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EFFECT OF *PLASMODIUM FALCIPARUM* ON BLOOD FEEDING BEHAVIOR OF NATURALLY INFECTED *ANOPHELES* MOSQUITOES IN WESTERN KENYA

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Abstract. Feeding behavior was compared between infected and uninfected field-collected groups of *Anopheles gambiae* sensu lato and *An. funestus* from western Kenya. A significantly greater percentage (81%) of *Plasmodium falciparum*–infected *An. gambiae* s.l. females probed on experimental hosts (hamsters) than did uninfected females (38%). Among those females that initiated probing, there was no effect of infection status on the ability to take a bloodmeal. *Plasmodium falciparum*–infected *An. gambiae* s.l. probed more often (mean = 4.0) and for a longer time (mean = 277 sec) than did their uninfected counterparts (mean = 2.4 probes and mean probing time = 214 sec). Results for the small number of *An. funestus* that fed followed the same trend. Among infected *An. gambiae* s.l. females, there was no effect of sporozoite density on either the number of probes made or the total probing time. Among uninfected females, there was no difference in feeding behavior between nulliparous and parous females. In laboratory experiments, female age had no effect on blood-feeding behavior. Our findings provide evidence that natural malaria infection modifies the feeding behavior of *Anopheles* females.

The influence of parasites on the feeding behavior of hematophagous arthropods in nature is not yet known. Laboratory studies indicate that parasites induce behavioral changes in their vectors that may enhance parasite transmission by prolonging vector-host contact. Blood feeding behavior of insect vectors has been shown to be affected by infection with *Leishmania*,[1] *Trypanosoma*,[2,3] and *Plasmodium*.[4,5] In all of these studies, parasite infection resulted in increased vector-host contact. Increased probing and extended host contact may also affect transmission dynamics by arousing host defenses, leading to interrupted feeding and multiple host contacts per gonotrophic cycle.[6] The purpose of our study was to investigate the influence of natural *Plasmodium falciparum* infection on the feeding behavior of wild *Anopheles gambiae* Giles sensu lato and *An. funestus* Giles, the principal vectors of human malaria in sub-Saharan Africa.

MATERIALS AND METHODS

Field observations

The collection site was located at Kisian (0°05′S and 34°40′E) approximately 10 km west of Kisumu in western Kenya. Malaria is holoendemic at Kisian and this site has been the focus of several previous studies.[7–9] Host-seeking mosquitoes were collected during biting catches inside houses three nights per week from December 1988 through June 1989.

Individual, non-bloodfed *An. gambiae* s.l. and *An funestus* were transferred to transparent plexiglas cages (2.5 × 2.5 × 5.0 cm), which were covered by nylon netting on two of the long sides so that mosquitoes could probe through them. Mosquitoes were held in a cage for 5–10 min before feeding to permit acclimatization. Mosquitoes were allowed to feed on hamsters that were anesthetized by intraperitoneal injection of 0.15 mg/kg of sodium pentobarbitone. The abdomen was shaved, a hamster was laid on its back, and the caged mosquitoes were exposed to the shaved abdomen.

Mosquito feeding behavior was observed for 10 min and observations were recorded on an audio cassette tape. Light was provided by a small flashlight and a kerosene lamp. Data was transcribed in the laboratory, and the following variables were considered: initiation of probing, the number of probes, total probing time, and whether or not a mosquito took blood. A probe was defined as the insertion of mouthparts into the skin. All probes subsequent to the first had to be preceded by desistance and complete removal of mouthparts from the skin before rein-

sertion at the same or, more commonly, a new site. Thus, separate probes are distinguished from blood vessel–searching behavior, wherein a female moves the mouthparts up and down within the skin without removing them. Total probing time was defined as the amount of time that mouthparts were inserted into the skin during the 10-min observation period, regardless of the number of probes made. Following exposure to the hamster, female mosquitoes were assigned to one of three classes; unfed, partially fed, or fully fed.

