EXPERIMENTAL INFECTION OF *ANOPHELES GAMBIAE S.S.*, *ANOPHELES FREEBORNI* AND *ANOPHELES STEPHENSI* WITH *PLASMODIUM MALARIAE* AND *PLASMODIUM BRASILIANUM*

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ABSTRACT. Susceptibility to infection of 2 strains of *Anopheles gambiae s.s.*, *An. freeborni* and *An. stephensi*, was determined for 2 closely related malaria parasites, *Plasmodium malariae* and *P. brasilianum*. Neither strain of *An. gambiae* supported development of oocyst densities as great as the other 2 anopheline mosquitoes. The ZAN strain of *An. gambiae s.s.* from Zanzibar was more susceptible to infection with the strain of *P. malariae* from Uganda than the G-3 strain of *An. gambiae s.s.* from The Gambia. All species and strains of mosquitoes supported complete development to the presence of sporozoites in the salivary glands.

INTRODUCTION

The susceptibility of different strains of *Anopheles gambiae* Giles to experimental infection with different geographic strains of *Plasmodium vivax* has been reported (Collins and Roberts 1991). Because of the major importance of this mosquito species in the transmission of malaria in Africa, studies were made on its susceptibility to infection with an indigenous strain of *Plasmodium malariae* and the closely related malaria parasite of New World monkeys, *Plasmodium brasilianum*. Reported here are the results of studies comparing strains of *An. gambiae s.s.* from East and West Africa with our 2 reference mosquito species, *Anopheles freeborni* Aitken and *Anopheles stephensi* Liston.

MATERIALS AND METHODS

The G-3 strain of *An. gambiae s.s.*, originally from The Gambia, was obtained from the London School of Hygiene and Tropical Medicine and has been maintained in our insectary since 1979. The Zanzibar (ZAN) strain was originally acquired from field collections in 1984. *Anopheles freeborni*, (F-1 strain originally from Marysville, California, U.S.A.), and *An. stephensi*, (originally from India), have been maintained in our insectary more than 10 years.

The Uganda I/CDC strain of *P. malariae* was obtained in 1982 from a blood donor who had come to the United States from Uganda 8 years previously. Blood was inoculated into a splenectomized chimpanzee. After the chimpanzee developed a patent parasitemia, blood was passaged to splenectomized *Aotus* and *Saimiri* monkeys and other chimpanzees (Collins et al. 1984, 1989a, 1989b, 1990a). The *P. brasilianum* strains (Peruvian I and III) originated from naturally acquired infections in *Aotus vociferans* and *Saimiri sciureus peruviensis* monkeys from Peru (Collins et al. 1985, 1990b). The strains were maintained by linear passage in *Saimiri* monkeys.

Heparinized blood infected with *P. malariae* was obtained from the chimpanzee by venapuncture and was fed to mosquitoes through parafilm membranes using blown glass feeders warmed with a circulating water pump (Rutledge et al. 1964). For feeding, mosquitoes were transferred to small screen-topped ice-cream-carton cages. Mosquitoes fed directly through the mesh on the membrane or on anesthetized monkeys. Infected mosquitoes were held in an incubator at 25°C and 60% RH until they were dissected 9 to 12 days after feeding and examined for oocysts. If oocysts were present, a portion of the group of mosquitoes was held to determine the completion of the sporogonic cycle (presence of sporozoites in the salivary glands).

For comparative feedings, the different mosquito species/strains were caged and fed simultaneously on the animal or membrane. Only lots of mosquitoes fed at the same time were compared. Usually, no infections were obtained. However, if either of the mosquito species was infected, the dissection results were used for comparison. Five chimpanzees and 16 *Aotus lemurinus griseimembra* monkeys served as infective donors for *P. malariae*. Eighteen *Saimiri sciureus boliviensis* or *Saimiri sciureus peruviensis* monkeys served as infective donors for *P.*

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brasilianum. All animals were splenectomized either before or during infection to stimulate the production of infective gametocytes.

