



RESEARCH ARTICLE

Variation in the effectiveness of insecticide treated nets against malaria and outdoor biting by vectors in Kilifi, Kenya [version 1; peer review: 4 approved with reservations]

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Abstract

Background: Insecticide treated nets (ITNs) protect humans against bites from the *Anopheles* mosquito vectors that transmit malaria, thereby reducing malaria morbidity and mortality. It has been noted that ITN use leads to a switch from indoor to outdoor feeding among these vectors. It might be expected that outdoor feeding would undermine the effectiveness of ITNs that target indoors vectors, but data are limited. **Methods:** We linked homestead level geospatial data to clinical surveillance data at a primary healthcare facility in Kilifi County in order to map geographical heterogeneity in ITN effectiveness and observed vector feeding behaviour using landing catches and CDC light traps in seven selected areas of high and low ITN effectiveness. **Results:** We observed 33% and 39% visits associated with positive malaria slides among ITN users and non-ITN-users, respectively; ITN use was associated with 22% protection from malaria (crude OR = 0.78, 95% CI: 0.72, 0.84). We obtained significant modification of ITN effectiveness by geographical area ($p=0.022$), and identified significant hotspots using the spatial scan statistic. Most biting occurred outdoors (62%) and was by *An. funestus* (76%), and appeared to be more frequent in low ITN effectiveness areas compared with high ITN effectiveness areas (69% vs. 26%, $p<0.001$), but this was due to a single outlying area. After excluding this outlying area, outdoor biting

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was similar in low vs. high ITN effectiveness area (69% vs. 75%, $p=0.76$).

Conclusion: Our data therefore do not support the hypothesis that outdoor biting undermines the effectiveness of ITNs in our study area.


Keywords

ITNs, outdoor, Anopheles mosquito, effectiveness, Kilifi, Kenya, KHDSS



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Introduction

Despite the recent scale-up effort to achieve control, malaria continues to cause morbidity and mortality, especially in sub-Saharan Africa. There are uncertainties in global estimates^{1–3}; however in 2015, the World Health Organization estimated global deaths due to malaria to be 438,000 (range: 236,000–635,000) and the burden of febrile illness at 214 million cases (range: 149–303 million)⁴. Modelling studies suggest that approximately 1.4 billion of the world's population live at risk of stable malaria and ~1.1 billion at risk of unstable malaria⁵.

The frontline tools for malaria control in sub-Saharan Africa, insecticide treated nets (ITNs) and indoor residual spray, are optimally effective if baseline transmission occurs indoors⁶. The major vectors of human malaria mostly feed indoors, and transmission can therefore be substantially reduced by these tools⁶. The proportion of the at risk population who have access to ITNs was modeled to have increased from 4% to 67% between 2004 and 2015⁷. ITNs operate in three ways: deterrence, excito-repellence and killing, thereby reducing the density, feeding frequency, feeding success, and survival of *Anopheles* mosquito vectors⁶. By reducing vector densities and vector survival, ITNs not only directly protect the individual ITN user, but also reduce the overall transmission intensity and protect the whole community when a particular threshold of bed net coverage is reached^{8–10}. The evidence base supports ITN use over a range of transmission intensities¹¹ and protective efficacy has been demonstrated against infection, clinical disease and mortality^{12–16}. However, residual malaria transmission is well described even after optimal ITN use, which could be caused by changes in the behaviour of the mosquito vector that allows them to evade fatal contact with these frontline tools of intervention^{17,18}. The most obvious behavioural change is the mosquito vector exhibiting exophagic tendencies – i.e. the vector feeds outdoors on humans.

Among malaria vectors in Africa, the two principal species complexes are: *Anopheles gambiae sensu lato* (s.l.) and *Anopheles funestus* group. Both species complexes feed primarily indoors; however both have exhibited behavioral shifts to outdoor biting or feeding in the early part of the evening following prolonged use of ITNs in some areas^{5,19,20}. This behavioral change might have resulted from one of three processes: (i) selection, either for species that more readily engages in outdoor feeding, for instance in favour of *An. arabiensis* rather than *An. gambiae sensu strictu* (s.s.); (ii) by selecting for evolutionary change within a species; or (iii) a response to inability to feed during the night in the absence of genetic variation²¹. In Western Kenya and South-eastern Tanzania there have been reports of a reduction in indoor feeding by *An. gambiae sensu strictu* (s.s.) and an increase in the relative abundance of *An. arabiensis*. The latter has a broader range of feeding times and biting behavior, including outdoor feeding^{8,21–23}. In northern Tanzania, where ITNs have been used for several years, the mosquitoes are biting more frequently during the hours of the early evening and early morning when people are more likely to be awake and vulnerable outside of their nets^{6,24}. The potential for ITNs to result in species switches was appreciated in earlier controlled trials^{21,22,25}, and is now reported more widely as ITN use is scaled up in Western Kenya and on the East African coast^{21,22}.

In Kilifi, Kenya, a switch in the most common vector, from *An. gambiae sensu strictu* (s.s.) to *An. arabiensis*, occurred during the period of ITN scale-up¹⁹. The increased ability of *An. arabiensis* to feed outdoors might be expected to result in a decrease in ITN effectiveness. However, there is little data to support this contention, and some data and models that are available suggest that ITNs continue to be effective despite outdoor feeding^{26,27}. The objectives of this study were (i) to examine whether there has been a shift in vector biting patterns and/or vector behaviour, during the period of intense ITN use along the Kenyan coast; (ii) to test for geographical heterogeneity in ITN effectiveness within the surveillance area of a primary healthcare facility in Kilifi County; and (iii) to assess whether outdoor vector biting is a potential explanation for the variation in ITN effectiveness.

Methods

Study area

The clinical surveillance study was conducted between January 2009 and December 2014 within a 6km radius of Pingilikani dispensary in Kilifi County on the Kenyan Coast (Figure 1): within the Kilifi Health and Demographic Surveillance System (KHDSS). All children under 13 years presenting for medical assessment to Pingilikani dispensary (except those with trauma as their only concern) were assessed by research staff and had finger-prick blood samples examined for malaria parasites. Thick and thin blood smears were stained with 10% Giemsa and examined at 1000X magnification for asexual *Plasmodium falciparum* parasites. Before slides could be considered negative, 100 fields were examined. Children with malaria positive slides were treated with co-artemether.

Transmission of malaria peaks after the long rains from April to June and the short rains from October to November each year, although transmission has been declining^{28–31}. The surveillance area was divided into 2.5x2.5 km regular polygons resulting in 21 geographical areas (Figure 2). As part of KHDSS, four-monthly enumeration rounds were conducted to identify births, deaths and migration events. Each inhabitant was described by their family relationships and their homestead of residence, with geospatial coordinates, and assigned a unique personal identifier³². These details were used to link children visiting Pingilikani dispensary to geospatial coordinates for the homestead of residence. Data on ITN use was collected once yearly during cross-sectional surveys integrated into the regular KHDSS enumeration since 2008. Questionnaires were used to collect household data on ITN ownership and use on the night prior to enumeration. Seven geographical areas were selected for mosquito sampling out of 21 areas for which clinical effectiveness estimates were determined (Figure 2). The basis of selecting the seven areas was (i) geographical areas with >60 homesteads available for randomization; (ii) areas representative of highest and lowest ITN effectiveness.