Individual mosquitoes were held in their feeding cages until the following morning when they were transported to the laboratory. Cages were kept in a refrigerator at 4°C until the mosquitoes were examined by dissection of the salivary glands for the presence of sporozoites. The number of sporozoites in dissection-positive salivary glands was graded as follows: +1 = 1–10, +2 = 11–100, +3 = 101–1,000 and +4 > 1,000. After dissection, salivary glands were stored at −70°C until tested by enzyme-linked immunosorbent assay (ELISA) for *P. falciparum* circumsporozoite protein.[10] Parous state was determined by examination of tracheolar skeins following the method of Detinova.[11]

Laboratory observations

Effect of age on blood-feeding behavior. Three age groups (6–8, 16–18, and 26–28 days old) of uninfected colony *An. gambiae* Giles sensu strictu (pink-eye strain) were tested at the same time. Females were starved overnight before testing. Individual females were acclimatized for 10 min in cages, and offered a shaved, anesthetized mouse as a host. Behaviors were recorded and analyzed as described above. The recorder was unaware of the age of the individual being tested.

Effect of mosquito species on bloodfeeding behavior. Feeding behaviors of individuals from laboratory colonies of *An. gambiae* s.s. (pink eye strain) and *An. arabiensis* Patton (Ahero strain) were compared. Comparisons of the two species were made at the same time for each of three age groups; 7–8-, 12-, and 17–21-day old females. Procedures followed those described above.

Statistical analysis

Data were examined for normality of distribution and equality of variances among treatments. In most cases, the $\log_{10}$ transformation insured both that data were normally distributed and that variances were equal among treatments. In these cases, data were analyzed by one-way analysis of variance. When neither original data nor transformed data were normally distributed or when variance differed among treatments, untransformed data were analyzed with the nonparametric Kruskal-Wallis test. Distributional data were analyzed by the chi-square test of independence. Statistical methods followed those described by Sokal and Rohlf.[12]

RESULTS

Field observations

Forty-two percent (136 of 321) of the *An. gambiae* s.l. initiated probing when offered a hamster. Of the 136 *An. gambiae* s.l. that probed, 30 females contained sporozoites in their salivary glands, of which 26 infections were identified as *P. falciparum* by ELISA.

A significantly greater proportion of *P. falciparum*–infected *An. gambiae* s.l. (26 of 32) attempted to feed than did uninfected females (110 of 289; $\chi^2 = 22.01$, degrees of freedom [df] = 1, $P < 0.001$). Among mosquitoes that initiated probing, there was no difference in the proportion that imbibed blood between infected (24 of 26) and uninfected (96 of 110) mosquitoes ($\chi^2 = 0.51$, df = 1, $P < 0.05$). Among females that fed, there was no difference in the ratio of full to partial blood meals taken by infected (19:5) and uninfected (68:28) mosquitoes ($\chi^2 = 0.67$, df = 1, $P > 0.05$).

Frequency distributions of the number of probes made by infected and uninfected *An. gambiae* s.l. are shown in Figure 1. Approximately 65% of infected females probed at least three times compared with only 27% of uninfected females. Infected mosquitoes probed nearly twice as often as uninfected mosquitoes ($F = 11.20$, df = 1,134, $P < 0.01$) (Table 1). The frequency distributions of total probing time of individual infected and uninfected *An. gambiae* s.l. are shown in Figure 2. Infected *An. gambiae* s.l. probed an average of 276.8 sec compared with 214.1 sec for uninfected females (Kruskal-Wallis statistic = 4.84, $P < 0.05$; untransformed data) (Table 2).

There was no effect of intensity of sporozoite infection, as measured by grade, on the number

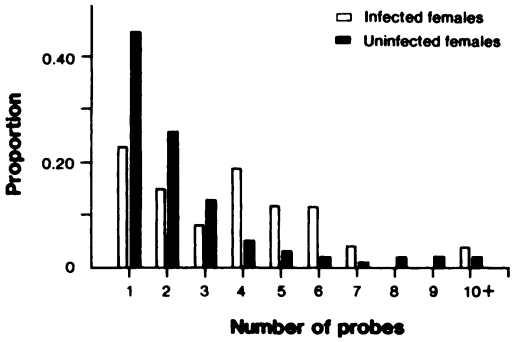

FIGURE 1. Frequency distribution (proportions) of the number of probes made by infected and uninfected *Anopheles gambiae* s.l.