The average number of oocysts per gut for a particular lot of mosquitoes was multiplied × 100 to give the gut infection index. The geometric mean gut infection index for the strain of *An. gambiae s.s.* was divided by that of a reference mosquito species to give the gut infection index ratio. The mean percent infection for the strain of *An. gambiae s.s.* was divided by that of a reference mosquito species to give the percent infection ratio. Differences between percentage infection were determined using normal approximation (z-test with variance). Because the number of mosquitoes varied by lot, the variance involves a weighted sum of variations about the mean. Differences between the gut infection indices were determined by t-test.

RESULTS

A total of 355 feedings (186 *P. malariae* and 169 *P. brasilianum*) were selected in which one or both of the G-3 or ZAN strains of *An. gambiae s.s.* or *An. freeborni* and *An. stephensi* were infected (Table 1 and Figures 1 and 2).

The Uganda I/CDC strain of *P. malariae* was readily infective to both the G-3 and ZAN strains of *An. gambiae s.s.* (Table 1, Fig. 1). When compared with *An. freeborni*, the ZAN strain had a higher mean percent infection ratio (43.9:100) than the G-3 mosquitoes (37.2:100). The same was true for the mean gut infection index ratios (24.1:100 for the ZAN:FRE comparison vs. 11.2:100 for the G-3:FRE comparison). When compared with *An. stephensi*, the ZAN strain also had higher mean percent infection and gut infection index ratios than the G-3 strain of *An. gambiae s.s.* (79.9:100 vs. 55.5:100 and 45.2:100 vs. 27.4:100, respectively), although the differences were less significant than with *An. freeborni* (Table 1). On the 15 occasions when both the G-3 and ZAN strains were fed together, the percent infection and the gut infection index ratios were 48.1:100 and 22.1:100, respectively, with the ZAN strain given the value of 100. Dissection of salivary glands of each of these species/strains of anophelines indicated the complete development of the sporogonic cycle to the presence of large numbers of sporozoites in the salivary glands.

The 2 strains of *P. brasilianum* were also readily infective to both the G-3 and ZAN strains of *An. gambiae s.s.* (Table 1, Fig. 2). When compared with *An. freeborni*, the G-3 strain hada higher mean percent infection ratio (30.9:100) than the ZAN mosquitoes (26.3:100). The same was true for the mean gut infection

Table 1. Comparative infectivity of *Plasmodium malariae* and *Plasmodium brasilianum* to 2 strains of *Anopheles gambiae s.s.* (G-3 and ZAN), An. freeborni (FRE), and An. stephensi (STE) mosquitoes.

Mosq. species comparison	No. lots comp.	Positive mosquitoes/no. dissected	Percent infection			Gut infection indices		
			Mean	*P*-value*	Ratio	Geometric mean	*P*-value**	Ratio
P. malariae								
G-3:FRE	38	133/645	21.4:57.5	<0.0001	37.2:100	23.3:208.6	<0.0001	11.2:100
ZAN:FRE	46	260/855	32.3:53.1	<0.0001	43.9:100	49.4:205.4	0.0004	24.1:100
G-3:STE	39	142/653	21.7:39.1	0.002	55.5:100	25.7:93.9	0.002	27.4:100
ZAN:STE	48	274/874	33.4:41.8	0.14	79.9:100	58.3:129.1	0.04	45.2:100
G-3:ZAN	15	52/302	19.1:39.7	0.016	48.1:100	24.3:110.2	0.008	22.1:100
P. brasilianum								
G-3:FRE	24	86/400	21.5:69.6	<0.0001	30.9:100	31.4:583.8	<0.0001	5.4:100
ZAN:FRE	41	106/552	18.5:70.4	<0.0001	26.3:100	21.0:657.8	<0.0001	3.2:100
G-3:STE	39	148/581	25.5:30.8	0.63	82.8:100	27.5:95.5	0.63	28.8:100
ZAN:STE	48	133/718	18.2:26.1	0.055	69.7:100	24.6:62.4	0.03	39.4:100
G-3:ZAN	17	45/224	20.1:22.8	0.70	88.2:100	19.6:42.1	0.30	46.6:100

* = z-test.
** = pooled t-test.