Mosquito sampling

Indoor and outdoor biting profiles of *An. gambiae s.l.* and the *An. funestus* group were estimated using human landing catches (HLC) and CDC-light traps (CDC-LT) by visiting randomly selected houses (random selection done by stratified sampling) between July and August 2016. For both indoor and outdoor

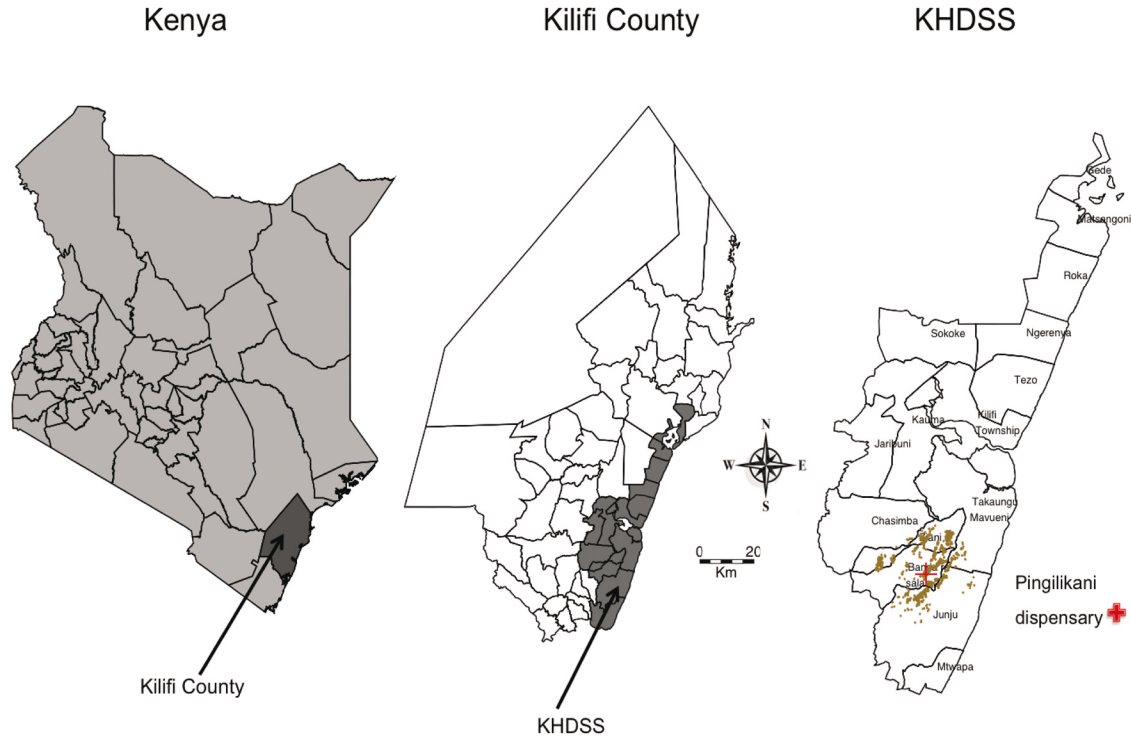


Figure 1. Situation of Kilifi County in Kenya and the map of Kilifi County showing the boundaries of the KHDSS. The map of KHDSS shows the locations and the situation of homesteads and Pingilikani dispensary where the study was conducted. The brown plotted point on the KHDSS map represents homesteads.

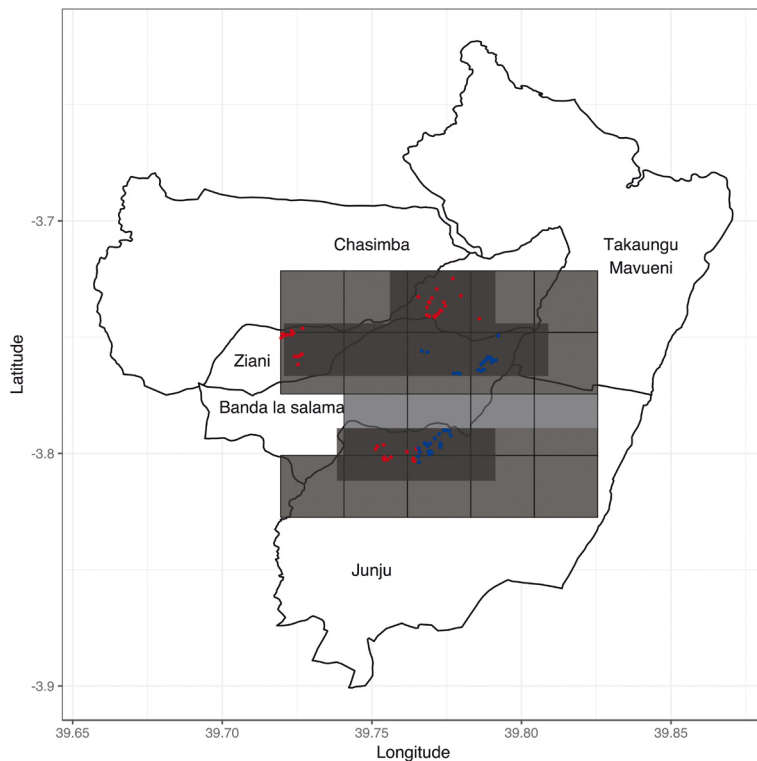


Figure 2. Map of the 2.5X2.5 km geographical areas (grids in light gray), the geographical areas where mosquito sampling was conducted (grids in dark gray) and the homesteads where mosquito sampling was done. Each plotted point represents an individual homestead, where color shading indicates ITN effectiveness, with red shading indicating low effectiveness and blue shading indicating high effectiveness.

mosquito collection, HLC was conducted by two pairs of trained male volunteers (one pair was located indoors and the other pair outdoors, but at the same homestead), who sat with their legs exposed and caught mosquitoes that attempted to bite them using an aspirator. HLC was conducted between 18:00hours and 06:00hours for 45 minutes each hour, allowing 15 minutes break for rest. The catches for each hourly interval were stored in separate collection cups. CDC-light traps were also set indoor and outdoor between 18:00hours and 06:00hours. The HLC and the CDC-LT collections took place in different houses. In each geographical area, sampling was conducted for at least 3 days in at least 16 houses; 8 houses for HLC and 8 houses for CDC-LT. In total, 26 days of sampling were conducted across 115 houses in the seven selected geographical areas within the surveillance area.

Mosquito processing

The mosquito samples were morphologically separated for sex and identified for species^{6,24}. The female *Anopheles* mosquitoes were tested for falciparum infection using a sandwich circumsporozoite protein (CSP) enzyme linked immunosorbent assay (ELISA)³³ (anti-CSP capture: Pf2A10-28 and conjugate : Pf2A10-CDC antibodies; KPL, Gaithersburg, MD, USA). Individual mosquitoes were stored at -20°C in micro-centrifuge tubes containing a small amount of desiccant (silica gel) separated from the mosquito by a thin layer of cotton prior to ELISA and molecular analysis for sibling species by polymerase chain reaction^{6,34}.

Statistical analysis

Statistical analyses were performed using STATA v13.1 (StataCorp, College Station, TX, USA). To assess for geographical heterogeneity, we used the logistic regression model to analyze data on over 20,000 visits from children attending Pingilikani dispensary. The outcome of interest was presence of malaria by microscopy on presentation to the dispensary. The potential risk factors included: ITN use, age of the child, year of presentation to the dispensary and the geographical area, as defined by the 2.5×2.5 km regular polygons. We assessed whether the effect of ITN use on malaria was altered by geographical area by including an interaction term between geographical area and ITN use. We also assessed whether the effect of ITN use was altered by the age of the child and whether geographical areas altered the effect of age. To assess the nonlinear effect of age in the regression models, multiple fractional polynomial transformation was used. Given that the hospital malaria episodes were clustered within patients, we allowed for clustering by using a logistic regression model with robust standard errors. The robust standard errors were used to account for the clustering effect in the estimation of the standard errors. The ratio of malaria in the non-ITN users to that in the ITN users was expressed as an odds ratio (OR) as determined by logistic regression. ITN effectiveness was calculated as $(1 - OR) \times 100$. Model fit was assessed by examining residuals against covariates. Spearman's rank correlation was used to assess the association between ITN effectiveness and prevalence of malaria. SaTScan software (version 9.4; <https://www.satscan.org/>), a spatial scan statistic developed by Kulldorf³⁵, was used to detect potential spatial variations of ITN effectiveness by identifying statistically significant geographical clustering of ITN effectiveness.

