Effect of Permethrin-Impregnated Nets on Exiting Behavior, Blood Feeding Success, and Time of Feeding of Malaria Mosquitoes (Diptera: Culicidae) in Western Kenya

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ABSTRACT The impact of permethrin-treated bednets on the feeding and house entering/exiting behavior of malaria vectors was assessed in two studies in western Kenya. In one study, matched pairs of houses were allocated randomly to receive bednets or no bednets. Exiting mosquitoes were collected in Colombian curtains hung around half of each house; indoor resting mosquitoes were collected by pyrethrum spray catches. The number of Anopheles gambiae Giles and An. arabiensis Patton estimated to have entered the houses was unaffected by the presence of bednets; Anopheles funestus Giles was less likely to enter a house if bednets were present. Anopheles gambiae and An. funestus were less likely to obtain a blood meal and significantly more likely to exit houses when bednets were present. No difference was detected in An. arabiensis rates of blood feeding and exiting. In a second experiment, hourly night biting collections were done on 13 nights during the rainy season to assess whether village-wide use of permethrin-treated bednets caused a shift in the time of biting of malaria vectors. A statistically significant shift was detected in the biting times of An. gambiae s.l., although the observed differences were small. No change was observed in the hourly distribution of An. funestus biting. Our study demonstrated that, at least in the short-term, bednets reduced human-vector contact and blood feeding success but did not lead to changes in the biting times of the malaria vectors in western Kenya.

KEY WORDS Anopheles gambiae complex, Anopheles funestus, bednets, behavior

The efficacy of insecticide-treated bednets and other materials in reducing morbidity and mortality due to malaria has been well documented (Alonso et al. 1991, Choi et al. 1995, Binka et al. 1996, Nevill et al. 1996). Insecticide-treated bednets likely act in three ways to reduce human-vector contact. First, intact nets provide a physical barrier to mosquitoes. Second, the insecticide has toxic effects on mosquitoes that attempt to feed (Miller et al. 1991, Curtis et al. 1996). Lastly, many insecticides such as permethrin have exito-repellent properties that affect the behavior of mosquitoes by reducing the rate of entry into houses and increasing the rate of early exit from houses (Lines et al. 1987, Miller et al. 1991). Although it is widely believed that untreated nets alone do not afford much protection against severe malaria to users (Snow et al. 1987, D’Alessandro et al. 1995), the relative importance of the toxic and repellent effects of insecticides such as permethrin is unclear. Studies have demonstrated that community wide use of permethrin-treated bednets and curtains cause a decline in parity and sporozoite rates as well as the overall vector population, indicating that mortality of vectors is an important mode of action for bednets (Magesa et al. 1991, Magbity et al. 1997, Cuzin-Ouattara et al. 1999). However, Miller et al. (1991) found that mortality of vectors within experimental huts did not increase in the presence of permethrin-treated nets compared with untreated nets. They concluded that permethrin-treated nets act largely through repellent effects, reducing the number of vectors that enter huts and increasing the number that exit before acquiring a blood meal (Miller et al. 1991).

Although reduced house entry and early exit may help to limit human-vector contact, mosquitoes also may respond to the use of insecticide treated bednets in ways that compromise their efficacy. Genetic changes in vector susceptibility to insecticides are particularly concerning (Chandre et al. 1999). However, changes in mosquito behavior also may reduce the efficacy of impregnated bednets. In a study in coastal Kenya, Mbogo et al. (1996) found that An. gambiae s.l. was more likely to bite earlier and outdoors in villages with bednets compared with villages without. A shift in the biting times or locations of malaria vectors potentially could undermine the effectiveness of insecticide-treated bednets.
Because vector behavior may influence the efficacy of permethrin-treated bednets, we initiated studies to determine how permethrin-treated bednets affected the rates of entry into and exit from houses by vector mosquitoes and if widespread use of bednets caused a shift in the biting times of malaria vectors.

Materials and Methods

Study Area. The study was conducted in Asembo Bay (34° 23’ E, 0° 11’ S), ~40 km west of Kisumu in western Kenya, where a community-wide efficacy trial of permethrin-treated bednets was ongoing. The Asembo Bay study area covers 200 km² and has a population of ~55,000 people, 95% of whom are of the Luo ethnic community. People live in traditional homesteads (compounds) consisting of one or more houses and surrounding agricultural fields. Houses usually are constructed of mud and wood, with a roof of grass thatch or iron sheets. The majority of residents farm maize, cassava, millet, and sorghum. Rainfall averages around 1,000 mm/yr with most occurring during the two annual rainy seasons that occur in March–June and November–December. The area has a high level of malaria transmission with the main vectors being An. gambiae s.s. and An. funestus Giles (Beier et al. 1990, Taylor et al. 1990).

