

P. 2

COMPARISON OF DIRECT AND MEMBRANE FEEDING METHODS TO INFECT *ANOPHELES ARABIENSIS* WITH *PLASMODIUM FALCIPARUM*

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Abstract. Two standard methods are available to infect mosquitoes with malaria parasites: direct feeding through the skin of the gametocyte carrier, and membrane feeding. *Anopheles arabiensis* collected at larval stages and reared in an insectary were fed in parallel by feeding on *Plasmodium falciparum* gametocyte carriers and by membrane feeding on venous blood of the same gametocyte carriers. Infection of mosquitoes was assessed at Day 7 post bloodmeal by oocyst count of the mosquito midguts. The following parameters were not significantly different between the two methods: the percentage of gametocyte carriers infective for at least one mosquito (52.4% through the skin versus 57.1% through the membrane), the mean infection rate of mosquitoes (10.0% versus 11.3%), the geometric mean oocyst number per mosquito (2.51 versus 3.83). In conclusion, infection of mosquitoes by membrane feeding was similar to infection by direct feeding. Most of the volunteers preferred venipuncture to mosquito bites.

INTRODUCTION

Infection of mosquitoes by *Plasmodium* occurs by the ingestion of gametocytes during a bloodmeal. Two methods are available to perform this infection: first, a natural bloodmeal taken by the mosquito through the skin of a gametocyte carrier and second, a venipuncture followed by ingestion of this blood by the mosquito through the membrane of a feeder apparatus.¹ These two methods differ in several ways. Ingested blood comes from capillaries for direct feeding but from veins for membrane feeding. In the membrane feeding method, an anticoagulant is added and blood is handled before being put in the feeder. Furthermore, during the course of the latter, blood undergoes chemical changes and its cellular components settle. It is not known whether or how these differences affect infectivity for the mosquito. This study compared the infection of *Anopheles arabiensis* by *Plasmodium falciparum* gametocytes by direct feeding through the skin and by feeding through a membrane. The maintenance and handling of mosquitoes were previously standardized.^{2,3}

MATERIALS AND METHODS

The study was performed at Thiès (14°47'N, 16°55'E), Sénégal, a town located 70 km east of Dakar. This is an endemic area in which malaria transmission is highly seasonal. The study was conducted between September and December 1998 when malaria cases were most frequent.

Gametocyte carriers. *Plasmodium falciparum* gametocyte carriers were identified among residents in the area of Thiès. Persons > 5 years old (or their parents) were informed of the protocol in which their participation was requested. Those who consented and did not harbor other *Plasmodium* species were included in the study. The protocol was approved by the Ministry of Health of Senegal.

Mosquitoes. *Anopheles arabiensis* were collected at larval stages in breeding sites (pools and wells) in Dakar.⁴ The larvae were reared in the Dakar insectary at 27–28°C. Adults were maintained at the same temperature and at 70–90% relative humidity, with a continuously available 5% sucrose solution. No bloodmeal was ingested before infection. Three-day-old females were placed without males in a paper cup covered with a mosquito net (volume = 60 ml; diameter = 6 cm; height = 2.5 cm) and starved for 5–6 hours before

the bloodmeal. Mosquitoes were transported in a box isolated from light and heat under a dampened floor cloth for the approximately 1.5 hr road trip to Thiès. The temperatures inside the box varied from 25.5–31.0°C with a mean difference between the minimum and the maximum of 1.32°C.

Blood handling and mosquito engorgement. Venipuncture at the antecubital fossa was performed on the human gametocyte carriers between 10 AM and 1 PM and the blood collected into a sterile lithium heparinate Vacutainer® tube (Becton Dickinson, Franklin Lakes, NJ). A membrane feeder (diameter 2.5 cm, surface area 5.0 cm² as described by Ponudurai and others)⁵ held at 37.5°C was immediately filled with 1.5 ml of blood using a sterile syringe. Mosquitoes were allowed to feed through a Baudruche membrane (Joseph Long Inc., Belleville, NJ) for 10 minutes in a dark place. Eighty female mosquitoes in 2 cups of 40 mosquitoes each were used, the first cup being placed under the membrane for ten minutes then a second cup under the same membrane for the next 10 minutes. After engorgement, the feeder and the Baudruche membrane were cleaned by rinsing in a large amount of water without detergent and immersed in 95% ethanol and dried before reuse.