In order to compare counts of female *Anopheles* captured, we determined the relative proportion of each mosquito species in each geographical area and ITN effectiveness levels (ITN effectiveness was divided into 2 levels based on the estimates obtained from the logistic regression above – i.e. high and low ITN effectiveness). Three areas with high ITN effectiveness and four areas with low ITN effectiveness were selected based on the findings of the scan statistic. We compared the proportion of vectors biting outdoors in each geographical area. We estimated the confidence intervals of these proportions using the binomial distributions, and tested for an association between biting preference and ITN effectiveness (at the level of geographical area).

Results

Geographical variations in ITN effectiveness

Between 2009 and 2014, there were 20,827 visits to Pingilikani dispensary made by 4,992 children aged between 3 months to 12 years (Supplementary Table 1). Of these visits, 7220 (35%) were classified as episodes of malaria, with a median number of 7 (IQR: 4,12) episodes per child during this time period. The number of children, cases of malaria and ITN use in the 21 geographical areas examined is summarized in detail in Supplementary Table 1. ITN use was consistently >50% in all geographical areas and the prevalence of ITN use in non-malaria cases was 74.9% (95% CI: 74.2, 75.6).

Among children who were ITN users, 33% (5045/15234) of the visits were associated with positive malaria slides, whereas among non-ITN-users 39% (2175/5593) of the visits were associated with positive malaria slides. ITN use was associated with a 22% protection from malaria; crude OR = 0.78, 95% CI: 0.72, 0.84 ($p < 0.001$). When geographical area was added to the model as an interaction term with ITN use, we obtained a statistically significant variation in ITN effectiveness between the geographical areas ($p = 0.014$). Geographical variation in ITN effectiveness remained robust ($p = 0.022$) even after adjusting for the year of visitation to the dispensary and plausible interactions (i.e. interactions between ITN use and nonlinear age, and between geographical area and nonlinear age). The stratum specific adjusted OR for the association of ITN use on malaria in the geographical areas was calculated and shown in the order of decreasing effectiveness (Figure 3 & Supplementary Table 1). Previous data have shown that ITN effectiveness is lower in areas of high malaria transmission^{11,36}. This did not appear to be the explanation for variation in effectiveness in this data (Supplementary Figure 1); the Spearman rho coefficient value for the association of ITN effectiveness and prevalence of malaria was 0.308, $p = 0.331$.

Hotspots

Using the logistic regression model, we estimated ITN effectiveness for each individual homestead where there was sufficient data to calculate a point estimate (i.e. >30 observations from homestead aggregated at a 2.5 km smoothing). Using SaTScan software, we identified 6 significant hotspots of low ITN effectiveness: $p = 0.001$ for 4 hotspots, $p = 0.002$ and $p = 0.014$ for a 5th and 6th hotspot (Figure 4). We concluded that spatial variation in ITN effectiveness was not due to random noise based on the 95% confidence intervals obtained from the logistic regression

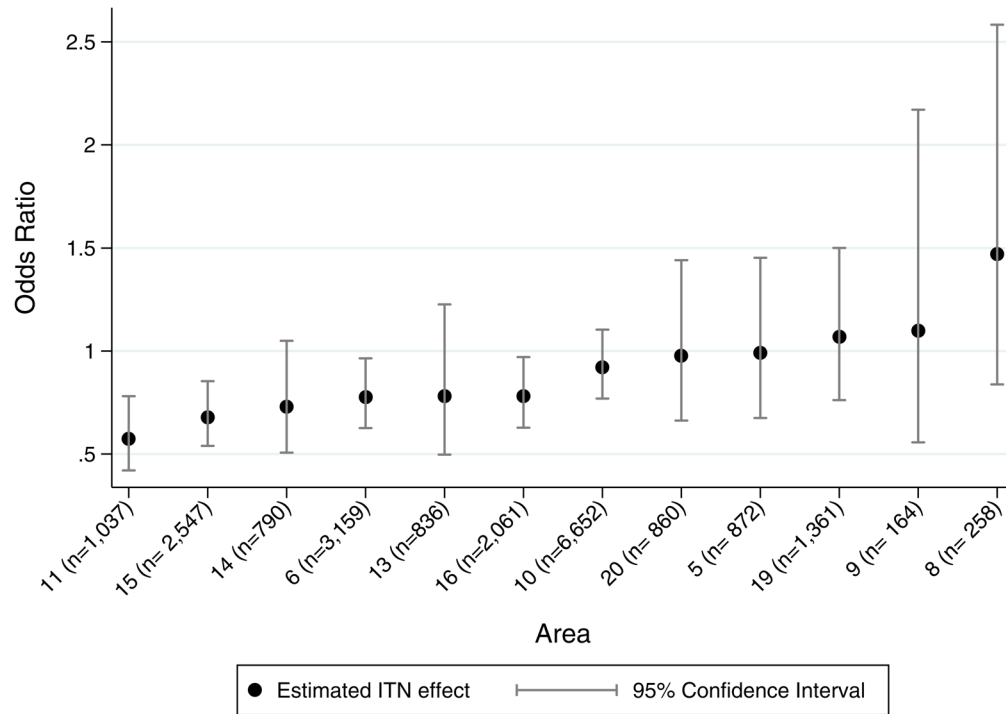


Figure 3. Scatter plot of stratum specific adjusted Odds Ratio and 95% confidence intervals of 12 geographical areas in order of decreasing effectiveness.

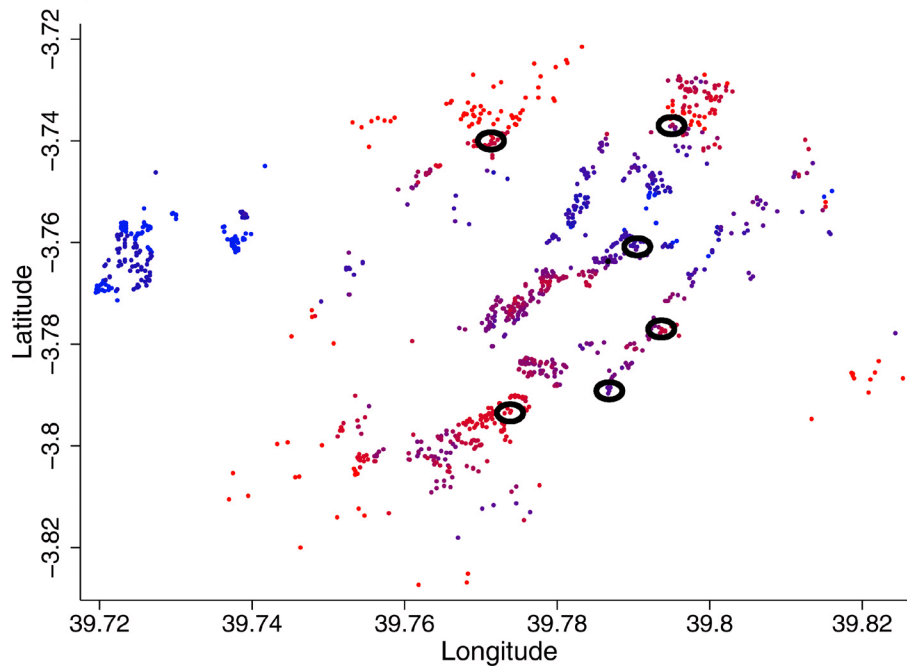


Figure 4. Scatter plot of estimated insecticide treated net (ITN) effectiveness for individual homesteads aggregated at a 2.5km smoothing. Each plotted point represents an individual homestead, where color shading indicates ITN effectiveness, with red shading indicating low effectiveness and blue shading indicating high effectiveness. The large black circles indicate the significant hotspots (analyzed without smoothing).

analysis for geographical areas and the existence of significant hotspots by SaTScan, and selected seven geographical areas for further entomological studies to represent a range of ITN effectiveness estimates.

Vector abundance

Over 26 nights, 411 female *Anopheles* mosquitoes were collected by both methods (i.e. 259 by HLC and 152 by CDC-LT), representing a mean of 15.8 mosquitoes per night. 63% of mosquitoes were collected using HLC. Of the 411 mosquitoes, 314 (76%, 95% CIs 72%, 80%) were *An. funestus* group, which was significantly greater than *An. gambiae s.l.* ($p < 0.001$). The proportion of *Anopheles* mosquitoes caught outdoors (62%; 95% CI: 57,67) was significantly greater than the proportion caught indoors ($p < 0.001$). There were more *Anopheles* mosquitoes collected outdoors in all geographical areas except area 6, where most of the mosquitoes were collected indoors (Table 1). The frequencies of species collected in each geographical area are summarized in Supplementary Table 2. *An. funestus* group was the most prevalent species in all areas.