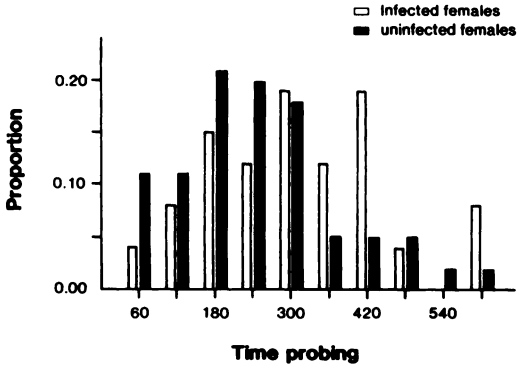

FIGURE 2. Frequency distribution (proportions) of the total probing time of infected and uninfected *Anopheles gambiae* s.l. Individual times were rounded to the nearest 60-sec interval (e.g., 1–60 sec = 60, 61–120 sec = 120, etc.).

of probes (Kruskal-Wallis statistic = 0.81, P = 0.847) or on total probing time (Kruskal-Wallis statistic = 3.23, P = 0.358) of infected *An. gambiae* s.l.

Parous state was determined for 252 uninfected *An. gambiae* s.l. There was no difference between nulliparous and parous females in the proportion that initiated probing (χ^2 = 0.34, df = 1, $P > 0.05$). There was also no difference between these two groups in the number of probes made (F = 0.45, df = 1,250, $P > 0.05$). Among females that probed, there was no difference between uninfected nulliparous and parous females in total probing time (F = 0.53, df = 1,95, $P >$ 0.05).

Only 44 *An. funestus* were tested. Of these, 15 (34%) attempted to feed, of which two were found to be infected, both by dissection and *P. falciparum* ELISA. The numbers of *An. funestus* were too small for meaningful analysis, but the trends were similar to those seen for *An. gambiae* s.l. Infected *An. funestus* probed more often and for a longer time than did their uninfected counterparts (Tables 1 and 2).

Laboratory observations

Effect of age on bloodfeeding behavior of *An. gambiae* s.s. There was no difference among age groups in either the number of probes (F = 1.22, df = 2,108, $P > 0.05$) or the total probing time (F = 0.02, df = 2,108, $P > 0.05$). Additionally, a 3 × 2 contingency table showed that there was no difference among age groups in the proportions probing and not probing (χ^2 = 3.35, df = 2, $P > 0.05$).

Effect of mosquito species on bloodfeeding behavior. Within the three age groups tested, there was no difference in total probing time between *An. gambiae* s.s. and *An. arabiensis* (age 7–8 days, Kruskal-Wallis statistic = 0.933, $P > 0.05$; age 12 days, F = 0.66, df = 1,33, $P > 0.05$; age 17–21 days, F = 0.29, df = 1,40, $P > 0.05$). For number of probes, there was no difference between the species within the 7–8-day age group (F = 0.38, df = 1,34, $P > 0.05$) and the 12-day age group (F = 0.05, df = 1,33, $P > 0.05$). However, there was a significant difference between

TABLE 1

Effect of Plasmodium falciparum *on the number of probes made by* Anopheles gambiae *s.l. and* An. funestus

Species	Salivary gland condition	n	No. of probes (mean ± SD)
An. gambiae s.l.	Infected	26	4.00 ± 0.68
	Uninfected	110	2.37 ± 0.21
An. funestus	Infected	2	3.50 ± 0.50
	Uninfected	13	2.62 ± 0.42

TABLE 2

Effect of Plasmodium falciparum *on the total probing time of* Anopheles gambiae *s.l. and* An. funestus

Species	Salivary gland condition	n	Probing time (sec) (mean ± SD)
An. gambiae s.l.	Infected	26	276.8 ± 26.4
	Uninfected	110	214.1 ± 11.9
An. funestus	Infected	2	238.0 ± 81.0
	Uninfected	13	207.1 ± 34.8

the species in the number of probes made by 17–21-day-old females ($F = 7.21$, $df = 1,41$, $P < 0.05$). *Anopheles arabiensis* females made an average of 2.13 probes compared with an average of 1.45 probes made by *An. gambiae* s.s.

