# EFFECT OF PERMETHRIN-TREATED BED NETS ON THE SPATIAL DISTRIBUTION OF MALARIA VECTORS IN WESTERN KENYA

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Abstract. The effect of insecticide (permethrin)-treated bed nets (ITNs) on the spatial distribution of malaria vectors in neighboring villages lacking ITNs was studied during a randomized controlled trial of ITNs in western Kenya. There was a trend of decreased abundance of Anopheles gambiae with decreasing distance from intervention villages both before (P=0.027) and after (P=0.002) introduction of ITNs, but this trend was significantly stronger after ITNs were introduced (P=0.05). For An. funestus, no pre-intervention trend was observed (P=0.373), but after the intervention, a trend of decreased abundance with closer proximity to intervention compounds developed (P=0.027). Reduction in mosquito populations in villages lacking ITNs was most apparent in compounds located within 600 meters of intervention villages. Sporozoite infection rates decreased in control areas following the introduction of ITNs (P<0.001) for both species), but no spatial association was detected between sporozoite rates and distance to nearest intervention village. We conclude that high coverage of ITNs is associated with a community-wide suppression of mosquito populations that is detectable in neighboring villages lacking ITNs, thereby affording individuals residing in these villages some protection against malaria.

### INTRODUCTION

Insecticide-treated bed nets (ITNs) are effective in reducing morbidity and mortality due to malaria in sub-Saharan Africa. 1-5 Entomologic monitoring during trials of ITNs has demonstrated reductions of entomologic inoculation rates by 78–95% in various African settings. 6-9 While ITNs cause mortality in many vector species, they also act as irritants or repellents, deterring some mosquitoes from entering houses and causing early exit of others. 10 It has been suggested that the primary mode of action for ITNs is to discourage mosquitoes from entering houses. 11 However, the effect of permethrin may follow a dose response: at higher concentrations mosquitoes are deterred from entering houses while at lower concentrations, mosquitoes are more likely to enter houses and acquire a lethal dose of insecticide. 12

In community-wide intervention trials, bed nets and insecticide have been provided without cost to ensure high coverage in intervention groups. In operational programs, coverage may be lower. In areas of sub-Saharan Africa where ITNs are available, the cost of nets and insecticide is often cited as a major barrier to the uptake and maintenance of insecticide treated nets. <sup>13–15</sup> In The Gambia <sup>16</sup> and coastal Kenya, <sup>17</sup> retreatment rates decreased considerably when people had to pay for insecticide. Social and cultural factors may also affect the level of ITN use in a community. Therefore, in practice, coverage of ITNs may be incomplete.

Incomplete coverage of ITNs could have several effects on mosquito distribution and behavior. Mosquitoes may be diverted from houses with ITNs to those without them, thus increasing malaria transmission and disease risk among people without ITNs. 18 It has been suggested that this may have occurred in trials of untreated bed nets and that observed differences in malaria morbidity were due to increased infections in unprotected persons rather than a decrease in morbidity among bed net users. 19 Alternatively, the use of ITNs may act to depress the population of vector mosquitoes in large areas. If large enough, this community effect would provide some protection to everyone living in a given region

including those who do not possess ITNs. Evidence of a community effect in mosquito populations has been observed on the Kenyan coast where compounds located within 400 meters of an intervention village had significantly fewer mosquitoes than compounds further away.<sup>20</sup> Studies in Tanzania and Burkina Faso suggested that ITNs or curtains reduced vector survival and that unprotected persons sleeping indoors or outdoors within intervention villages experienced fewer mosquito bites than persons sleeping in control villages.<sup>6,8</sup> In two studies, these community effects had a measurable impact on child morbidity<sup>21</sup> and mortality.<sup>22</sup> However, several studies in The Gambia indicated no evidence for a community effect.<sup>23–25</sup>

In this report, we used a geographic information system to test the hypothesis that widespread ITN use affects the distribution and abundance of mosquitoes in adjacent areas without ITNs. Specifically, we tested whether control compounds that neighbor intervention villages experience a change, whether an increase or decrease, in the abundance of blood fed malaria vectors found inside houses. Through repeated measures of the vector population and precise measurements of household locations, we determined the degree to which abundance of malaria vectors changed as a function of distance from houses with ITNs.

