Korean	*Aotus* (7) Chimpanzee (1)	North Korea	1953	Collins et al. 1985b
Chesson	*Aotus* (7) *Saimiri* (2) Chimpanzees (5)	New Guinea	1944	Ehrman et al. 1945
New Guinea I/OCDC	*Aotus* (7)	Papua New Guinea	1979	Collins et al. 1985a
ONG/CDC	*Aotus* (22)	Indonesia	1980	Collins et al. 1982
NAM/CDC	*Aotus* (3)	Indonesia	1979	Collins et al. 1982
Indochina I/CDC	*Aotus* (1)	Thailand/ Kampuchea	1980	Centers for Disease Control 1980

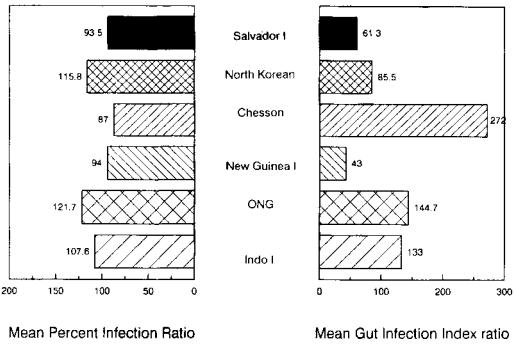

Fig. 2. Mean percent infection and Gut Infection Index ratios for 187 comparative feedings of *Anopheles gambiae* G-3 and *An. stephensi* on animals infected with 6 different isolates of *Plasmodium vivax*.

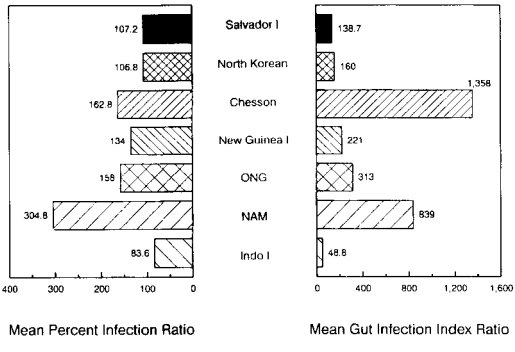

Fig. 3. Mean percent infection and Gut Infection Index ratios for 151 comparative feedings of *Anopheles gambiae* G-3 and *An. dirus* on animals infected with 7 different isolates of *Plasmodium vivax*.

strains, but less heavily infected with the North Korean and NAM strains than the G-3 strain.

The mean infection ratios indicated that the G-3 strain was more susceptible to the Salvador I, Chesson and New Guinea I strains, but less susceptible than *An. stephensi* to the North Korean, ONG and Indochina I strains (Fig. 2). A comparison of the GII ratios indicated that the Chesson strain of *P. vivax* was much more infectious to *An. stephensi* than were the other 5 strains of *P. vivax*. The G-3 strain was more heavily infected than *An. stephensi* for the Salvador I, North Korean and New Guinea I strains. No comparative feedings were made with the NAM strain.

The mean infection ratios indicated that the G-3 strain mosquitoes were more frequently infected than *An. dirus* and were more heavily infected only with the Indochina I strain (Fig. 3). The number of *An. dirus* mosquitoes infected with oocysts of the Chesson and NAM strains far exceeded those in the G-3 strain mosquitoes.

Eighty-nine paired comparisons were made between 2 strains of *An. gambiae*, one from West Africa (G-3) and the other from East Africa (KWA) (Fig. 4). The G-3 strain had a higher infection ratio with the Salvador I and New Guinea I strains than *An. gambiae* KWA. With the North Korean, ONG, NAM and Indochina I strains, the percentage of infection was similar. The GII ratios indicate that the intensity of oocyst infection in the G-3 was much greater with the Salvador I and New Guinea I strains of *P. vivax*, slightly greater with the NAM and Indochina I strains, and slightly less with the North Korean and ONG strains. No comparative feedings were made with the Chesson strain.

