IMPACT OF PERMETHRIN-TREATED BED NETS ON ENTOMOLOGIC INDICES IN AN AREA OF INTENSE YEAR-ROUND MALARIA TRANSMISSION

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Abstract.   The effect of permethrin-treated bed nets (ITNs) on malaria vectors was studied as part of a large-scale, randomized, controlled trial in western Kenya. Indoor resting densities of fed *Anopheles gambiae s.l.* and *An. funestus* in intervention houses were 58.5% ($P = 0.010$) and 94.5% ($P = 0.001$) lower, respectively, compared with control houses. The sporozoite infection rate in *An. gambiae s.l.* was 0.8% in intervention areas compared with 3.4% ($P = 0.026$) in control areas, while the sporozoite infection rates in *An. funestus* were not significantly different between the two areas. We estimated the overall transmission of *Plasmodium falciparum* in intervention areas to be 90% lower than in control areas. Permethrin resistance was not detected during the study period. As measured by densities of *An. gambiae s.l.*, the efficacy of bed nets decreased if one or more residents did not sleep under a net or if bed nets had not been re-treated within six months. These results indicate that ITNs are optimally effective if used every night and if permethrin is reapplied at least biannually.

INTRODUCTION

Insecticide-treated bed nets (ITNs) or curtains have a profound impact on malaria transmission in experimental trials in sub-Saharan Africa, as shown by reduction of various entomologic indices,[1–4] and more importantly, by diminution of morbidity and mortality in humans.[5–8] However, the efficacy of ITNs in areas of intense, perennial transmission has never been assessed. Therefore, a community-based, randomized, controlled trial of permethrin-treated bed nets was initiated in western Kenya.[9,10] Malaria transmission in this area is intense-residents receive up to 300 infectious bites per person per year- and occurs year round, with peaks just after the two annual rainy seasons (Hawley WA, unpublished data).[11]

As part of the efficacy trial, we assessed some standard entomologic parameters: the number of indoor resting mosquitoes, house exiting behavior, the proportion of mosquitoes infected with *Plasmodium falciparum* sporozoites, and insecticide resistance. We also assessed the effect of several human factors on the number of blood fed mosquitoes found resting indoors. The most important of these is adherence, which is whether the individual owning the net actually slept under a properly deployed net during the night before resting mosquitoes were collected. We also measured the impact of the number of bed nets in houses and the time since re-treatment of nets with insecticide on numbers of fed mosquitoes.

MATERIALS AND METHODS

Study site. The Asembo study site lies on the northern shore of Lake Victoria, 50 km west of Kisumu town in western Kenya. The area encompasses 200 km^2 characterized by gently rolling hills (elevation = 1,080–1,230 meters) that are drained by several small streams. Total annual rainfall since 1990 has averaged around 1,400 mm per year. Rainfall occurs year-round with the heaviest rains in March to April and with a second, smaller peak in November and December. Malaria is holoendemic in the region and estimates of entomologic

inoculation rates have ranged from 0 to 5 infectious bites per person per night with an average of between 0.65 and 0.79.[11–13] The primary vectors are *Anopheles gambiae*, *An. arabiensis*, and *An. funestus*.[11] *Plasmodium falciparum* is the primary species of human malaria in the region.[11,14]

Asembo is divided into 79 villages largely defined by clan relationships. Most inhabitants of Asembo live in traditional houses with mud walls and thatched roofs. The eaves of most houses are open, allowing for unimpeded entry and exit of mosquitoes. Family compounds, consisting of one or more houses, are separated from each other by surrounding farmland. Most of the population practices subsistence agriculture, with the main crops being maize, millet, cassava, and groundnuts. In August 1996, 40 of the 79 villages in Asembo were randomly selected by public lottery to receive bed nets. In November and December 1996, bed nets (polyester, 156 denier; Siamdutch Mosquito Netting Co., Bangkok, Thailand) were distributed to cover all sleeping spaces in the intervention villages. Bed nets were pretreated with permethrin (Peripel®; AgrEvo, Berlin, Germany) at a dose of 500 mg/m^2 and re-treated at approximately six-month intervals. Details of the study area and design are provided by Phillips-Howard and others.[9,10]