In 1995, residents were recruited to participate in a study of the efficacy of permethrin-treated bednets in reducing all-cause child mortality. Villages were randomly allocated to receive bednets in the first round (bednet villages) or 2 yr later (control villages). Bednets were retreated to the target dose at approximately 6-mo intervals thereafter.

Biting Pattern. In December 1997 and January 1998, we assessed the impact of village-wide use of permethrin-treated bednets on vector biting times. Four compounds were selected, two from a bednet village and two from a control village. In each compound and upon informed consent, five volunteers slept under bednet traps outside the houses. Volunteers were provided prophylaxis as well as appropriate treatment if they presented with symptoms of malaria. Bednet traps were located outside houses to minimize the direct effects that bednets might have had on mosquito biting pattern in intervention villages. The traps remained in place from 1800 to 0700 hours. An experienced collector aspirated mosquitoes at one-hour intervals from the bednet traps. Hourly catches for each trap were placed in separate prelabeled paper cups and mosquitoes provided with sugar solution soaked in cotton pads until processed. Volunteers sleeping under the traps rotated each night among the twenty collection sites. Collections were suspended if rain fell at any time during the night.

Processing of Mosquitoes. In the laboratory, the mosquitoes were killed with chloroform and identified morphologically using the keys of Gilles and De Meillon (1968) and Gilles and Coetzee (1987). Abdominal stages were classified as fed, gravid, half-gravid or unfed. Mosquitoes were desiccated over anhydrous calcium sulfate and stored at room temperature until processed. For the study of house entering/exiting, all An. gambiae s.l. were identified to species by polymerase chain reaction (PCR) (Scott et al. 1993). A subset of An. gambiae s.l. from the study of mosquito biting times were identified to species.

Statistical Analysis. The total number of each species entering a house was estimated as the number collected inside by pyrethrum spray catch plus two times the number collected in the curtain. For all analyses, the number of mosquitoes collected in the Colombian curtain was doubled to account for those that exited on the sides not covered by the curtain. To estimate the deterrent effect on the total number of each species entering a house, a Wilcoxon signed-rank test was performed. The proportion of each species that exited during the night and the proportion that had successfully blood fed were analyzed using a paired t-test. Only pairs in which at least one mosquito was collected from each house were included for these analyses. Proportions first were transformed by the arcsine of the square root. For the proportions at the extreme ends of the range of possible values (i.e., near 0 and 1), the arcsine transformation was improved by replacing 0/n with 1/4n and n/n with 1–1/4n (Zar 1984). The differences between the arcsine-transformed proportions were tested for normality before performing a paired t-test. To estimate the effect of bednets on the biting times of mosquitoes, empirical distribution functions were calculated for biting times in bednet and control villages and a Kolmogorov–Smirnov test was used to compare the two distribution functions.
Anopheles gambiae

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portion exiting, proportion blood fed and proportion unfed

s.l., 159 An. gambiae (An. funestus 556 anopheline mosquitoes were captured; 337 were
Anopheles arabiensis

nets (enteredhouseswithbednetsthanhouseswithoutbed-
forquitoes entering houses was not signiﬁcantly different
of house entering behavior. The total number of mos-
species in relation to bednet use is shown in
summary of the average number of mosquitoes col-
collected, exiting behavior and blood feeding success of
proportion that failed to feed increased for both An. gambiae (t = 2.94, df = 21, P = 0.008) and An. funestus (t = 2.41, df = 27, P = 0.023). No difference was observed in the proportion of An. arabiensis that had failed to feed (t = 1.58, df = 5, P = 0.175).