# MATERIALS AND METHODS

**Study site.** Asembo Bay is located in Bondo district (formerly Siaya district) 50 km west of Kisumu in western Kenya along the shores of Lake Victoria. The study area encompasses an area of approximately 70 km<sup>2</sup> of gently rolling hills (elevation = 1,080–1,230 meters) drained by several small streams. The region experiences a bimodal pattern of rainfall, with the heaviest rains falling from March through May and with a smaller peak occurring in November and December. The predominant malaria vectors in the area are *Anopheles gambiae* and *An. funestus*. Sporozoite infection rates vary from 5% to 10% and entomologic inoculation rates have been estimated to be between 0.65 and 0.79 infectious bites per

person per night.<sup>26–28</sup> The primary species of human malaria in the region is *Plasmodium falciparum*.<sup>26,29</sup>

The study population consisted of approximately 17,000 inhabitants, most of whom are members of the Luo ethnic group. The population of the study area is dispersed among approximately 2,500 family compounds distributed throughout the study area. Compounds are defined as one or more houses (usually 3-5 houses) and surrounding farmland. Most houses are constructed with stick and mud walls and a thatched roof. Smaller numbers are constructed with brick walls and/or corrugated tin roofs. Most inhabitants practice subsistence farming with maize the staple crop. Family compounds in the study area were grouped into 19 villages ranging in size from 46 to 242 compounds and 225 to 1,714 inhabitants. In July 1995, this area was enrolled as part of a largescale trial of ITNs and, in December 1996, enough ITNs (Siamdutch Mosquito Netting Co., Bangkok, Thailand) were distributed to cover all sleeping places (beds or mats) in 9 of 19 villages. Villages receiving ITNs (intervention villages) were assigned by lottery; the 10 villages that did not receive ITNs in December 1996 (control villages) were given nets in early 1999. Bed nets were pre-treated with 0.5 g of permethrin/m<sup>2</sup> of netting and were re-treated with that target dose approximately every six months.

**Geographic information system.** The mapping of the study area using a differential global positioning system (GPS) has been described in detail elsewhere. Briefly, all family compounds, market areas, schools, health facilities, and potential mosquito breeding sites (dams and burrow pits) were mapped using carrier phase GPS processing with differential correction. All roads and streams were mapped using mobile point GPS processing. Mapping was done with Magellan (Magellan System Corp., San Dimas, CA) Pro Mark X GPS units. A

base station was constructed at the Kenya Medical Research Institute field station in Kisian, 10 km west of Kisumu. The position of the base station was estimated by taking the average of several readings. The base station took readings every second throughout the day and saved them into a control file. At the end of each day, control files and remote files were downloaded to a personal computer for post-processing. Postprocessing of point and mobile data was done using Magellan software to calibrate positions of the remote files. Points from mobile files then were replaced with smoothed lines using AutoCAD (Autodesk, Inc., San Raphael, CA). Resulting files were imported into AtlasGIS (Environmental Systems Research Institute, Redlands, CA) to create a base map of the study area that incorporated the location (latitude, longitude, and elevation) of each compound sampled (Figure 1). Compound locations are accurate to within a few meters.