Entomologic surveillance. Mosquito collections were conducted in conjunction with quarterly adherence monitoring,[15] beginning in March 1997, three months after the distribution of bed nets to intervention villages. Houses for sampling were selected by two-stage cluster sampling. Each quarter, 10 intervention and 10 control villages were randomly selected with a probability proportional to size and, within each village, one house was randomly selected for sampling. The nine nearest neighbors were also included in the sample. Additional houses were sampled when time permitted while fewer than 10 houses were sampled if the collections were not completed by 10:00 AM. Observations were made in 853 houses from 40 intervention villages and 682 houses from 36 control villages. Thus, samples were taken from 76 of 79 villages. Between 4:30 AM and 6:00 AM, houses were visited to observe ITN use by the residents. The number of bed nets and people

sleeping in the house was recorded, along with the number of people directly observed to be sleeping under bed nets. The entomology team followed-up the adherence monitoring with a pyrethrum spray collection (PSC) between 7:00 AM and 10:00 AM. Briefly, white sheets were laid upon the floor and over the furniture within the house. Two collectors, one inside the house and one outside, sprayed around the eaves with 0.025% pyrethrum emulsifiable concentrate with 0.1% piperonyl butoxide in kerosene. The collector inside the house then sprayed the roof and walls. The house was closed for 10–15 minutes, after which dead mosquitoes were collected from the sheets and transferred to the laboratory on moist filter paper inside petri dishes.

Evaluation of house exiting rates. Since PSC collections may be biased by differential exit rates,[16–18] we tested the hypothesis that blood fed mosquitoes are more likely to exit households with ITNs compared with those without ITNs. Compounds were selected that had two houses that could be matched based upon size, construction, and the number of occupants. One house was randomly selected to receive bed nets. On the night before collections were made, a Colombian curtain[19] was hung around half of each house so that mosquitoes could enter and exit on two sides while exiting mosquitoes would be captured on the remaining two sides. Exiting mosquitoes were collected from the curtain at midnight and just before dawn. Indoor resting mosquitoes were collected from inside the house the next morning by PSC.

Laboratory processing. All anophelines were identified and sorted by abdominal status (fed, unfed, gravid, half-gravid). Mosquitoes were then placed individually in tubes and desiccated over calcium sulfate. Dried mosquitoes were tested for the presence of *P. falciparum* sporozoite antigen by enzyme linked immunosorbent assay (ELISA).[20] Due to constraints on time and personnel, a random sample accounting for 65% of all anophelines collected was selected for testing. A sample of *An. gambiae s.l.* was identified to species by a polymerase chain reaction (PCR) as *An. gambiae s.s.* or *An. arabiensis*.[21]

Insecticide susceptibility/resistance. During the rainy seasons of 1997, indoor resting mosquitoes were collected using hand aspirators from intervention and control houses for resistance monitoring. Because so few mosquitoes were collected from intervention houses, fed and gravid mosquitoes were allowed to lay eggs and assays were performed on females of the F_1 generation using World Health Organization test kits.[22] When adult females of the F_1 generation were three days old, they were exposed to 0.25% permethrin test paper for periods ranging from 10 to 90 minutes. Each test paper was used no more than four times and permethrin concentrations were confirmed by gas chromatography on every third paper used. At least 150 mosquitoes were assayed from intervention and control areas. After exposure, mosquitoes were transferred to paper cups and provided 5% sugar solution. Delayed mortality was recorded at 24 hours post-exposure. Similar procedures were performed using untreated paper to act as controls. Results were discarded if mortality was > 20% in the controls.
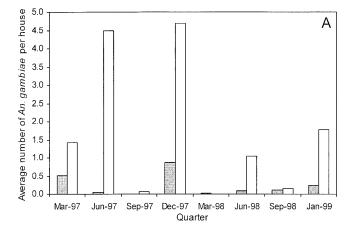
Bioassays. Bioassays were performed on a random sample of ITNs at approximately three-month intervals to assess the killing efficacy of the insecticide residues on a laboratory colony of *An. gambiae s.s.* with known susceptibility to permethrin. A bioassay cone[23] was attached to one side of the net

and mosquitoes were introduced into the cone. Mosquitoes were exposed for three minutes and then removed to paper cups. The mosquitoes were provided with sugar water and delayed mortality was assessed at 24 hours post-exposure. Two replicates with 10 mosquitoes per replicate were done on each net. During each bioassay, a similar procedure was performed twice on an untreated net to serve as a control. If control mortality was > 20%, results from the bioassays were discarded. At baseline and again after the first round of redipping, 10 nets were randomly selected and insecticide residues measured by gas chromatography on 10 cm × 10 cm samples from the side and top of each net. All gas-liquid chromatographic analyses were performed by extracting samples in 10 mL of 0.3% didecylphthalate in chloroform and agitating for 30 minutes. One microliter of extract was injected into a gas-liquid chromatography system using a megabore capillary column of fused-silica quartz and a flame ionization detector. For quantification, relative responses were compared with internal standards.[24]