DISCUSSION

Our data provide field evidence for the alteration of vector feeding behavior by a human pathogen. The results support laboratory evidence presented for several insect-parasite systems, including viruses,[13] bacteria,[14] and protozoa.[2-4, 15] Extended vector-host contact resulting from infection may be a common parasite-mediated mechanism that maximizes transmission success.

The mechanism responsible for the changes observed in blood-feeding behavior is not known. In *Aedes aegypti* (L.), salivary gland apyrase functions as an anticoagulant that minimizes the time needed to take a blood meal.[16, 17] Salivary apyrase activity is reduced in *P. gallinaceum*–infected *Ae. aegypti*, which increases the probing time of sporozoite-infected mosquitoes.[4] Salivary apyrase is also found in *Anopheles* vectors of human malaria,[18] so that the same mechanism may be responsible for increased host contact of infected *An. gambiae* s.l. Since there was no relationship in our study between intensity of sporozoite infection and bloodfeeding behavior, the mechanism that mediates these behavioral changes appears to be independent of parasite density.

Infected mosquitoes are old mosquitoes and it is possible that we were assessing the effect of age on blood feeding behavior and not the effect of malaria infection. This explanation is unlikely, however, since age had no effect on either the number of probes or total probing time of uninfected *An. gambiae* females in laboratory experiments. In addition, among wild uninfected *An. gambiae* s.l., there were no differences in feeding behavior between nulliparous females and parous females, the latter of which are usually older.

Anopheles gambiae Giles sensu strictu and *An. arabiensis* Patton are the only members of the *An. gambiae* complex that occur at our study site.[7, 8, 19] During the study period, *An. arabiensis* comprised 18% of 1,129 uninfected *An. gambiae* s.l. and 0% of 72 sporozoite-infected *An. gambiae* s.l. identified from Kisian (Copeland RS, unpublished data). Thus, of the wild mosquitoes examined in the present study, most or all of the infected *An. gambiae* s.l. were probably *An. gambiae* s.s. Our laboratory observations indicated minimal differences in feeding behavior between uninfected *An. gambiae* and *An. arabiensis*. Behaviors were the same in five of six comparisons. Only the oldest *An. arabiensis* probed more often than *An. gambiae* s.s. of the same age. If older, uninfected *An. arabiensis* probe more often in the wild than *A. gambiae* s.s., then inclusion of some *An. arabiensis* in the analysis of wild *An. gambiae* s.l. would artificially reduce real differences between uninfected and infected *An. gambiae* s.s.

Of those mosquitoes that initiated probing, similar proportions of wild infected and wild uninfected females fed successfully. When tested, however, confined mosquitoes were exposed to a single anesthetized host. Under natural conditions, irritation resulting from an immediate hypersensitivity reaction may induce a defensive host-response resulting in an abortive attempt by the mosquito to secure a blood meal.[6, 20] Thus, extended vector-host contact could result in multiple host contacts by infected females per gonotrophic cycle. This hypothesis could be tested in houses whose human inhabitants have different blood types, by comparing the proportions of mixed blood meals in infected and uninfected mosquitoes found resting indoors. A proportion of females is known to take multiple blood meals in nature,[21, 22] but the relative contributions of infected and uninfected females are unknown.

Malaria transmission by *Anopheles* may be enhanced both by an increase in vector contact with individual hosts and by multiple host contacts per gonotrophic cycle. If so, the current model of malaria transmission, which assumes a constant biting rate over the life of a mosquito, irrespective of infection,[23] would need to be revised.

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