Data collection. Weekly mosquito collections were done in all houses where children who were enrolled in a longitudinal study of the development of natural immunity to malaria resided.31 All children less than five years of age residing in the study area were eligible for enrollment. Because new children were continually enrolled in the study and older children continually exited the study, different houses were sampled throughout the study period. However, sampling was done repeatedly in many houses. Routine collections began in all villages in 1994 and were stopped in the nine villages receiving ITNs in December 1996. Collections continued in the 10 remaining control villages until October 1997. Female mosquitoes that had fed the previous night were collected using a simple bed net trap hung approximately 6 cm above sleeping spaces of study participants (Hawley WA and others, unpublished data).<sup>32</sup> Mosquitoes were aspirated from the traps early in the morning and returned to the laboratory where they

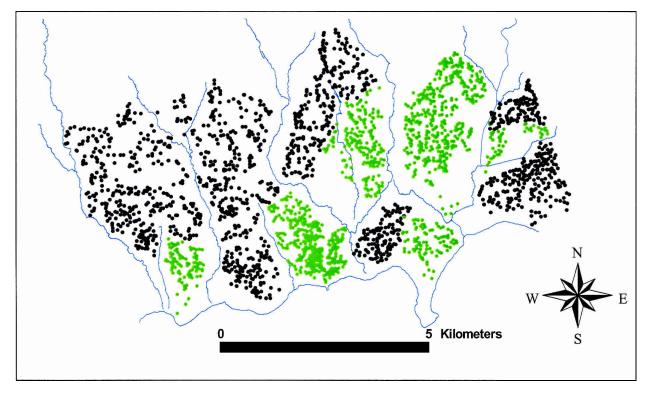


FIGURE 1. Map of the Asembo Bay study area showing intervention (green) and control (black) compounds.

were killed with chloroform, identified to species, and desiccated over anhydrous calcium sulfate. A collection therefore represented a single trap-night from one bed net. Dried specimens were tested for sporozoite infection using a sporozoite enzyme-linked immunosorbent assay (ELISA).<sup>33</sup> A subset of all specimens identified morphologically as *An. gambiae s.l.* were identified to sibling species by the polymerase chain reaction (PCR).<sup>34</sup> Only primers for *An. gambiae* and *An. arabiensis* were used because these are the only members of the *Anopheles gambiae* complex known to occur in Asembo Bay. Rainfall and maximum and minimum temperatures were recorded daily at four stations located within the study area.

**Data analysis.** Minimum distances from compounds in control villages to compounds in intervention villages were calculated using SAS statistical software (SAS Institute, Inc., Cary, NC). Minimum distances also were calculated from compounds in control villages to potential mosquito breeding sites: dams, burrow pits, the lakeshore, and streambeds. Compounds were categorized by distance to lakeshore as 0–999 meters, 1,000-1,999 meters, 2,000-2,999 meters, or  $\ge 3,000$ meters. For the other distance measures, compounds were assigned to one of four categories: 0-299 meters, 300-599 meters, 600-899 meters, and ≥900 meters. Continuous variables (rainfall and temperature) were centered around the mean. Statistical significance of the effect of distance to nearest intervention compound upon the abundance of An. gambiae and An. funestus was tested by repeated measures Poisson regression using the GENMOD procedure in SAS. The statistical test used an autocorrelative structure with the house as the repeated subject. This analysis incorporates an assumption that the correlation between measurements on the same house decreased as the time between measurements increased. Interaction terms between time (pre-intervention versus intervention periods) and distance from intervention compounds were included in all models. This allowed for estimation of the effect of distance to the nearest compound in an intervention village in 1997 while adjusting for preintervention abundance in 1996. Additional factors controlled for in the model of An. gambiae abundance were the distance to the lakeshore, rainfall 1-4 weeks before collection and average daily maximum temperature 1-4 weeks before collection. The model also included a significant interaction between rainfall 1-4 weeks before collection and year. The model for An. funestus was similar but included rainfall 5-8 weeks before collection because this was a stronger predictor of An. funestus abundance than rainfall 1-4 weeks before collection. The An. funestus model did not include the interaction between rainfall and year because this interaction was not significant. Distances to the nearest river, dam, or burrow pit were not significant and, therefore, were not included in the final models. Each model was run twice. First, dummy variables for distance from intervention villages were created using abundance at distances ≥900 meters from an intervention village as the baseline. This allowed for comparisons of the effect of distance at each distance category. Second, models were run treating the distance from intervention village as a continuous categorical variable to test for trends. The sporozoite rates for An. gambiae and An. funestus were modeled with repeated measures logistic regression using the GENMOD procedure in SAS. Only distance to the nearest compounds in an intervention village and time were included in these models.