Statistical analysis. The number of fed indoor resting mosquitoes in intervention houses was compared with the number of fed indoor resting mosquitoes in control houses using repeated measures Poisson regression. The analysis used an exchangeable correlation structure that assumed correlations were the same among all pairs of houses within the same village and quarter of collection. Separate univariate analyses were done using the same methods to assess how the number of ITNs within a house, the time since last re-treatment (or initial bed net distribution), and adherence of the residents (defined as the number of household residents who slept under their ITNs the night before the collection was made) affected the number of indoor resting mosquitoes. Due to the high colinearity among variables ($P < 0.001$ by Pearson's correlation analysis for all comparisons), a single multivariate analysis was not performed. The proportion of *An. gambiae s.s.* versus *An. arabiensis*, the exit rate of blood fed mosquitoes, and the sporozoite infection rates in intervention and control areas were compared by a chi-square test.

RESULTS

A total of 5,053 mosquitoes were obtained from 1,535 houses. Culicine mosquitoes accounted for 61.8% of all mosquitoes collected. Most of these were *Culex quinquefaciatus* Say. *Anopheles gambiae s.l.* accounted for 28.1% of all mosquitoes collected, while the remaining 10.1% were *An. funestus*. Of 165 specimens identified by PCR, 73 (44%) were *An. gambiae s.s.,* the remainder were *An. arabiensis*. Since unfed, gravid, and half-gravid mosquitoes were unlikely to have fed the previous night, the analyses of the effect of bed nets on indoor resting densities for *An. gambiae s.l.* and *An. funestus* included only fed mosquitoes. A total of 613 fed *An. gambiae s.l.* and 198 fed *An. funestus* were captured over the two-year period. The average numbers of fed *An. gambiae s.l.* and *An. funestus* per house in intervention and control houses during each of the eight quarters are shown in Figure 1. Overall, the number of fed anophelines captured indoors was 71.5% lower in intervention villages than in control villages ($P < 0.001$). Densities of fed *An. gambiae s.l.* were 58.5% lower ($P = 0.010$), while densities of fed *An. funestus* were 94.5% lower ($P < 0.001$) in intervention houses compared with control houses. The results were similar when the total number of
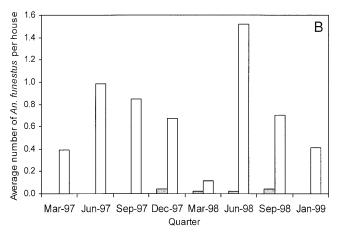
FIGURE 1. Indoor resting densities of fed **A**, *Anopheles gambiae s.l.* and **B**, *An. funestus* by quarter. Shaded bars represent intervention houses and open bars represent control houses.

quefaciatus was not significantly affected by the presence of ITNs (reduction = 24.3%; P = 0.433).

Poisson regression was used to model separately the effects of the number of ITNs in houses, the time since insecticide re-treatment, and adherence of house residents on indoor resting densities of fed mosquitoes. For each model, the number of indoor resting mosquitoes in control houses was the referent. The results for *An. gambiae s.l.* and *An. funestus* are shown in Table 1. The percent reduction was calculated as 1 minus the relative risk as estimated by Poisson regression. For *An. gambiae s.l.*, adherence, the time since re-treatment and the number of ITNs within a house had a strong effect on the degree of reduction of indoor resting densities. In intervention houses, if everyone in the house used an ITN the previous night, the number of indoor resting fed *An. gambiae s.l.* was reduced 77.8% compared with control houses ($P < 0.001$). If at least one person, but not all, used an ITN the previous night, there were 23.8% fewer *An. gambiae s.l.* compared with control houses (P = 0.498). If no one in the house used an ITN the previous night, the average number of *An. gambiae s.l.* was only 5.2% lower than the average number found in control houses (P = 0.907). The time since last re-treatment also affected indoor resting densities of *An. gambiae s.l.* The reduction in indoor resting densities compared with control houses was 82.0% within 90 days of re-treatment ($P < 0.001$), 63.4% between 90 and 180 days after re-treatment (P = 0.077), and only 16.9% more than 180 days (maximum time = 215 days) after re-treatment (P = 0.694). Increasing numbers of ITNs within a house resulted in fewer *An. gambiae s.l.* captured by PSC. Compared with control houses, indoor resting densities of *An. gambiae s.l.* were 77.0% lower in houses with three or more nets ($P < 0.001$), 62.9% lower in houses with two nets (P = 0.014), and 42.6% lower in houses with one net (P = 0.120).