The species and proportion of mosquitoes collected in areas of high vs. low ITN effectiveness are summarised in Table 2 and Supplementary Figure 2. Overall, the proportion of outdoor biting was higher in the low ITN effectiveness areas (69% vs. 27%, $p < 0.001$), but this apparent significance was due to a single area (labelled area 6), which was an outlier for indoor biting (Figure 5). When we excluded area 6, the proportion of outdoor biting in the low vs. high ITN effectiveness areas was non-significant (69% vs. 75%, $p = 0.76$). Moreover, when analysed by individual geographical area there was not a visually obvious trend associating increasing outdoor biting with decreasing ITN effectiveness in the seven geographical areas (Figure 5). The Spearman rho coefficient value for the association of ITN effectiveness and proportion of mosquitoes collected outdoors was -0.464 , $p = 0.302$.

Table 1. Proportion of *Anopheles* mosquitoes collected indoors and outdoors.

	Number collected	% Indoor (CI)	% Outdoor (CI)
All	411	38 [33, 43]	62 [57, 67]
Species			
<i>An. gambiae s.l.</i>	76	17 [9, 27]	83 [73, 91]
<i>An. funestus</i>	314	46 [40, 52]	54 [48, 60]
Other anopheles	21	0	100 [84, 100*]
Geographical area			
5	171	46 [38, 53]	54 [47, 62]
6	59	83 [71, 92]	17 [8, 29]
13	9	44 [14, 79]	56 [21, 86]
15	6	33 [4, 78]	67 [22, 96]
16	6	17 [0.4, 64]	83 [36, 99]
19	113	12 [7, 20]	88 [80, 93]
20	47	19 [9, 33]	81 [67, 91]

*CI: Confidence Interval, %: Proportion per 100

Discussion

Malaria is an important public health problem in sub-Saharan Africa, and many countries, including Kenya, have attempted to reduce this burden by increasing ITN ownership and usage^{37,38}. However, previous reports have shown that prolonged ITN use leads to behavioral shifts in the mosquito vector from indoor to outdoor biting or feeding in the early part of the evening^{6,19,24}. This shift in mosquito feeding behavior might be expected to result in a decrease in ITN effectiveness. We identified statistically significant

Table 2. Composition of the mosquito species in areas of high and low ITN effectiveness.

Trap type	Species	Low ITN effectiveness areas			High ITN effectiveness area		
		Total (N)	Outdoor (n)	Outdoor (%)	Total (N)	Outdoor (n)	Outdoor (%)
HLC	<i>An. gambiae</i>	57	45	78.9	5	4	80.0
	<i>An. funestus</i>	152	69	45.4	24	10	41.7
	Other Anopheles	21	21	100	0	0	0
	Total	230	135	58.7	29	14	48.3
CDC-LT	<i>An. gambiae</i>	14	14	100	0	0	0
	<i>An. funestus</i>	96	86	89.6	42	5	11.9
	Other Anopheles	0	0	0	0	0	0
	Total	110	100	90.9	42	5	11.9

*HLC: Human landing catches, CDC-LT: CDC light trap, %: Proportion per 100, N & n: number of mosquitoes collected.

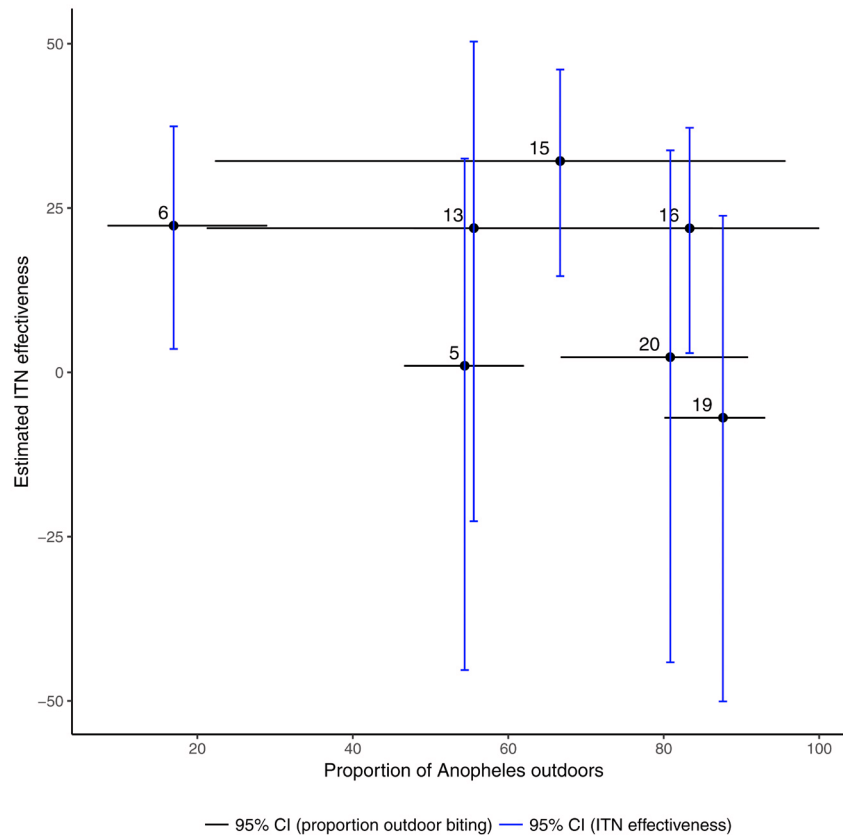


Figure 5. Scatter plot of estimated insecticide treated net (ITN) effectiveness and the proportion of *Anopheles* mosquitoes collected outdoors and 95% confidence intervals of these estimates.

geographical variation in the effectiveness of ITN and identified areas where ITN effectiveness was found to be consistent with the 50% estimate reported in the literature^{11,39,40}, and other areas where ITNs were less effective (Figure 3). This variation could conceivably have arisen as a result of variations in quality of ITNs, patterns of use, host resistance, insecticide resistance or other factors, including random variation. We investigated whether variations in outdoor vector biting was a potential explanation.

We found that *An. funestus* was more prevalent than *An. gambiae s.l.* species complex, consistent with a previous report¹⁹. We observed small-scale spatial variability in vector abundance (Table 1), which is consistent with previous reports on the Kenyan Coast^{20,41}. We also observed a higher proportion of mosquito vectors collected outdoors than indoors, in areas of both high and low ITN effectiveness (Figure 5). On first principles one would expect that outdoor biting would lead to ITNs becoming ineffective. However, despite seeing consistent outdoor biting throughout the study area this did not appear to be associated with an overall reduction in ITN effectiveness. We may have observed an apparently statistically significant increase in the prevalence of outdoor biting in areas of low ITN effectiveness. However, this was due to a single

outlying geographical area and there was no variation in prevalence of outdoor biting after this area was excluded. This suggests the statistical significance of the initial comparison may have been due to ecological confounding, where a geographical area with high ITN effectiveness happened to have more indoor mosquitoes, but this relationship was not confirmed in other areas (Figure 5).

How should we interpret the finding that outdoor feeding does not consistently lead to a reduction in ITN effectiveness? It is possible that the higher proportion of mosquitoes biting outdoors represents a behavioral response to unsuccessful feeding attempts made indoors during the night, and therefore it may simply be a marker of successful ITN use. This avoidance behavior may exert a cost on the vector, and so ITNs may in fact still be protective in areas where outdoor biting is observed, as has been suggested previously²⁷.