The direct feeding was started at the same time as the membrane feeding. It involved two cups of 35 female mosquitoes each simultaneously placed in contact with the calves of the gametocyte carrier for 15 minutes in the dark. After direct feeding, a painkilling ointment was applied.

Blood remaining in the blood collection set was used to make thick blood films. They were Giemsa-stained and 200 microscopic oil-immersion fields were examined. The gametocyte density was estimated on the basis of 8,000 leukocytes/mm³ blood.

Treatment of mosquitoes. After engorgement, mosquitoes were transported by road to the Dakar insectary. Mosquitoes were individually examined and those not fully engorged were removed. Fully fed mosquitoes (no more than 15 per cup) were maintained in paper cups at 26–28°C and 70–90% relative humidity without any further bloodmeals. Cotton wool impregnated with sucrose solution was put on each cup and replaced twice a day. Seven days after the bloodmeal, surviving mosquitoes were dissected for microscopic examination of the oocysts in the midgut using one drop of 1% mercurochrome.

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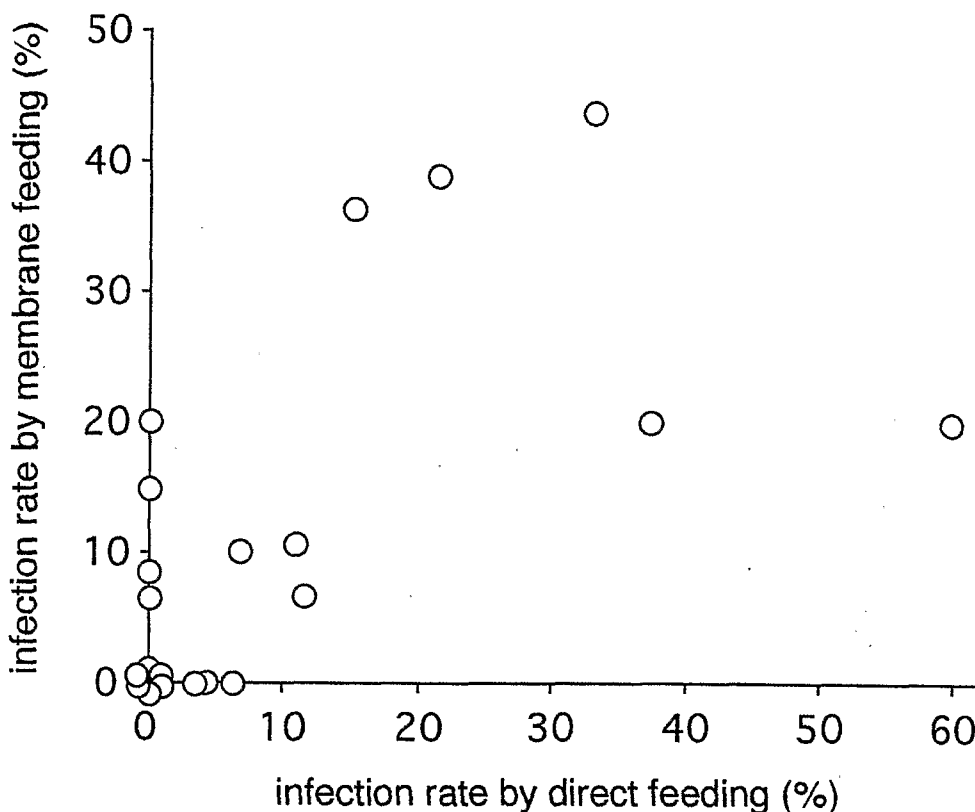


FIGURE 1. Relationship between infection rates of *Anopheles arabiensis* fed with the blood of 21 *Plasmodium falciparum* gametocyte carriers using two methods. The 3 circles on the horizontal axis designate gametocyte carriers who infected mosquitoes only by direct feeding. The 4 circles on the vertical axis indicate mosquitoes infected only by membrane feeding. The 8 circles in the central part of the figure, mosquitoes infected by both methods. The 6 circles on the origin of the two axis, mosquitoes not infected by the two methods.