Similar patterns were observed for *An. funestus*, but the effect of reduced adherence was much less pronounced. For houses in which all residents had slept under their bed nets the previous night, the reduction in indoor resting *An. funestus* compared with control houses was 97.0% ($P < 0.001$). In houses in which at least one person had slept under their bed net, the reduction was 96.9% (P = 0.008). If no one used their

mosquitoes (including gravid, half-gravid, and unfed mosquitoes) was included in the analysis, although the total number of *An. gambiae s.l.* was more strongly affected by the presence of ITNs (reduction = 71.0%; $P < 0.001$) than the number of fed *An. gambiae s.l.* The number of indoor resting *Cx. quin-*

TABLE 1

Univariate poisson regression analyses of indoor resting densities of fed *Anopheles gambiae s.l.* and *An. funestus* with living in an intervention village, adherence with insecticide-treated bed net (ITN) use, time since insecticide re-treatment, and the number of ITNs in the house as predictor variables.*

	An. gambiae s.l.		An. funestus	
	% Reduction	P	% Reduction	P
Residence in intervention village	58.5	0.010	94.5	<0.001
Adherence of house residents (number sleeping under an ITN) during the previous night				
All in house	77.8	<0.001	97.0	<0.001
At least one person	23.8	0.498	96.9	0.008
No one in house	5.2	0.907	82.2	0.031
Time since treatment (days)				
<90	82.0	<0.001	98.9	<0.001
90–180	63.4	0.077	96.9	<0.001
>180	16.9	0.694	84.2	0.001
Number of ITNs in house				
1	42.6	0.120	92.4	<0.001
2	62.9	0.014	95.0	<0.001
≥3	77.0	<0.001	100.0	†

* All comparisons are made to the number of fed mosquitoes in control houses.
† The poisson regression model failed to converge because no *An. funestus* were captured in houses with ≥3 ITNs. Estimates of the % reduction in houses with 1 or 2 nets were obtained by a poisson regression in which houses with ≥3 ITNs were excluded.

bed net in the house the previous night, there were still 82.2% ($P = 0.031$) fewer *An. funestus* compared with control houses. The time since bed nets were re-treated also had little effect on the degree of reduction of indoor resting densities of *An. funestus*. Compared with control houses, there were 98.9% fewer *An. funestus* in houses with ITNs that had been re-treated less than 90 days before the date of collection ($P < 0.001$), 96.9% fewer in houses with bed nets that had been re-treated 90–180 days before the date of collection ($P < 0.001$), and 84.2% fewer in houses with bed nets that had been re-treated more than 180 days (maximum time = 215 days) before the date of collection ($P = 0.001$). Increasing the number of nets within a house reduced the number of *An. funestus*, with none captured in houses with three or more nets. However, the presence of at least one ITN reduced the numbers of this species by more than 90% compared with control houses.

Exiting rates of blood fed mosquitoes were not significantly different in houses with ITNs compared with houses without them for either *An. gambiae s.s.* (0% versus 14.5%; $\chi^2 = 1.00$, $P = 0.316$) or *An. arabiensis* (25% versus 14.3%: $\chi^2 = 0.39$, $P = 0.348$). However, blood fed *An. funestus* were significantly more likely to exit houses with bed nets than those without bed nets (33.3% versus 4.3%: $\chi^2 = 14.3$, $P < 0.001$). Based upon a subset of *An. gambiae s.s.* identified to species by a PCR, the proportion of *An. gambiae s.s.* was higher in intervention villages compared with control villages, but this difference was not statistically significant ($P = 0.192$). In intervention houses, significantly fewer *An. gambiae s.l.* were found infected with *P. falciparum* sporozoites (0.8% versus 3.4%; $P = 0.026$). The sporozoite rate in *An. funestus* was higher in intervention villages (5.0%) than in control villages (3.0%). However, only 20 *An. funestus* from intervention villages were tested for the presence of *P. falciparum* sporozoite antigen and the difference was not statistically significant ($P = 0.624$). The sporozoite rate for all species combined was 1.2% in intervention villages compared with 3.4% in control villages ($P = 0.054$).