Biting Pattern. Using bednet trap collections, a total of 17,112 anopheline mosquitoes was captured during 13 nights of sampling. In the intervention village, 10,341 An. gambiae s.l. and 697 An. funestus were collected, whereas 5,853 An. gambiae and 221 An. funestus were collected from the control village. A total of 1,588 An. gambiae s.l. was identiﬁed to sibling species, 1,201 from the intervention villages and 387 from the control villages. Proportions of An. gambiae were 73.6% from the intervention area compared with 79.6% from the control area (χ² = 1.2, df = 1, P = 0.253). The proportions of An. gambiae and An. arabiensis did not differ signiﬁcantly among times of biting for both the control (D = 0.040, P = 0.999) and intervention (D = 0.083, P = 0.124) areas. Because the ratios of An. gambiae to An. arabiensis over the times of biting were consistent in both the control and the intervention villages, numbers were pooled for An. gambiae s.l. for comparison between the two areas. The biting cycle in An. gambiae s.l. and An. funestus for both the inter-
vention and the control villages is shown in Fig. 1. There was a statistically signiﬁcant difference in the biting pattern of An. gambiae s.l. in the bednet village

<table>
<thead>
<tr>
<th>Table 1. Average number of mosquitoes entering houses, proportion exiting, proportion blood fed and proportion unfed</th>
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<tr>
<td>Houses with</td>
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<td>Anopheles gambiae</td>
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<td>No. entering house</td>
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<td>% exiting house</td>
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<td>% blood fed</td>
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*a See text for calculation of total number entering houses.

Results

House Entering/Exiting Behavior and Feeding Success. Sixty pairs of houses were sampled in which 556 anopheline mosquitoes were captured; 337 were An. funestus and 219 were An. gambiae s.l. Of the An. gambiae s.l., 159 An. gambiae and 42 Anopheles arabiensis Patton were identiﬁed using PCR, whereas 18 (8%) were unidentiﬁed and excluded from further analysis. Mosquitoes captured by pyrethrum spray catches were considered to be resting inside the house, whereas those captured by the Colombian curtains were considered to have exited the house. A summary of the average number of mosquitoes collected, exiting behavior and blood feeding success of each species in relation to bednet use is shown in Table 1.

All 60 pairs of houses were included in the analysis of house entering behavior. The total number of mosquitoes entering houses was not signiﬁcantly different for An. gambiae (S = 30.5, P = 0.591) or An. arabiensis (S = 2.5, P = 0.940). Signiﬁcantly fewer An. funestus entered houses with bednets than houses without bednets (S = 142.5, P = 0.045). For comparisons of proportion exiting and blood fed, only pairs of houses in which at least one mosquito was collected from each house were used in the analysis. There were 22 pairs available for the analysis of An. gambiae, six for the analysis of An. arabiensis, and 27 for the analysis of An. funestus. The proportion of mosquitoes exiting was signiﬁcantly higher from houses with impregnated bednets than from houses without bednets for both An. gambiae (t = 5.17, df = 21, P < 0.001) and An. funestus (t = 5.27, df = 26, P < 0.001). There was no difference in the proportion of An. arabiensis exiting (t = 0.42, df = 5, P = 0.695). In houses with permethrin-impregnated bednets, signiﬁcantly lower proportions of An. gambiae (t = 5.58, df = 21, P < 0.001) and An. funestus (t = 2.81, df = 26, P = 0.029) had successfully blood fed. No difference was detected in the proportion of An. arabiensis that successfully blood fed (t = 0.34, df = 5, P = 0.611). The proportion that failed to feed increased for both An. gambiae (t = 2.94, df = 21, P = 0.008) and An. funestus (t = 2.41, df = 27, P = 0.023). No difference was observed in the proportion of An. arabiensis that had failed to feed (t = 1.58, df = 5, P = 0.175).

Fig. 1. Frequency of biting by time for An. gambiae s.l. (A) and An. funestus (B). Solid lines indicate collections made in a bednet village while the dashed lines indicate collections made in a control village.
Discussion

The use of a matched design allowed us to assume that a similar number of blood seeking mosquitoes were available to enter both houses of each pair, thereby allowing us to make direct comparisons of house entering and exiting behavior. The presence of permethrin-treated bednets in households increased the rate of early exit of An. gambiae and An. funestus in western Kenya. Similar results have been found using experimental huts in The Gambia and Tanzania (Lines et al. 1987, Miller et al. 1991, Curtis et al. 1996). Anopheles funestus was less likely to enter homes that had bednets, but no impact was observed on the house entering behavior of An. gambiae as has been shown in some experimental hut trials (Lindsay et al. 1991, Miller et al. 1991). The demonstration of a deterrent effect in earlier trials was somewhat unexpected given the low vapor pressure of permethrin (Wells et al. 1986), which usually acts as a contact insecticide. Our study indicated that the deterrent effect may be due to early exit of mosquitoes rather than a decreased rate of entry.