Comparative feedings between the *An. gambiae* G-3 and *An. gambiae* ZAN were only made with the Salvador I and the New Guinea I strains of *P. vivax* (Fig. 5). As shown, the G-3 had a slightly higher infection ratio and intensity of oocyst infection with the Salvador I strain. The reverse was true with the New Guinea I strain.

In nearly all of the paired feedings, when one species had a high number of oocysts, so did the other. Regression lines were prepared for each of the various comparisons to investigate this

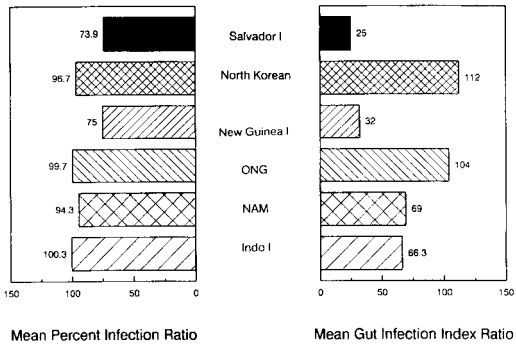

Fig. 4. Mean percent infection and Gut Infection Index ratios for 89 comparative feedings of *Anopheles gambiae* G-3 and *An. gambiae* KWA on animals infected with 6 different isolates of *Plasmodium vivax*.

Fig. 5. Mean percent infection and Gut Infection Index ratios for 78 comparative feedings of *Anopheles gambiae* G-3 and *An. gambiae* ZAN on animals infected with 2 different isolates of *Plasmodium vivax*.

relationship. Figure 6 presents the results of 168 paired feedings of the G-3 strain of *An. gambiae* and *An. freeborni* and is modeled by $\ln y = 2.747 + 0.5419(\ln x)$. The r^2 value (0.42) suggests only a moderate relationship, probably due to an excess number of negative (uninfected) *An. freeborni* feedings. Figure 7 presents the results of 187 paired feedings of G-3 and *An. stephensi* and is modeled by $\ln y = 1.49 + 0.6651(\ln x)$. The relationship is similar to that of *An. gambiae* and *An. freeborni* in Fig. 6 ($r^2 = 0.41$). Figure 8 models the results of 151 paired feedings of G-3 and *An. dirus* with $\ln y = 3.028 + 0.522(\ln x)$. The rather low r^2 (0.40) is influenced by an excessive number of negative feedings for *An. dirus*. Figure 9 (G-3 vs. KWA, $n = 89$, $\ln y = 0.884 + 0.077(\ln x)$, $r^2 = 0.53$) and Fig. 10 (G-3 vs. ZAN, $n = 78$, $\ln y = 1.072 + 0.77 (\ln x)$, $r^2 = 0.67$) suggest a stronger association exists within the species than between the species.

DISCUSSION

Anopheles gambiae is recognized as one of the most important vectors of human malaria. However, most of the population within its range is not susceptible to infection with *P. vivax* because of the negative genotype *FyFy* in the population (Race and Sanger 1975, Mourant et al. 1976, Welch et al. 1977). These studies with strains of *P. vivax* from widely separated geographic regions indicate the marked capacity for

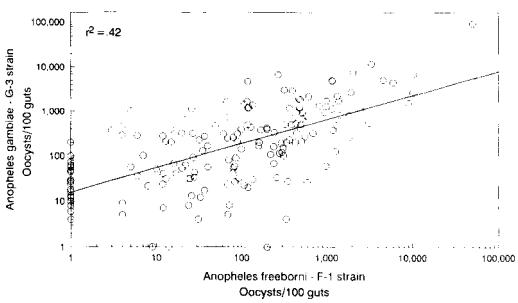

Fig. 6. Regression line for oocysts per 100 guts in 168 paired feedings of *Anopheles gambiae* G-3 and *An. freeborni* on 7 different isolates of *Plasmodium vivax*.

