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RESPONSE OF WILD *ANOPHELES FUNESTUS* TO REPELLENT-PROTECTED VOLUNTEERS IS UNAFFECTED BY MALARIA INFECTION OF THE VECTOR[1]

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ABSTRACT. A field experiment was conducted to compare the biting behavior of *Plasmodium falciparum*-infected and uninfected *Anopheles* on humans using mosquito repellents. Repellent formulations (5% [wt/vol] *N,N*-diethyl-3-methylbenzamide [deet] or 5% [wt/vol] AI3-37220, a piperidine compound, both in 100% ethanol, or 100% ethanol alone [as a control]) were applied to the lower legs of 3 collectors in each of 4 houses. Collectors caught mosquitoes over 6 collection nights. Mosquitoes were collected into vials after they initiated probing on the leg of a collector. Infected females made up the same proportion of the *Anopheles funestus* populations biting either repellent-protected or unprotected individuals. We conclude that repellent formulations are equally effective against *Plasmodium*-infected and uninfected *An. funestus*.

INTRODUCTION

The biting behavior of hematophagous insects may be affected by infection with protozoan parasites (Jenni et al. 1980, Anez and East 1984, Beach et al. 1985), bacteria (Cavanaugh 1971), and viruses (Grimstad et al. 1980). Among mosquito species with high natural levels of salivary gland apyrase, malaria infection causes local salivary gland pathology resulting in an increase in total time spent probing while attempting to feed (Rossignol et al. 1984, Wekesa et al. 1992) and in the number of probes made (Wekesa et al. 1992) compared to uninfected conspecifics. Additionally, in a study of a natural population of *Anopheles gambiae sensu lato* (*s.l.*) mosquitoes in western Kenya, infected *An. gambiae s.l.* were significantly more likely than their uninfected counterparts to secure a blood meal when offered an anesthetized hamster (Wekesa et al. 1992). This observation suggested that infected *Anopheles* may be more avid or persistent feeders at a blood source. The small number of *Anopheles funestus* Giles examined in that study showed the same trends in altered feeding behavior (Wekesa et al. 1992). If host-seeking *Anopheles* also show infection-dependent differences that affect their behavior in the presence of repellents, strategies for personal protection in malaria endemic areas may need to be revised. Previously, only the work of Robert et al. (1991) has addressed this question. In their study, the response of colonized *Anopheles stephensi* Liston to repellent formulations of *N,N*-diethyl-3-methylbenzamide (deet) and permethrin was independent of infection with either *Plasmodium falciparum* or *Plasmodium berghei*. However, *An. stephensi* is characterized by low concentrations of salivary apyrase (Ribeiro et al. 1984), and does not show infection-dependent differences in feeding behavior (Li et al. 1992).

In the present study, in conjunction with a field trial of mosquito repellents, we examined natural field populations of *An. funestus* and *An. gambiae s.l.*, species whose feeding behavior has been shown previously to be affected by infection with *P. falciparum* malaria. The following analysis was done, therefore, to determine if malaria infection affects the behavioral response of host-seeking *Anopheles* in the presence of hosts with and without repellent protection.

MATERIALS AND METHODS

The study was conducted in Nyanza Province, western Kenya, adjacent to the Ahero rice irrigation scheme. *Anopheles* ecology and vector dynamics have been characterized in this area by Githeko et al. (1993). A complete description of methods and efficacy of repellents is given in Walker et al. (1996). Briefly, 2 repellents, deet, 100% (Morflex Inc., Greensboro, NC) and 1-(3-

[1] The views of the authors do not purport to reflect the position of the U.S. Department of the Army or the Department of Defense, or the Government of Kenya. Mention of a commercial product does not constitute an endorsement of the product by the Department of Defense. The volunteers gave informed consent to participate in the study. This paper is published with the permission of the directors, Kenya Medical Research Institute and Walter Reed Army Institute of Research.

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cyclohexen-1-yl-carbonyl)-2-methylpiperidine (AI3-37220), 99% (synthesized by T. P. McGovern, Insect Chemical Ecology Laboratory, USDA-ARS, Beltsville, MD) were evaluated against an absolute ethanol control. Repellents were prepared as a 5.0% (wt/vol) solution in absolute ethanol with a concentration of 50 mg active ingredient (AI)/ml diluent and applied at a rate of 0.1 mg AI/cm^2 of lower leg surface area. Controls received absolute ethanol. Twelve local adult male residents of the area were selected as volunteers. The lower legs, between the ankles and knees, were used as the sites of repellent and control treatments. Volunteers wore hooded screened jackets to restrict mosquito feeding to the lower legs. Treatments were applied *ca.* 1 h before the start of a test. Collections of biting mosquitoes were made inside local houses between 2000 and 0545 h, over 6 collection nights. Volunteers were randomly selected for treatment and house so that for each collection night there were 4 participants per treatment and 3 treatment participants per house (deet, AI3-37220, and control). For 45 min during each collection hour, volunteers captured all mosquitoes biting them on their lower legs. Mosquitoes were collected individually into 20-ml scintillation vials.