Spatial heterogeneity in malaria exposure has been described at micro-epidemiological level at varying transmission settings⁴² and is responsible for variations in disease risk within small geographical areas and is evidenced by local clustering of malaria infections. Within the 2.5 km squared geographical areas, ITN effectiveness

appears to have been spatially heterogeneous (Figure 4); however, we were unable to demonstrate a significant association between ITN effectiveness and outdoor biting at the level of seven small geographical areas. The observed geographical variation in ITN effectiveness therefore remains unexplained. Possibilities include insecticide resistance, or geographical variations in human behaviour in terms of ITN use.

Our study has some limitations. Data on ITN use may have been incorrectly reported, as we did not require each resident to be present to respond to the ITN ownership and use questions. We attempted to minimize this by instructing data collecting teams to interview only residents of the same homestead regarding ITN ownership and usage. There may have been some misclassification as we did not ascertain ITN use during hospital visitation but instead used the yearly ITN data collected by the KHDSS. The results may also be biased and confounded by other unmeasured factors (e.g., variation in the quality and type of ITN, urbanization, socio-economic status and mother's education). Therefore, the estimates obtained could be an overestimation or underestimation of the true effectiveness. It is likely that we underestimated the protection afforded by the use of high-quality ITN because we included all ITNs, regardless of quality. The vast majority of ITNs in the area are long-lasting insecticidal nets, hence we do not expect substantial variation in insecticidal efficacy. The accuracy of the human landing catches may be affected by the inter-individual differences in attracting mosquitoes. The size of our study limits power: with a sample size of 411, and the proportion of mosquitoes biting outdoors at 69% in low ITN effectiveness areas we therefore had >90% power to detect a reduction to 27% or lower in high ITN effectiveness areas. Our study was therefore powered to detect only a large difference in the proportion of vectors caught outdoors. However, we reasoned that reductions of ITN effectiveness to less than half of the previously documented efficacy of 50% would require a doubling of the proportion of mosquitoes feeding outdoors. Hence our study was powered to detect large variations in the frequency of outdoor biting. Furthermore, since the proportion of vectors collected outdoors was high throughout the study area despite preserved ITN effectiveness in many areas, we conclude that the pattern of outdoor feeding identified in our site does not undermine ITN effectiveness.

In summary, we found no evidence that the currently observed switch from indoor to outdoor biting leads to reduced ITN effectiveness. The outdoor biting observed may therefore have been the result of high levels of ITN use leading to unsuccessful attempts at indoor feeding. It remains possible that selection pressures might lead to the emergence of populations of mosquitoes that are better adapted to outdoor feeding. Outdoor feeding is becoming more common in parts of Africa⁴³ and may represent evolutionary change in some areas, with a potential to undermine ITN effectiveness. Therefore, malaria control programs require monitoring

to assess the impact of ITNs on vector populations and vector behavioral change as well as monitoring ITN effectiveness as vectors evolve^{6,21,22,24,25}. Detailed studies of vector bionomics, continuous monitoring and malaria transmission dynamics are essential for predicting disease outbreaks and vector control in the region.

Ethical approval

This study was approved by the Kenya Medical Research Institute Scientific Ethics Review Unit (KEMRI/SERU/CGMR-C/024/3148). Written informed consent was obtained from the parents/guardians of the children attending the dispensary.

Data availability

Data that support the findings of this study (hospital surveillance, ITN community surveys and mosquito collection) are available from the KEMRI Institutional Data Access/Ethics Committee, for researchers who meet the criteria for access to confidential data. Details of the criteria can be found in the KEMRI-Wellcome [data sharing guidelines](#). The data includes homestead level coordinates as an essential component and these are personally identifiable data. Access to data is provided via the KEMRI-Wellcome Data Governance Committee: Data_Governance_Committee@kemriwellcome.org; Tel, +254708 587 210; Contact person, Marianne Munene (Secretary; Tel, +254709 983 436).

Author contributions

AK oversaw field implementation of the study, analyzed and interpreted the data and drafted the manuscript. JMM, MKR and PB conceived the study, helped with the field implementation of the study, and reviewed and revised the manuscript. PM, IO, JM, and JAGS reviewed and revised the manuscript. All authors approved the final manuscript, as submitted.

Competing interests

The authors declare that they have no competing interests.

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Supplementary material

Supplementary Table 1: Description of insecticide treated net (ITN) use, cases of malaria and ITN effectiveness in the 2.5x2.5 km geographical areas. Data includes the number of children observed, number visits made to Pingilikani hospital by the children, the number and proportion of malaria among ITN use or non-ITN-users in the 21 geographical areas, the stratum specific adjusted Odds Ratio (aOR) and the Confidence Interval (95% CI); ‡ areas with fewer than 35 observations were excluded from the logistic regression due to perfect prediction and/or collinearity.

[Click here to access the data.](#)

Supplementary Table 2: Composition of the mosquito species in seven geographical areas. Data includes number of *Anopheles* mosquitoes collected by human landing catches (HLC) and CDC light trap (CDC-LT) indoor or outdoor in the seven geographical areas, and the overall proportion.

[Click here to access the data.](#)

Supplementary Figure 1: Scatter plot of the log odds ratio of insecticide treated net (ITN) effect and 95% confidence interval of malaria positivity in 12 geographical areas against malaria prevalence among children presenting to Pingilikani dispensary.

[Click here to access the data.](#)

Supplementary Figure 2: Bar graph of the proportion of *Anopheles* mosquito species collected in areas of low and high insecticide treated net (ITN) effectiveness.

[Click here to access the data.](#)

References

- Snow RW, Guerra CA, Noor AM, *et al.*: The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature*. 2005; **434**(7030): 214–217. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Nkumama IN, O'Meara WP, Osier FH: Changes in Malaria Epidemiology in Africa and New Challenges for Elimination. *Trends Parasitol*. 2017; **33**(2): 128–140. [PubMed Abstract](#) | [Publisher Full Text](#)
- Cibulskis RE, Aregawi M, Williams R, *et al.*: Worldwide incidence of malaria in 2009: estimates, time trends, and a critique of methods. *PLoS Med*. 2011; **8**(12): e1001142. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- WHO: World malaria report. *World Health Organization*. 2015. [Reference Source](#)
- Gething PW, Patil AP, Smith DL, *et al.*: A new world malaria map: *Plasmodium falciparum* endemicity in 2010. *Malar J*. 2011; **10**: 378. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Russell TL, Govella NJ, Azizi S, *et al.*: Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malar J*. 2011; **10**: 80. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Walker PG, Griffin JT, Ferguson NM, *et al.*: Estimating the most efficient allocation of interventions to achieve reductions in *Plasmodium falciparum* malaria burden and transmission in Africa: a modelling study. *Lancet Glob Health*. 2016; **4**(7): e474–484. [PubMed Abstract](#) | [Publisher Full Text](#)
- Gimnig JE, Vulule JM, Lo TQ, *et al.*: Impact of permethrin-treated bed nets on entomologic indices in an area of intense year-round malaria transmission. *Am J Trop Med Hyg*. 2003; **68**(4 Suppl): 16–22. [PubMed Abstract](#)
- Howard SC, Omumbo J, Nevill C, *et al.*: Evidence for a mass community effect of insecticide-treated bednets on the incidence of malaria on the Kenyan coast. *Trans R Soc Trop Med Hyg*. 2000; **94**(4): 357–360. [PubMed Abstract](#) | [Publisher Full Text](#)
- Lindblade KA, Eisele TP, Gimnig JE, *et al.*: Sustainability of reductions in malaria transmission and infant mortality in western Kenya with use of insecticide-treated bednets: 4 to 6 years of follow-up. *JAMA*. 2004; **291**(21): 2571–2580. [PubMed Abstract](#) | [Publisher Full Text](#)
- Lengeler C: Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database Syst Rev*. 2004; (2): CD000363. [PubMed Abstract](#) | [Publisher Full Text](#)
- Binka FN, Hodgson A, Adjuik M, *et al.*: Mortality in a seven-and-a-half-year follow-up of a trial of insecticide-treated mosquito nets in Ghana. *Trans R Soc Trop Med Hyg*. 2002; **96**(6): 597–599. [PubMed Abstract](#) | [Publisher Full Text](#)
- ter Kuile FO, Terlouw DJ, Phillips-Howard PA, *et al.*: Impact of permethrin-treated bed nets on malaria and all-cause morbidity in young children in an area of intense perennial malaria transmission in western Kenya: cross-sectional survey. *Am J Trop Med Hyg*. 2003; **68**(4 Suppl): 100–107. [PubMed Abstract](#)
- Phillips-Howard PA, Nahlen BL, Kolczak MS, *et al.*: Efficacy of permethrin-treated bed nets in the prevention of mortality in young children in an area of high perennial malaria transmission in western Kenya. *Am J Trop Med Hyg*. 2003; **68**(4 Suppl): 23–29. [PubMed Abstract](#)
- Noor AM, Moloney G, Borle M, *et al.*: The use of mosquito nets and the prevalence of *Plasmodium falciparum* infection in rural South Central Somalia. *PLoS One*. 2008; **3**(5): e2081. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Snow RW, Rowan KM, Greenwood BM: A trial of permethrin-treated bed nets in the prevention of malaria in Gambian children. *Trans R Soc Trop Med Hyg*. 1987; **81**(4): 563–567. [PubMed Abstract](#) | [Publisher Full Text](#)
- Killeen GF, Govella NJ, Lwetoijera DW, *et al.*: Most outdoor malaria transmission by behaviourally-resistant *Anopheles arabiensis* is mediated by mosquitoes that have previously been inside houses. *Malar J*. 2016; **15**: 225. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Govella NJ, Chaki PP, Killeen GF: Entomological surveillance of behavioural resilience and resistance in residual malaria vector populations. *Malar J*. 2013; **12**: 124. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Mwangangi JM, Mbogo CM, Orindi BO, *et al.*: Shifts in malaria vector species composition and transmission dynamics along the Kenyan coast over the past 20 years. *Malar J*. 2013; **12**: 13. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Mbogo CN, Baya NM, Ofula AV, *et al.*: The impact of permethrin-impregnated bednets on malaria vectors of the Kenyan coast. *Med Vet Entomol*. 1996; **10**(3): 251–259. [PubMed Abstract](#) | [Publisher Full Text](#)
- Bayoh MN, Mathias DK, Odiere MR, *et al.*: *Anopheles gambiae*: historical population decline associated with regional distribution of insecticide-treated bed nets in western Nyanza Province, Kenya. *Malar J*. 2010; **9**: 62. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