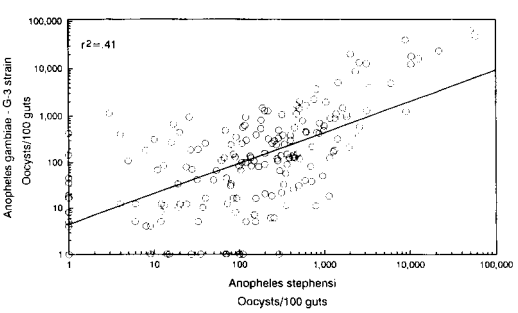

Fig. 7. Regression line for oocysts per 100 guts in 187 paired feedings of *Anopheles gambiae* G-3 and *An. stephensi* on 7 different isolates of *Plasmodium vivax*.

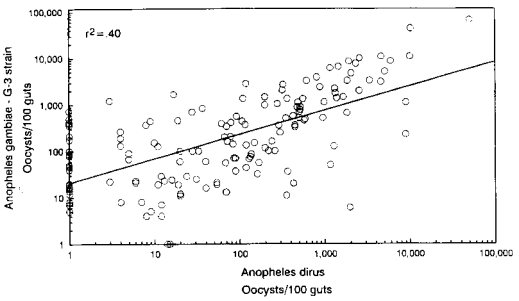

Fig. 8. Regression line for oocysts per 100 guts in 151 paired feedings of *Anopheles gambiae* G-3 and *An. dirus* on 7 different isolates of *Plasmodium vivax*.

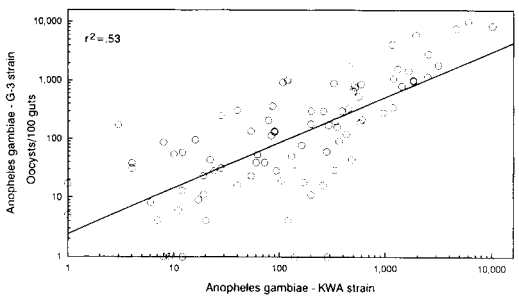

Fig. 9. Regression line for oocysts per 100 guts in 89 paired feedings of *Anopheles gambiae* G-3 and *An. gambiae* KWA on 6 different isolates of *Plasmodium vivax*.

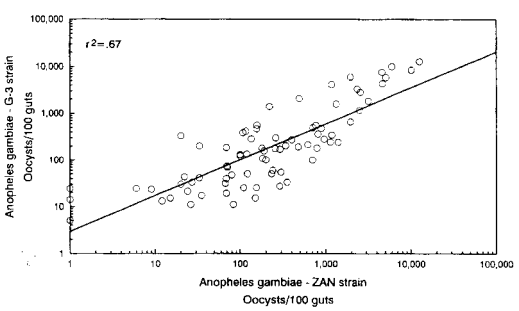

Fig. 10. Regression line for oocysts per 100 guts in 78 paired feedings of *Anopheles gambiae* G-3 and *An. gambiae* ZAN on 2 different isolates of *Plasmodium vivax*.

infection with *P. vivax* not only of the G-3 strain of *An. gambiae* from The Gambia, but also the KWA and ZAN strains from East Africa. This occurs even though *An. gambiae* usually does not have the opportunity to transmit this malarial parasite. It suggests that *An. gambiae* could readily transmit *P. vivax* if the vector was established in other geographic areas where humans are more susceptible to this parasite.

In the search for suitable vectors for laboratory studies on the human plasmodia, *An. gambiae* appears to be a very useful host, not only for the naturally occurring species of *Plasmodium* found in this mosquito in nature, but also for *P. vivax*. In addition, the G-3 strain has served as a model for the genetic selection of *Plasmodium*-refractory mosquitoes (Collins et al. 1986). *Anopheles dirus* require force-mating and are difficult to rear in large numbers. *Anopheles freeborni* is self-mating but has proven difficult to rear on large scale. These strains of *An. gambiae* and *An. stephensi* are self-mating and both easily reared in large numbers. As shown in Fig. 2, percentage infection is similar between the G-3 strain of *An. gambiae* and *An. stephensi*, although there were some marked differences in the number of oocysts per mosquito.

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