Bednets also decreased the proportion of mosquitoes that successfully blood fed during the previous night. Experimental hut studies have shown a reduction in feeding success (Miller et al. 1991), and entomologic monitoring during intervention trials has shown a reduction in the proportion of indoor resting mosquitoes that were blood fed in bednet villages (Mbogo et al. 1996). It is not known what happens to mosquitoes that fail to blood feed and exit early. Those mosquitoes may shift to feeding on other hosts, although several studies indicated a shift in host selection due to bednets is unlikely (Magesa et al. 1991, Lindsay et al. 1993, Mbogo et al. 1996). There is some concern that mosquitoes exiting houses with bednets before feeding may simply move to the nearest house and feed on unprotected persons (Lindsay et al. 1991).

In community-wide trials of insecticide treated bednets, evidence suggests that divergence of mosquitoes is unlikely and that depressed mosquito populations benefit everyone, including those who do not use bednets (Magesa et al. 1991, Mbogo et al. 1996). However, in these trials, bednets were supplied free of charge to most residents. Under operational conditions, few people will purchase and use bednets, and the divergence of mosquitoes to nonusers may become a serious problem.

A statistically significant change in the biting times of An. gambiae s.l. was observed in the current study. However, the difference observed in the frequency of biting over time was very small and the large sample size likely accounted for the finding of a statistically significant difference. Only one study has demonstrated clear changes in the biting time of An. gambiae (Mbogo et al. 1996). Other studies have found either no evidence or inconclusive evidence of a shift in biting times (Magesa et al. 1991, Zoulani et al. 1994). Mbogo et al. (1996) suggested that their observations may have been due to changes in species composition rather than changes in mosquito behavior. Our study and a study in west Africa (Lemasson et al. 1997) showed no differences in the biting times of An. gambiae and An. arabiensis. Possibly, the presence of Anopheles merus Dönitz in coastal Kenya could account for the shift in biting times. Alternatively, the method of collection may influence the biting pattern of Anopheles mosquitoes. Our traps were set outdoors to avoid direct effects of permethrin on biting behavior.

Increased early biting could be induced in three ways. First, An. gambiae females have been shown to have a consistent diurnal pattern, with a peak of biting after midnight (Bockarie et al. 1996, Lemasson et al. 1997). This pattern is likely regulated by light, temperature and humidity but also the physiological status of mosquitoes (Clements 1963, Kloowden 1994). If females fail to obtain a blood meal during the previous night, they may be more likely to commence host seeking earlier in the night. Second, mosquitoes biting earlier are more likely to be nulliparous than those biting later (Bockarie et al. 1996). Adult survivorship may be reduced in areas with widespread bednet use (Magesa et al. 1991), altering the age structure toward younger females that may feed earlier in the night. Alternatively, changes in the genetic structure of local populations may shift to those that are likely to commence feeding early. However, a genetically determined behavioral change is likely very complex and may require many years of intensive bednet use for it to occur. Although no shift in biting times was observed, data from the current study indicated that a shift could potentially occur as mosquitoes are less likely to successfully obtain a blood meal and more likely to exit houses early. Also, bednets may induce a mass effect on mosquito populations (J.E.G., unpublished data), which would tend to bias the mosquito population toward nulliparous females that tend to feed earlier. The failure to observe such a shift indicates that the stimuli to commence host-seeking are not well characterized or that An. gambiae and An. funestus behavioral patterns are not as rigid as previously reported.

A potential flaw in our study design is that we did not account for mosquito mortality inside houses. If permethrin-treated bednets increase mosquito mortality, our estimates of exit rates and blood feeding success may be biased. However, we do not believe that bednets significantly increased the mortality of mosquitoes for three reasons. First, experimental hut trials in The Gambia did not show any difference in mosquito mortality in huts with permethrin-treated bednets compared with those with untreated bednets (Miller et al. 1991). Second, the entry rate in houses with bednets was similar to that of houses without bednets, at least for An. gambiae and An. arabiensis.
Lastly, our observations indicated that the mortality rate of mosquitoes in houses is very low, whether a bednet is present or not (J.E.G., unpublished data).

In summary, bednets reduce the blood feeding success and increase the rate of early exit of malaria vectors. No effect was seen on the nocturnal biting pattern of either An. gambiae s.l. or An. funestus. Thus, bednets reduce the degree of human-vector contact and malaria transmission, and malaria vectors are unlikely, at least in the short-term, to shift their biting times to periods before people retire to bed.

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