Data treatment and statistical analysis. The blood-feeding rate is the proportion of female mosquitoes which were fully fed after a bloodmeal. The survival rate is the proportion of engorged females which survived 7 days after the bloodmeal. The infection rate is the proportion of mosquitoes which had one or more oocysts. The arithmetic mean observed in various experiments was calculated. The geometric mean of Williams [$= \text{exponential}(\text{arithmetic mean}(\ln(x_i + 1))) - 1$] was used to calculate oocyst mean number to allow the use of zeros.⁶

Calculations and infection rates were only performed for experiments in which at least 10 mosquito midguts were examined by the two methods. The means of paired series were compared using the non-parametric rank sum test of Wilcoxon. Fisher's exact probability test was used to compare the proportions of gametocyte carriers infective for mosquitoes.

RESULTS

Data were obtained using 60 *P. falciparum* gametocyte carriers and 9,000 *An. arabiensis* mosquitoes of which 4,335 were fully engorged. The mean blood-feeding rate was 65.2% after direct feeding versus 32.7% after membrane feeding ($P < 0.0001$ by Wilcoxon test, $n = 60$ pairs). The mean survival rate seven days after engorgement was 34.3% after direct feeding versus 28.1% after membrane feeding ($P = 0.006$, $n = 57$ pairs).

Comparison of the direct feeding method to the membrane

feeding method in terms of mosquito infectivity was made on 21 gametocyte carriers. The mean age of the gametocyte carriers was 20.3 years (range, 7-48; median, 17). Eighteen carriers had already received antimalarial treatment (12 with quinine, 4 with chloroquine, 2 with quinine plus chloroquine or sulfadoxine-pyrimethamine). Five had fever (body temperature $> 37.5^\circ\text{C}$) at the time the mosquitoes were taking a bloodmeal. Twenty mentioned a recent malaria attack and 6 had an asexual parasitemia (geometric mean = $166/\mu\text{L}$; maximum, $1,310/\mu\text{L}$). The geometric mean gametocytemia was $214/\mu\text{L}$ blood (range = 6-1,369). The proportion of gametocyte carriers who infected at least one mosquito was 71% (15/21). This value was 52.4% (11/21) after direct feeding versus 57.1% (12/21) after membrane feeding ($P = 0.77$). The degree of concordance between the two methods was 66.7% (14/21). The difference between the discordant pairs (4/21 versus 3/21) was not significant ($P = 0.70$). The mean infection rate was 10.0% after direct feeding versus 11.3% after membrane feeding ($P = 0.43$ by Wilcoxon test) with a maximum of 60% after direct feeding and 44% after membrane feeding (Figure 1). A strong correlation existed between direct and membrane feeding ($r = 0.605$, $n = 21$, $P = 0.0029$). The maximum number of oocysts observed on one midgut was 60 after direct feeding versus 252 after membrane feeding. The geometric mean number of oocysts per mosquito was 2.51 after direct feeding versus 3.83 after membrane feeding ($P = 0.16$). A strong correlation existed

for the geometric means of direct and membrane feeding ($r = 0.869$, $n = 21$, $P < 0.0001$).

The carrier's age, body temperature, recent antimalarial treatment, and asexual parasitemia were not linked to the infection rate of mosquitoes or the mean number of oocysts. When fifty of the volunteer gametocyte carriers were asked whether they preferred to provide blood by venipuncture or by the direct feeding method, 94% preferred the former and 6% the latter.

DISCUSSION

This study demonstrates concordance between two widely used methods to infect anopheline mosquitoes with *P. falciparum* gametocytes. The results have two implications: first, studies of experimental infections performed by membrane feeding⁷⁻¹⁰ and by direct feeding¹¹⁻¹³ have epidemiological relevance and second, trials to evaluate the efficacy of a transmission-blocking vaccine can use either method. Volunteers appear to favor providing blood by venipuncture for the membrane feeding method rather than directly to mosquitoes feeding on their skins.