Table 1

Number of trap-nights and number of mosquitoes collected in Asembo Bay, 1996–1997

	1996	1997
Anopheles gambiae s.l.	8,835	3,312
Anopheles funestus	10,956	3,203
Number of houses sampled	353	242
Number of trap-nights	7,449	4,412

Ethical clearance. The bed net trial and the longitudinal cohort study were reviewed and approved by the institutional review boards of the Kenya Medical Research Institute (Nairobi, Kenya) and the Centers for Disease Control and Prevention (Atlanta, GA). Informed consent was obtained from all caregivers after the study was explained in the local language.

## **RESULTS**

Summary data of the number of mosquitoes collected in 1996-1997 are shown in Table 1. Of 277 An. gambiae s.l. identified by PCR during the two-year period, 94.2% were An. gambiae s.s. In the intervention year, average numbers were lower compared with the pre-intervention year for An. gambiae (P = 0.001) but not for An. funestus (P = 0.157). In trend tests (Table 2), there was a significant trend for decreasing An. gambiae abundance with decreasing distance from intervention villages in both the pre-intervention year (P =0.027) and the intervention year (P = 0.002). Comparison between years indicated a significantly stronger trend in the intervention year (P = 0.05). No trend for An. funestus abundance with distance from an intervention village was observed in the pre-intervention year (P = 0.373). In the intervention year, a significant trend for decreasing abundance with decreasing distance from an intervention village was detected (P = 0.027). Comparison between years indicated a significant difference in the trends (P = 0.014).

Results of the regression analysis to assess differences in mosquito abundance by distance category are shown in Table 3. For each species and year, the risk ratio indicates the difference in mosquito abundance relative to the reference group (houses ≥900 meters from the nearest intervention

TABLE 2
Summary of main effects (distance plus distance × year interaction) for models testing trends of *Anopheles gambiae* and *An. funestus* abundance by distance from an intervention village\*

	Risk ratio	95% Confidence interval	P
Anopheles gambiae			
Pre-intervention	0.88	0.78 - 0.99	0.027
Intervention	0.77	0.61 - 0.97	0.002
Interaction (distance $\times$ year)	0.83	0.69 - 1.00	0.05
Anopheles funestus			
Pre-intervention	1.06	0.93 - 1.21	0.373
Intervention	0.73	0.56 - 0.94	0.014
Interaction (distance × year)	0.77	0.61 - 0.97	0.027

<sup>\*</sup>A risk ratio less than 1 indicates a decreasing trend with decreasing distance from intervention villages. For the interaction risk ratio, the pre-intervention year is held as the baseline so that estimates less than 1 indicate a lower risk ratio in the intervention year compared to the pre-intervention year. A significant interaction term indicates that the trends were significantly different between years.

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Table 3
Summary of main effects (distance plus distance × year interaction) for models of *Anopheles gambiae* and *An. funestus* abundance by distance category\*

Distance to intervention village (meters)	Pre-intervention	Intervention	P
Anopheles gambiae			
0–299	0.74 (0.51–1.07)	0.48 (0.29-0.81)	0.102
300-599	0.71 (0.53–0.94)	0.44 (0.27–0.73)	0.045
600-899	0.79 (0.61–1.03)	0.68 (0.43–1.06)	0.451
≥900	1	1	Reference
Anopheles funestus			
0–299	1.11 (0.74–1.68)	0.42 (0.21-0.84)	0.010
300-599	1.15 (0.81–1.65)	0.62 (0.31–1.24)	0.102
600-899	0.98 (0.67–1.42)	1.10 (0.56–2.16)	0.734
≥900	1	1 ′	Reference