Multiplying indoor resting densities of fed mosquitoes by the sporozoite rates is not a true entomologic inoculation rate, but does provide a relative assessment of transmission intensity in intervention and control areas. Based upon these calculations, we estimated that transmission by *An. gambiae s.l.* was reduced by 91.8% while transmission by *An. funestus* was reduced by 90.6%. Overall, transmission of *P. falciparum* in intervention villages during the two-year period was estimated to be 90.9% lower than in control villages.

Bioassays with *An. gambiae s.s.* indicated that bed nets impregnated with permethrin less than six months before the test were effective in killing an average of 86.2% (range = 48–100%) of mosquitoes. The killing effect decreased to 55.4% (range = 14–95%) in bed nets that had been treated 6–8 months previously. Analysis by gas chromatography after the first redipping indicated our re-treatment procedure attained permethrin doses of approximately 500 mg/m² on all bed nets examined.

The degree of insecticide resistance was evaluated during the first year of intervention using probits to model the relationship between exposure time and mortality and F_1 females. The exposure time required to achieve 50% mortality (LT_{50}) was estimated to be less than 10 minutes for mosquitoes collected in either control or intervention villages.

DISCUSSION

There was a clear impact of ITNs upon the indoor resting densities of both *An. gambiae s.l.* and *An. funestus*. Overall, there was a 71.5% reduction in the indoor resting densities of fed anopheline mosquitoes in intervention areas compared with control areas. Studies in The Gambia,[1] Burkina Faso,[4] and coastal Kenya[2,3] have demonstrated reductions in indoor anopheline densities by more than 80% in the presence of ITNs or curtains. Comparisons with other studies are often difficult due to the different sampling methods used in each. Estimated changes in mosquito abundance or biting rates often differ based upon the method of collection. In The Gambia and Sierra Leone, studies have demonstrated clear reductions in indoor resting densities in houses with ITNs based upon PSC, while comparisons based upon light trap or human bait collections indicated little or no change in the anopheline densities.[1,25–28] Similarly, indoor resting densities in coastal Kenya decreased in intervention houses, but outdoor biting rates actually increased in intervention villages after bed nets were introduced.[2] Indoor collections may be biased estimates of mosquito population sizes in areas using ITNs because the exito-repellancy of permethrin may cause mosquitoes to exit houses early,[16–18] while outdoor collections may be affected by the presence of members of the *An. gambiae* complex that do not normally enter houses and feed on humans. Comparisons were also difficult since we elected to include only fed mosquitoes in the analysis, whereas other studies have included mosquitoes in all abdominal stages. When gravid, half-gravid, and unfed mosquitoes were included in the analysis, the impact upon indoor resting *An. gambiae s.l.* was comparable to that observed in other studies (reduction = 71%; $P < 0.001$).

The ITNs did not affect all mosquito species to the same degree, with *An. funestus* the most sensitive and *Cx. quinquefaciatus* the least sensitive. *Anopheles funestus* has been noted to be very susceptible to chemical control, and spraying campaigns have reported apparent eradications of this species from small foci.[29] Similarly, this species was most strongly affected by the presence of ITNs, although we cannot discount the possibility that our estimates of *An. funestus* in intervention houses were biased by the higher exit rate from houses with ITNs. In contrast to previous studies,[16–18] Mathenge and others[30] found that *An. gambiae s.s.* was equally likely to enter houses with ITNs compared with those without them, although this species was more likely to exit before obtaining a blood meal. In the current study, we did not find any evidence of an increase in the exit rates of blood fed *An. gambiae s.s.* from houses with bed nets. These observations suggest that *An. gambiae s.s.*, unlike *An. funestus*, is affected by the insecticide only at very close ranges. Similar to observations in coastal Kenya[3] and Tanzania,[31] bed nets had little impact on indoor resting densities of *Cx. quinquefaciatus*. Field and laboratory studies have demonstrated this species to be less susceptible to permethrin than anopheline mosquitoes.[32,33]

A change was also noted in the sporozoite rates of anophelines collected in intervention areas. The overall sporozoite rate was 3.4% in control villages and 1.2% in intervention villages. Most of the change in sporozoite rates was due to a reduction in the sporozoite rate in *An. gambiae s.l.* Only 20 specimens from intervention areas were captured for testing for sporozoite antigen and data were insufficient to

evaluate the effect of bed nets on the sporozoite rate in *An. funestus*. Changes in the sporozoite or parity rates have been interpreted as evidence that ITNs have a community level effect upon the vector population. Gimnig and others[34] demonstrated that indoor biting rates were lower in control houses located near intervention villages compared with control houses located further from intervention villages, suggesting that ITNs caused a reduction in the vector population in and around intervention villages. The decrease in sporozoite rates also suggests that widespread use of ITNs in the Asembo Bay study area had an impact on the vector population.[34]