The overall significance of the current study is limited by two factors. First, blood-feeding and survival rates were relatively weak. This was mainly due to the use of wild vectors who had not been selected for maintenance and survival in the environmental conditions in the insectary. This use of wild-caught vector material along with naturally-infected humans provide data that reflect both the infectiousness of the parasite and the susceptibility of its local vector in the Dakar area. After several generations in an insectary, the ability of mosquitoes to be infected with *Plasmodium* may be altered compared to the original wild generation.¹⁴ Second, the infection rate of mosquitoes was also relatively low. In our study, the mean percentage of infected mosquitoes was 11%, which is slightly lower than found in comparable reports (12% in the Bobo-Dioulasso area of Burkina Faso;⁷ 19% in Yaounde, Cameroon;¹⁰ 25% in a village in Liberia;¹² and 26% in the Madang area of Papua New Guinea).¹⁵ Nevertheless, most of the blood-feedings on gametocyte carriers in these studies did not result in the infection of mosquitoes.

In the current study, 71% of gametocyte carriers were infective for mosquitoes. This percentage is slightly higher than those found in similar studies (53% in Burkina Faso;⁷ 38% or 62% in Cameroon;^{8,10} 45% in Papua New Guinea).¹⁵ This difference may be related to the large proportion of recent malaria attacks in our gametocyte carrier sample.

Graves observed a small decline in the gametocyte infectivity after 10 minutes in the feeder, and a marked decline after 30 minutes.¹⁶ However, Ponnudurai and others who employed an improved feeder (the same one used in our study) observed that infectivity remained stable during the first 30 minutes period of feeding.⁵ We therefore consider our observations to be valid up to 20 minutes after blood was put into the feeder.

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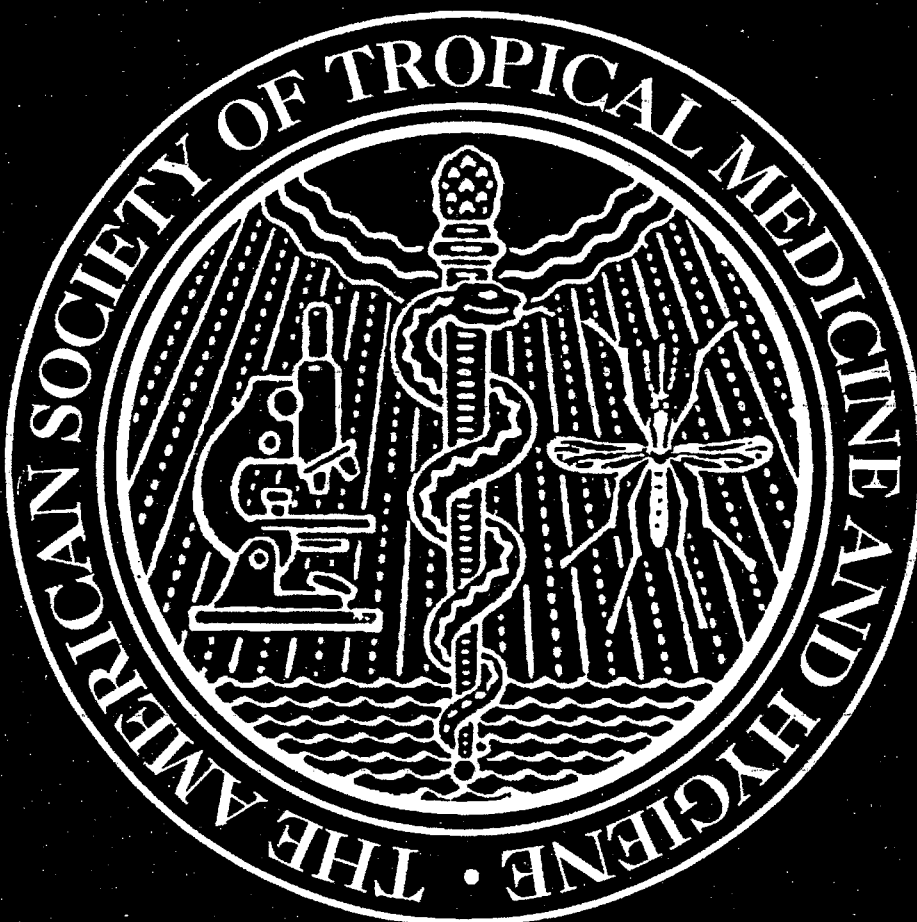
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