<sup>\*</sup>For each species and year, risk ratios relative to number of mosquitoes collected in houses ≥900 meters from an intervention village are presented. 95% confidence intervals are in parentheses. The P value indicates the significance level for the interaction term. A significant interaction term indicates that the risk ratios were significantly different between years.

village). Risk ratios less than 1 indicate lower numbers relative to the reference group and differences are statistically significant when the confidence intervals do not overlap one. One minus the risk ratio is an estimate of the relative difference in abundance compared with houses that were ≥900 meters from an intervention village. The P value listed in Table 3 is the probability level for the interaction term. A significant interaction term indicates that the risk ratios for the pre-intervention year and the intervention year are significantly different. In the pre-intervention year, the average number of An. gambiae was 21% lower in houses 600-899 meters from an intervention village compared with houses  $\geq$ 900 meters from an intervention village (P = 0.089). The average number of An. gambiae was 29% lower in houses 300–599 meters from an intervention village (P = 0.017) and 26% lower in houses 0-299 meters from an intervention village (P = 0.103). The difference was statistically significant at 300-599 meters. During the intervention year, An. gambiae abundance was 32% lower 600-899 meters from the intervention villages (P = 0.118), 56% lower at 300–599 meters (P =0.002), and 52% lower at 0-299 meters (P = 0.005). The differences were statistically significant at 0-299 meters and 300-599 meters when compared with mosquito numbers ≥900 meters from the intervention village. Comparing between years, the risk ratios were all lower in the intervention year. The difference in risk ratios was statistically significant at 300-599 meters (P = 0.045). The difference in risk ratios 0-299 meters from an intervention village was not statistically significant (P = 0.102), but difference was similar to that observed in the 300-599 meter distance category, and the higher P value may reflect a smaller sample size in the 0–299 meter distance category.

For An. funestus, no clear pattern of abundance by distance from intervention villages existed in the pre-intervention year. In the pre-intervention year, the average number of An. funestus was 2% lower in houses 600-899 meters from an intervention village compared with houses  $\geq 900$  meters from an intervention village (P=0.910). The average number of An. funestus was 15% higher in houses 300-599 meters from an intervention village (P=0.428) and 11% higher in houses 0-299 meters from an intervention village (P=0.587). In the intervention year, An. funestus numbers increased with in-

creasing distance from intervention villages. Relative to control houses that were  $\geq 900$  meters from intervention villages, An. funestus numbers were 10% higher in houses 600–899 meters from an intervention village (P=0.739), 38% lower in houses 300–599 meters (P=0.181) and 58% lower in houses 0–299 meters (P=0.013). The difference was statistically significant at 0–299 meters. Comparing between preintervention and intervention years, the risk ratios were significantly lower in the 0–299 meters distance category in the intervention year (P=0.010).

There was a marked decrease in sporozoite rates in both  $An.\ gambiae$  and  $An.\ funestus$  following the introduction of the ITNs in 1997. Overall, pre-intervention infection rates were 5.81% for  $An.\ gambiae$  and 5.09% for  $An.\ funestus$ . Infection rates were 0.56% and 0.41%, respectively, during the intervention period. The effect of year on the probability of infection was statistically significant for both  $An.\ gambiae$  (P < 0.001) and  $An.\ funestus$  (P < 0.001). However, logistic regression analysis showed no significant association between the distance from intervention villages and the risk of infection in the vectors in either the pre-intervention or the intervention years.

### **DISCUSSION**

The current study demonstrated a decrease in numbers of An. gambiae and An. funestus in control houses that were close to intervention villages compared with those more distant from intervention villages. The effects were strongest for An. funestus, in which no obvious pattern in mosquito numbers was observed pre-intervention. For An. gambiae, inferring an effect of the proximity to intervention villages was more difficult because there were fewer mosquitoes collected near intervention villages even before the ITN intervention. However, the relative number of An. gambiae collected near intervention villages compared with the number ≥900 meters from an intervention village was lower in the intervention year compared with the pre-intervention year. The evidence supports the hypothesis that a community effect reduces the overall vector population, and that persons lacking ITNs who live near the compounds of those using ITNs are afforded some protection from vector mosquitoes. No evidence was found for divergence of mosquitoes from intervention compounds to control compounds. Further evidence of a community effect was observed in health outcomes in children. Children residing in control villages but within 300 meters of an intervention village had a lower risk of malaria parasitemia, high-density parasitemia, anemia, and death relative to children who lived near the center of a control village.<sup>35</sup>