By multiplying the number of fed indoor resting mosquitoes by the sporozoite rates, we were able to estimate the relative transmission intensity for intervention and control areas. For *An. gambiae s.l.*, we estimated a decrease of 91.8% in the number of infected, indoor resting females that had fed the night before. We estimated a 90.6% decrease in the number of infected, indoor resting *An. funestus* that had fed the night before. Overall, we estimated that ITNs reduced transmission by more than 90% in the Asembo study area.

The linking of entomologic surveillance data with adherence monitoring allowed us to assess how adherence, the time since redipping, and the number of nets within a house affect the efficacy of ITNs against vector mosquitoes. Adherence, the time since redipping, and the number of ITNs within a house all had a significant impact on indoor resting densities of fed *An. gambiae s.l.* Experimental hut trials in The Gambia indicated that when an unprotected person slept in a house with another person who was using an ITN, that person received fewer mosquito bites than they would have if they had been alone, suggesting that the presence of a treated bed net may confer protection to the occupants of a house.[16] While this appeared to be true for *An. funestus*, there was no difference in the indoor resting densities of *An. gambiae s.l.* in houses with ITNs that were not in use compared with control houses. This indicates that proper and consistent use of ITNs is necessary to receive their full benefit. Houses in which bed nets had been treated more than six months before the collection date had similar numbers of *An. gambiae s.l.* compared with controls. The dose response of decreasing mosquito densities with increasing numbers of nets was somewhat unexpected. Although houses with more nets had more total insecticide, there were usually more people in those houses who would be expected to attract more mosquitoes. Separate analyses indicated a weak, non-significant trend of increasing numbers of *An. gambiae s.l.* with increasing numbers of residents in control houses. However, this trend was reversed in houses with ITNs, suggesting that the repellent effects of the insecticide had a stronger effect on indoor resting densities than the attractive volatiles given off by large numbers of people. The effect of increasing numbers of ITNs is also likely modulated by the size of the house. In larger houses, the nets and insecticide are less concentrated, and increasing the number of nets within larger houses might have a less pronounced effect on *An. gambiae s.l.* indoor resting densities. The number of indoor resting *An. funestus* was lower in intervention houses whether or not people were compliant and whether or not the bed nets had been treated recently. *Anopheles funestus* was also strongly affected by ITNs if at least one was present within the house. These observations indicate that the effectiveness of bed nets may be determined not only by the willingness of people to treat their bed nets and sleep under them, but also on the vector species present in a given area. In areas where *An. funestus* is the predominant vector, the mere presence of bed nets may be enough to significantly reduce transmission whereas, in areas where *An. gambiae s.l.* is the predominant vector, steps must be taken to ensure that bed nets are re-treated every six months and that people deploy them regularly.

Assessment of insecticide susceptibility/resistance was possible during only the first study year, since very few mosquitoes were collected in intervention areas during the second year. Vulule and others[35] found evidence for reduced susceptibility to permethrin in a small study less than 50 km from the Asembo study area, although subsequent studies in the same area suggested that selection for permethrin tolerance by treated bed nets was minimal.[36] The LT_{50}s were less than 10 minutes for mosquitoes collected from control and intervention houses. This is lower than the LT_{50} of 13 minutes found at the baseline of the study conducted by Vulule and others, indicating high susceptibility in the first year.[35] Insecticide use in this area, for either public health or agriculture, was low prior to this study and selection for resistance was minimal. The low numbers of indoor resting adult anophelines in intervention houses during the second year of the study suggest that resistance levels remained low. However, continued monitoring is necessary to ensure that permethrin remains effective against anophelines in this area.

This study has shown that ITNs significantly reduce indoor resting densities of malaria vectors and sporozoite rates in an area of intense, perennial transmission. Permethrin tolerance of malaria vectors was low. However, this study also demonstrated that ITNs are less effective if not used consistently and if not re-treated regularly. Malaria control programs that incorporate permethrin-treated bed nets as an integral part of a malaria control strategy must take steps to educate communities on the need to use ITNs every night and to re-treat their nets appropriately.

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