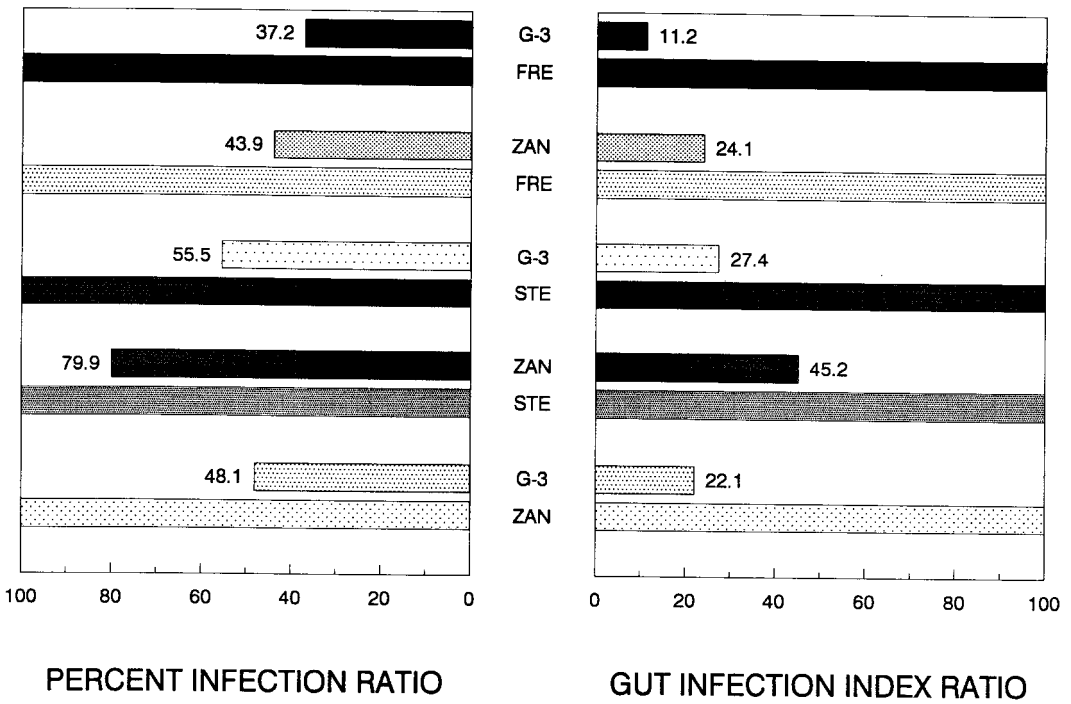

Fig. 1. Percent infection ratios and gut infection index ratios of *Anopheles gambiae s.s.* (G-3 and ZAN strains) and *An. freeborni* (FRE) and *An. stephensi* (STE) for *Plasmodium malariae.*