22. Mutuku FM, King CH, Mungai P, *et al.*: **Impact of insecticide-treated bed nets on malaria transmission indices on the south coast of Kenya.** *Malar J.* 2011; **10**: 356. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
23. Lindblade KA, Gimnig JE, Kamau L, *et al.*: **Impact of sustained use of insecticide-treated bednets on malaria vector species distribution and culicine mosquitoes.** *J Med Entomol.* 2006; **43**(2): 428–432. [PubMed Abstract](#)
24. Killeen GF, Kihonda J, Lyimo E, *et al.*: **Quantifying behavioural interactions between humans and mosquitoes: evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania.** *BMC Infect Dis.* 2006; **6**: 161. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
25. Gimnig JE, Kolczak MS, Hightower AW, *et al.*: **Effect of permethrin-treated bed nets on the spatial distribution of malaria vectors in western Kenya.** *Am J Trop Med Hyg.* 2003; **68**(4 Suppl): 115–120. [PubMed Abstract](#)
26. Charlwood JD, Alcantara J, Pinto J, *et al.*: **Do bednets reduce malaria transmission by exophagic mosquitoes?** *Trans R Soc Trop Med Hyg.* 2005; **99**(12): 901–904. [PubMed Abstract](#) | [Publisher Full Text](#)
27. Govella NJ, Okumu FO, Killeen GF: **Insecticide-treated nets can reduce malaria transmission by mosquitoes which feed outdoors.** *Am J Trop Med Hyg.* 2010; **82**(3): 415–419. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
28. O'Meara WP, Bejon P, Mwangi TW, *et al.*: **Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya.** *Lancet.* 2008; **372**(9649): 1555–1562. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
29. Okiro EA, Alegana VA, Noor AM, *et al.*: **Malaria paediatric hospitalization between 1999 and 2008 across Kenya.** *BMC Med.* 2009; **7**: 75. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
30. Mogeni P, Williams TN, Fegan G, *et al.*: **Age, Spatial, and Temporal Variations in Hospital Admissions with Malaria in Kilifi County, Kenya: A 25-Year Longitudinal Observational Study.** *PLoS Med.* 2016; **13**(6): e1002047. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
31. Snow RW, Kibuchi E, Karuri SW, *et al.*: **Changing Malaria Prevalence on the Kenyan Coast since 1974: Climate, Drugs and Vector Control.** *PLoS One.* 2015; **10**(6): e0128792. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
32. Scott JA, Bauni E, Moisi JC, *et al.*: **Profile: The Kilifi Health and Demographic Surveillance System (KHDS).** *Int J Epidemiol.* 2012; **41**(3): 650–657. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
33. Wirtz RA, Zavala F, Charoenvit Y, *et al.*: **Comparative testing of monoclonal antibodies against Plasmodium falciparum sporozoites for ELISA development.** *Bull World Health Organ.* 1987; **65**(1): 39–45. [PubMed Abstract](#) | [Free Full Text](#)
34. Scott JA, Brogdon WG, Collins FH: **Identification of single specimens of the Anopheles gambiae complex by the polymerase chain reaction.** *Am J Trop Med Hyg.* 1993; **49**(4): 520–529. [PubMed Abstract](#)
35. Kulldorff M: **A spatial scan statistic.** *Commun Stat Theory.* 1997; **26**(6): 1481–1496. [Publisher Full Text](#)
36. Bermejo A, Veeken H: **Insecticide-impregnated bed nets for malaria control: a review of the field trials.** *Bull World Health Organ.* 1992; **70**(3): 293–296. [PubMed Abstract](#) | [Free Full Text](#)
37. Noor AM, Mutheu JJ, Tatem AJ, *et al.*: **Insecticide-treated net coverage in Africa: mapping progress in 2000–07.** *Lancet.* 2009; **373**(9657): 58–67. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
38. Fegan GW, Noor AM, Akhwale WS, *et al.*: **Effect of expanded insecticide-treated bednet coverage on child survival in rural Kenya: a longitudinal study.** *Lancet.* 2007; **370**(9592): 1035–1039. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
39. Nevill CG, Some ES, Mung'ala VO, *et al.*: **Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast.** *Trop Med Int Health.* 1996; **1**(2): 139–146. [PubMed Abstract](#) | [Publisher Full Text](#)
40. Phillips-Howard PA, Nahlen BL, Alaii JA, *et al.*: **The efficacy of permethrin-treated bed nets on child mortality and morbidity in western Kenya I. Development of infrastructure and description of study site.** *Am J Trop Med Hyg.* 2003; **68**(4 Suppl): 3–9. [PubMed Abstract](#)
41. Mbogo CM, Mwangangi JM, Nzovu J, *et al.*: **Spatial and temporal heterogeneity of Anopheles mosquitoes and Plasmodium falciparum transmission along the Kenyan coast.** *Am J Trop Med Hyg.* 2003; **68**(6): 734–742. [PubMed Abstract](#)
42. Kreuels B, Kobbe R, Adjei S, *et al.*: **Spatial variation of malaria incidence in young children from a geographically homogeneous area with high endemicity.** *J Infect Dis.* 2008; **197**(1): 85–93. [PubMed Abstract](#) | [Publisher Full Text](#)
43. Githeko AK, Adungo NI, Karanja DM, *et al.*: **Some observations on the biting behavior of Anopheles gambiae s.s., Anopheles arabiensis, and Anopheles funestus and their implications for malaria control.** *Exp Parasitol.* 1996; **82**(3): 306–315. [PubMed Abstract](#)

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Version 1

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Sarah J. Moore

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The authors have conducted a study addressing the hypothesis that outdoor feeding of mosquitoes undermines the effectiveness of ITNs.