The introduction of ITNs appeared to have a stronger impact upon the distribution of *An. funestus* compared with *An. gambiae*. The stronger impact upon *An. funestus* was not entirely unexpected. Estimates of the direct impact of ITNs in the current trial indicated that *An. funestus* was significantly reduced in all intervention homes, even when residents did not use their nets. This species has proven highly susceptible to chemical control measures and slow to recolonize areas from which it has been eliminated. These results suggest that the degree of community effect may depend partly upon the species of malaria vectors present in a given area.

Data from the current and previous trials of ITNs indicate that the community effect upon mosquito abundance also re-

sults in improved health outcomes for children residing near intervention villages. In Ghana, child mortality increased by 6.7% for every increase of 100 meters from an intervention compound,<sup>22</sup> and studies in coastal Kenya indicated that for a child not sleeping under a bed net, the risk of malaria decreased with increasing use of bed nets in the area surrounding that child.21 In the current trial, rates of parasitemia, highdensity parasitemia, anemia, and mortality were lower in children who did not use nets but lived within 300 meters of an intervention village.35 For these health outcomes, the protective effect of living near an intervention village was nearly as strong as that for children living within the intervention villages. This was somewhat surprising given that the reduction in mosquito abundance in control houses located near intervention villages was not nearly as strong as that observed in houses with ITNs.9 This difference may simply reflect the difficulty in modeling mosquito abundance over a large geographic area for an extended time, as well as the uncertainty in the exact relationship between transmission intensity and health outcomes.

The degree of protection conferred to persons not sleeping under ITNs is likely related to the proportion of persons sleeping under nets within a given area. Studies in Burkina Faso, which indicated a stronger community effect than observed in the current study, were based upon collections made near the geographic center of intervention villages and up to 4 km from the nearest control compound.8 In The Gambia, evidence for community effect was observed only in one village which was geographically isolated from other villages and had a high level of net usage.<sup>24</sup> Hawley and others<sup>35</sup> showed that the community effect appeared to follow a dose response with increasing coverage, with little or no community effect observed when coverage was less than 25%. The community effects observed in these trials suggest that estimates of the protective efficacy of bed nets may have been underestimated because some control houses may indirectly benefit from their proximity to intervention villages.

Although there was evidence for a community effect rather than divergence of mosquitoes, it should be emphasized that the design of community-wide intervention trials will not reflect the true picture of ITN use under operational settings. In intervention trials, ITNs usually are allocated to entire villages and steps are taken to ensure high compliance. In reality, ITN use may be sparse within villages. In this situation, ITN coverage may not be high enough to achieve a community effect. It is possible that very low coverage of ITNs with a random distribution within villages may have different effects, causing diversion of mosquitoes to nearby compounds and increasing malaria risk among the residents of those compounds. Continued monitoring of malaria control programs that implement ITNs as part of their control strategy is necessary to estimate the degree of coverage required to obtain a community effect and to monitor whether lower levels of coverage actually cause divergence of vectors to unprotected persons. Lastly, the type of insecticide used and the dose at which it is applied to nets may affect the strength of any community effect. The insecticide used in the current study was permethrin at a target dose of 500 mg/m<sup>2</sup> of netting. In the future, other insecticides such as alphacypermethrin, lambdacyhalothrin, or deltamethrin likely will be used with increasing frequency. These insecticides have higher killing activity and lower repellency compared with permethrin. 37,38

Thus, the probability of divergence of mosquitoes due to these chemicals may be lower and any community effect may be higher than that observed in the current study.

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