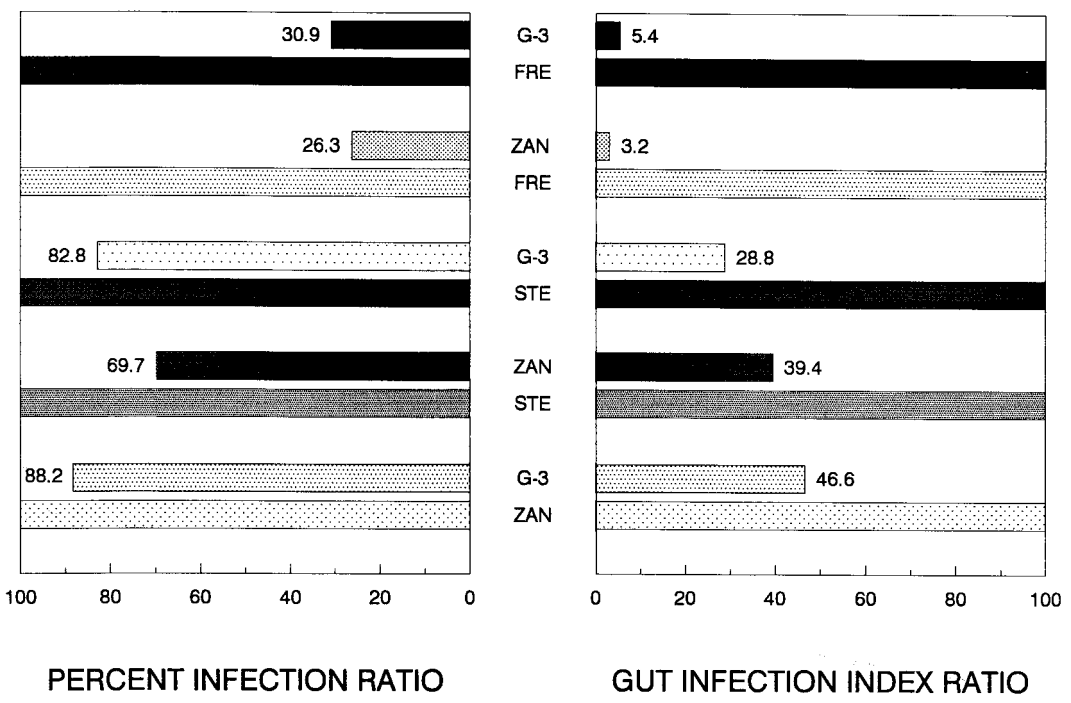

Fig. 2. Percent infection ratios and gut infection index ratios of *Anopheles gambiae s.s.* (G-3 and ZAN strains) and *An. freeborni* (FRE), and *An. stephensi* (STE) for *Plasmodium brasilianum.*

index ratios (5.4:100 for the G-3:FRE comparison vs. 3.2:100 for the ZAN:FRE comparison). When compared with *An. stephensi*, the ZAN and G-3 strains were essentially the same for the mean percent infection ratio (69.7:100 for the ZAN:STE comparison vs. 82.8 for the G-3:STE comparison). However, the gut infection index ratios indicated that the ZAN strain was more heavily infected (39.4:100 vs. 28.8:100 for the G-3). On the 17 occasions when both the G-3 and ZAN strains were fed together, the percent infection and gut infection index ratios were 88.2:100 and 46.6:100, respectively, with the ZAN strain given the value of 100. No significant differences in these values were observed. Salivary gland examination indicated the complete development of the sporogonic cycle in each of these species/strains of anophelines.

DISCUSSION

The Uganda I/CDC strain of *P. malariae* originated from Uganda and can be considered an East African parasite. The ZAN strain of *An. gambiae* from Zanzibar was more susceptible to infection than the West African G-3 strain of *An. gambiae*. The results suggest that strains of *P. malariae* may be more infective to coindigenous strains of *An. gambiae s.s.* Confirmation studies must await the adaptation of additional strains of *P. malariae* from other geographic areas of Africa.

Plasmodium brasilianum is morphologically very similar to *P. malariae* and may have resulted from direct adaptation of *P. malariae* into South American monkeys from human infections (Coatney et al. 1971). Molecular studies on the circumsporozoite proteins of these 2 parasites indicate a high level of homology (Lal et al. 1988a, 1988b). Our results show that *An. gambiae s.s.* can readily serve as a host for the development of *P. brasilianum* as well as for *P. malariae*. However, neither of the strains of *An. gambiae s.s.* had percentage of infection rates or the number of oocysts per gut as high as the *An. freeborni* or *An. stephensi* mosquitoes.

In our search for efficient hosts for experimental vector or parasite studies, *An. gambiae s.s.* has some advantages. It is readily reared in the laboratory, feeds well on animals and through parafilm membranes, and is relatively long living. Its usefulness will be enhanced as its susceptibility to additional strains/species of *Plasmodium* is demonstrated.

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