The entomology presented in the paper is inadequate to answer the hypothesis presented for the following reasons:

1. 5 years of clinical data are presented (2009-2014) but only one month of mosquito sampling is conducted in 2016, two years after the last piece of clinical data was collected.
2. No PCR speciation was reported. In this area there are a number of cryptic species that look the same but differ in both their behaviour and their ability to transmit malaria. No molecular techniques were used to test the mosquito species. So you could have a switch from *An. gambiae* s.s. that bites indoors and has high vectorial competence to *An. arabiensis* that bites outdoors and has lower vectorial competence. The same is true in the *An. funestus* complex that is comprised of a number of outdoor biting species like *An. lesoni* or *An. rivulorum*.
3. The authors reported that PCR (polymerase chain reaction) was done on the mosquitoes yet I cannot find data in the paper reporting the outcome of the PCR. All data reports *An. gambiae* s.l. and *An. funestus* group.

The paper explores changing mosquito behaviour with lowered effectiveness of nets but only used one month of vector collections two years after the clinical data was collected to test this link and the actual species present are not reported. I therefore find this a big stretch of the data. Vector density, composition and behaviour varies throughout the year and these collections were made for a short time. I therefore don't think the data are sufficient to accept or reject the hypothesis.

That being said the rest of the data is very useful and nicely presented. The data do demonstrate that there is substantial outdoor biting in June/July, and I should like to see the species composition in the area seeing as the authors report that the PCR was done. Outdoor biting may not increase malaria if the

vectors doing the outdoor biting are not very competent for malaria.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical entomology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 06 Jan 2018

Alice Kamau, Centre for Geographic Medicine Research-Coast, Kilifi, Kenya

We are grateful for this review and the helpful comments and suggestions that have been made. We have included a point-by-point response (in bold) to the issues raised.

Q1) 5 years of clinical data are presented (2009-2014) but only one month of mosquito sampling is conducted in 2016, two years after the last piece of clinical data was collected.

A1: We have updated the clinical surveillance data to December 2016 and updated the manuscript accordingly.

Q2) No PCR speciation was reported. In this area there are a number of cryptic species that look the same but differ in both their behaviour and their ability to transmit malaria. No molecular techniques were used to test the mosquito species. So you could have a switch from *An. gambiae* s.s. that bites indoors and has high vectorial competence to *An. arabiensis* that bites outdoors and has lower vectorial competence. The same is true in the *An. funestus* complex that is comprised of a number of outdoor biting species like *An. leesonii* or *An. rivulorum*.

A2: We have included data obtained from the ELISA-CSP and molecular analysis in the

results section. The mosquitoes were differentiated to species as shown under the result section. However, we do not have molecular data for the *An. funestus* group. We have included this as a limitation in the discussion section as shown below

Result section

“Over 26 nights, 415 female *Anopheles* mosquitoes were collected by both methods (i.e. 272 by HLC and 143 by CDC-LT), representing a mean of 16 mosquitoes per night. 66% of mosquitoes were collected using HLC. Of the 415 mosquitoes morphologically identified, 311 (75%) were *An. funestus* group, 84 (20%) were *An. gambiae* s.l. and 20 (5%) were other *Anopheles* i.e. *An. protoriensis*, *An. coustani*, *An. moucheti* and *An. squamosus* (Table 2). The *An. funestus* group was significantly greater than *An. gambiae* s.l. ($p < 0.001$). Out of the 84 amplified samples of *An. gambiae* s.l., 68 (81%) were *An. Arabiensis* and 16 (19%) were *An. gambiae* s.s. The proportion of *Anopheles* mosquitoes caught outdoors (60%; 95% CI: 55%, 65%) was significantly greater than the proportion caught indoors ($p < 0.001$). There were more *Anopheles* mosquitoes collected outdoors in all geographical areas except area 6, where most of the mosquitoes were collected indoor (Table 2). The frequencies of vectors collected in each geographical area are summarized in Supplementary Table 2. *An. funestus* group was the most prevalent vector in all areas. Of the 272 mosquitoes collected by HLC, 3.3% (9/272) tested positive for *P. falciparum* sporozoites. Higher sporozoite rate was observed among the *An. funestus* group (7/9). The rate of indoor and outdoor biting estimated by HLC was 19.8 and 25.5 bites per person per night, respectively.”

Discussion section

“Lack of explicit molecular data for distinguishing sibling species and molecular forms within the *An. funestus* group introduces ambiguity into the interpretation of the results of the study.”

Q3) The authors reported that PCR (polymerase chain reaction) was done on the mosquitoes yet I cannot find data in the paper reporting the outcome of the PCR. All data reports *An. gambiae* s.l. and *An. funestus* group.

A3: We have addressed this comment as shown above.

Q4) The paper explores changing mosquito behaviour with lowered effectiveness of nets but only used one month of vector collections two years after the clinical data was collected to test this link and the actual species present are not reported. I therefore find this a big stretch of the data. Vector density, composition and behaviour varies throughout the year and these collections were made for a short time. I therefore don't think the data are sufficient to accept or reject the hypothesis.

A4: We have updated the clinical surveillance data to December 2016 and updated the manuscript accordingly. We have included the limitation of the one month vector collection in the discussion section as shown below.

Discussion section

“The size of our study limits power: with a sample size of 415, and the proportion of mosquitoes biting outdoors at 67% in low ITN effectiveness areas we therefore had >90% power to detect a reduction to 27% or lower in high ITN effectiveness areas. Our study

was therefore powered to detect only a large difference in the proportion of vectors caught outdoors. However, we reasoned that reductions of ITN effectiveness to less than half of the previously documented efficacy of 50% would require a doubling of the proportion of mosquitoes feeding outdoors. Hence our study was powered to detect large variations in the frequency of outdoor biting. In addition, the accuracy of mosquito sampling data is limited as only one month of sampling was conducted in this study, we recommend sampling for a longer duration of time.”

Q5) That being said the rest of the data is very useful and nicely presented. The data do demonstrate that there is substantial outdoor biting in June/July, and I should like to see the species composition in the area seeing as the authors report that the PCR was done. Outdoor biting may not increase malaria if the vectors doing the outdoor biting are not very competent for malaria.

A5: We have addressed this comment as shown above.

Competing Interests: No competing interests were disclosed.

Reviewer Report 18 May 2017

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Gerry F Killeen 

Environmental Health and Ecological Sciences Thematic group, Ifakara Health Institute, Dar es Salaam, Tanzania

Apart from some unfortunately important exceptions, the data for this study are meticulously collected and analysed. However, many of the most important results are either over-interpreted or misinterpreted so these exceptions are substantive. In fact, the correct interpretation may well be almost the exact opposite of that presented here: That LLINs are consistently effective across a landscape where transmission is dominated by a vector that primarily attacks people indoors at night while they are asleep.

1. The biggest single problem with this paper is that the indoor and outdoor biting rate estimates come from stationary, fully exposed human volunteers exhibiting artificial experimental behaviours, without adjusting them for normal human behaviours that mean most of us are indoors asleep during the peak biting hours of nocturnal African malaria vector mosquitoes. This is an understandable and common mistake, but a very important one. Like *Anopheles funestus* in most locations across Africa, the 55-45 distribution of biting location preference for this population is essentially indiscriminate, so it is the behaviour of humans that determines where exposure actually occurs. So unless everyone in coastal Kenya sleeps half indoors and half outdoors throughout the night, simply comparing indoor versus outdoor HLCs is misrepresentative and greatly exaggerates the contribution of outdoor biting to transmission by this species. Once adjusted for human behaviour patterns, >90% of human biting exposure to this key vector species is consistently estimated to occur indoors in the absence of some protective measures at locations

scattered all across Africa [1]. Unless human behaviour on the coast of Kenya is far more exophilic (everyone sleeps outdoors?) than all the other human populations we have data for, there is nothing in the data presented that is unusual or that convince me this vector population behaves differently from *Anopheles funestus* elsewhere. The logical conclusion of this paper (albeit with some additional data and analyses to support it) is that, unsurprisingly, there is little difference in the effectiveness of nets across landscapes dominated by the same vector that primarily encounters people indoors at night while they are asleep and can use a net.