Mosquitoes were transported to the Kenya Medical Research Institute, Vector Biology and Control Research Centre at Kisian for processing and identification based on morphological characteristics. After identification, mosquitoes were held at −70°C. Later, individual mosquitoes were cut at the junction of the thorax and abdomen, and thoraces were tested by ELISA for the presence of *P. falciparum* circumsporozoite protein antigen (Beier et al. 1987). Mosquitoes were considered positive if they had optical densities at least twice that of the mean of 8 negative controls (uninfected females). Abdomens were saved for mosquitoes identified as members of the *An. gambiae* complex. Twenty percent (every 5th mosquito) of these individuals were identified to species following extraction of DNA and its amplification by the polymerase chain reaction (Taylor et al. 1993). Previous work at the study site showed that *Anopheles arabiensis* Patton comprised nearly 100% of mosquitoes identified as members of the *An. gambiae* complex (Githeko et al. 1993).

RESULTS

Both deet and AI3-37220 provided significantly more protection against mosquito biting than the ethanol control, and these results are reported elsewhere (Walker et al. 1995). Over the 6 collection nights 5,038 *Anopheles* were collected. Of these, 3,677 (73%) were *An. funestus* and 1,361 (27%) were *An. gambiae s.l.* One hundred percent of tested *An. gambiae s.l.* (n = 272) were identified as *An. arabiensis.* We assumed, therefore, that all mosquitoes identified morphologically as *An. gambiae s.l.* were *An. arabiensis.*

Table 1. Numbers of *Plasmodium falciparum*-infected and uninfected *Anopheles funestus* biting repellent-protected and unprotected individuals.

Treatment	No. infected	No. uninfected	% infected
AI3-37220	10	580	1.7
Deet	18	1,020	1.7
100% ethanol	38	2,011	1.9

None of 1,361 *An. arabiensis* was positive by ELISA for *P. falciparum.* Therefore, we were unable to evaluate the potential effect of malaria infection on biting behavior of this species in the presence of repellents. Of the *An. funestus,* 2,049 (56%) were captured while attempting to feed on controls, whereas 1,038 (28%) and 590 (16%) were captured on collectors who were using deet and AI3-37220, respectively. Sixty-six of 3,677 *An. funestus* (1.8%) were infected with *P. falciparum.* Infected females made up the same proportion of the *An. funestus* populations biting either repellent-protected or unprotected individuals (deet, 1.7%; AI3-37220, 1.7%; or 100% ethanol, 1.9%) (Table 1; $\chi^2 = 0.09$, df = 2, $P > 0.05$).

DISCUSSION

Malaria infection of wild *Anopheles* increases the probability of successful bloodfeeding on a restrained hamster, even though feeding by infected mosquitoes requires a greater number of probes and a longer time spent probing (Wekesa et al. 1992). If natural malaria infection increases vector persistence at the host, one effect may be to cause an apparent decrease in sensitivity of infected mosquitoes to repellents, compared to uninfected mosquitoes. In such a case, the proportion of the total bites made on repellent-protected humans by infected *Anopheles* ought to be higher than the same proportion made on unprotected individuals. Our data revealed no differences in these proportions among 2 repellent treatments and the control, and provided no evidence for infection-dependent differences in the response of *An. funestus* to repellent-protected individuals. Based on the data collected in this study, repellents remain an important option for disease prevention in malarious regions.

We were unable to examine the effect of infection status on bloodfeeding behavior for either *An. gambiae sensu stricto* (*s.s.*), the most important malaria vector in Africa, or *An. arabiensis*, whose importance as a vector is highly variable. No *An. gambiae s.s.* were collected at our study site, and captured *An. arabiensis* were uniformly uninfected. Both *An. gambiae* and *An. arabiensis* have high levels of salivary apyrase (Cupp et al. 1994), and naturally occurring *An. gambiae s.l.* (probably *An. gambiae s.s.*) showed marked infection-dependent changes in bloodfeeding behavior (Wekesa et al. 1992). The responses of infected individuals of both species to repellent-protected and unprotected volunteers should be evaluated in future repellent trials.

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