2. The most important data clearly missing from the characterization of the study scenario are (a) spoorozoite rates (mentioned in the methods but not the results) and EIR estimates, to confirm that *Anopheles funestus* group mosquitoes are the most important vectors of malaria in this area, (b) quantitative estimates of where and when humans are exposed to these two major vector taxa (not species unless PCR data are added) that weight the biting estimates by surveys of human behaviour [2-5]. These are increasingly common calculations applied to data from all over the tropics [6-13], and vitally important to conduct before making any quantitative statements about proportional contributions of outdoor biting exposure.
3. There is no evidence of any “shift” in behaviours over time presented here, so the term “undermines” is unjustified and seems to create an argument that hasn’t been made. Most behaviours that enable residual malaria transmission despite LLIN use are pre-existing, although plastic, and often it’s just the vector population composition that shifts 14, so the term “limits” is more appropriate.
4. While indeed there is no evidence here that outdoor transmission contributes to ongoing transmission, there is also no evidence that it does not. Such outdoor fractions of transmission can only be expected to become epidemiologically detectable once larger quantities of indoor transmission (which I’m convinced is the case here as explained above) have been tackled. So the phraseology of conclusions needs to be tempered using words like “yet”, and explain how these currently minor fractions of transmission may emerge as important contributors to sustained endemicity once further progress has been made with indoor control [14,15].
5. In any case, LLINs clearly fall a long way short of being 100% efficacious with 22% personal protection estimated here, so there clearly are considerable limitations to this technology that need explanation. To get a better handle on whether outdoor exposure does contribute to residual transmission, in our experience it’s necessary to test as a function of individual human behavioural profiles weighted by activity patterns for the most dominant local vectors [13]. Indeed human behaviour is the primary driver of where and when exposure occurs [1] and is far more variable than the mosquito behaviours that matter within a single vector species [15].
6. In any case, for many of the surveyed locations, very few mosquitoes were caught (Supplementary Table 2) and CDC light traps catches indoors and outdoors are not comparable, so reporting these data as indicators of the degree of exophagy or endophagy is going too far and overstretching very little entomological data.
7. The fact that these are not differentiated to species (again, though this is mentioned in the methods but no results are presented) also means that areas with apparently different mosquito behaviours are probably areas that simply have different relative abundances of primary vector, secondary vector and non-vector species within the *Anopheles funestus* group and within the *Anopheles gambiae* complex. For example, greater outdoor feeding at dawn and dusk is a known

characteristic of *Anopheles rivulorum* and *Anopheles parensis*, originally discovered in this region on the basis of their distinctive behaviours and much weaker vectorial capacities.

8. The term “species” is used very loosely and interchangeably with other taxonomic classification levels, resulting in some misleading over-interpretation. While *Anopheles gambiae sensu lato* is indeed a complex, *Anopheles funestus sensu lato* is a group (not a complex, as stated in the introduction) and neither can be described as a species, unless one is talking about unambiguously identified individual specimens of the nominate species, which are by far the most efficient species within each taxon.
9. All of these most important limitations seem to be missing from the paragraph opening with the sentence “Our study has some limitations”.
10. What is called “effectiveness” here refers only to the relatively minor personal protection effect of bednets, and does not capture any variations in community-level impact. All fine but please explain this study limitation clearly.
11. Correspondingly, doesn’t capture how big a change this transmission picture is relative to the same setting 10 to 15 years ago when nominate *Anopheles gambiae* were still quite abundant. The explanations about the relative abundance of vector taxa (not species) is accurate but rather static and lacking in long term context, demonstrating the much bigger overall impact on vector populations and endemicity. This is a pity when this contemporary study has been conducted in an area with so much historical entomological literature, so please enrich the narrative.
12. While I agree with the closing statement about enhancing entomological surveillance, in my experience many groups are under-interpreting or misinterpreting the data they already have, so perhaps that capacity limitation merits some emphasis as a priority.

References

1. Huho B, Briët O, Seyoum A, Sikaala C, et al.: Consistently high estimates for the proportion of human exposure to malaria vector populations occurring indoors in rural Africa. *Int J Epidemiol*. 2013; **42** (1): 235-47 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Killeen GF, Kihonda J, Lyimo E, Oketch FR, et al.: Quantifying behavioural interactions between humans and mosquitoes: evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania. *BMC Infect Dis*. 2006; **6**: 161 [PubMed Abstract](#) | [Publisher Full Text](#)
3. Seyoum A, Sikaala CH, Chanda J, Chinula D, et al.: Human exposure to anopheline mosquitoes occurs primarily indoors, even for users of insecticide-treated nets in Luangwa Valley, South-east Zambia. *Parasit Vectors*. 2012; **5**: 101 [PubMed Abstract](#) | [Publisher Full Text](#)
4. Killeen GF: A second chance to tackle African malaria vector mosquitoes that avoid houses and don't take drugs. *Am J Trop Med Hyg*. 2013; **88** (5): 809-16 [PubMed Abstract](#) | [Publisher Full Text](#)
5. Killeen GF: Characterizing, controlling and eliminating residual malaria transmission. *Malar J*. 2014; **13**: 330 [PubMed Abstract](#) | [Publisher Full Text](#)
6. Bradley J, Lines J, Fuseini G, Schwabe C, et al.: Outdoor biting by *Anopheles* mosquitoes on Bioko Island does not currently impact on malaria control. *Malar J*. 2015; **14**: 170 [PubMed Abstract](#) | [Publisher Full Text](#)
7. Moiroux N, Damien GB, Egrot M, Djenontin A, et al.: Human exposure to early morning *Anopheles funestus* biting behavior and personal protection provided by long-lasting insecticidal nets. *PLoS One*. 2014; **9** (8): e104967 [PubMed Abstract](#) | [Publisher Full Text](#)

8. Geissbühler Y, Chaki P, Emidi B, Govella NJ, et al.: Interdependence of domestic malaria prevention measures and mosquito-human interactions in urban Dar es Salaam, Tanzania. *Malar J.* 2007; **6**: 126 [PubMed Abstract](#) | [Publisher Full Text](#)
9. Govella NJ, Okumu FO, Killeen GF: Insecticide-treated nets can reduce malaria transmission by mosquitoes which feed outdoors. *Am J Trop Med Hyg.* 2010; **82** (3): 415-9 [PubMed Abstract](#) | [Publisher Full Text](#)
10. Bugoro H, Cooper RD, Butafa C, Iro'ofa C, et al.: Bionomics of the malaria vector *Anopheles farauti* in Temotu Province, Solomon Islands: issues for malaria elimination. *Malar J.* 2011; **10**: 133 [PubMed Abstract](#) | [Publisher Full Text](#)
11. Russell TL, Govella NJ, Azizi S, Drakeley CJ, et al.: Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malar J.* 2011; **10**: 80 [PubMed Abstract](#) | [Publisher Full Text](#)
12. Russell TL, Beebe NW, Bugoro H, Apairamo A, et al.: Frequent blood feeding enables insecticide-treated nets to reduce transmission by mosquitoes that bite predominately outdoors. *Malar J.* 2016; **15**: 156 [PubMed Abstract](#) | [Publisher Full Text](#)
13. Msellemu D, Namango HI, Mwakalinga VM, Ntamatungiro AJ, et al.: The epidemiology of residual *Plasmodium falciparum* malaria transmission and infection burden in an African city with high coverage of multiple vector control measures. *Malar J.* 2016; **15** (1): 288 [PubMed Abstract](#) | [Publisher Full Text](#)
14. Govella NJ, Chaki PP, Killeen GF: Entomological surveillance of behavioural resilience and resistance in residual malaria vector populations. *Malar J.* 2013; **12**: 124 [PubMed Abstract](#) | [Publisher Full Text](#)
15. Killeen GF, Marshall JM, Kiware SS, South AB, et al.: Measuring, manipulating and exploiting behaviours of adult mosquitoes to optimise malaria vector control impact. *BMJ Global Health.* 2017; **2** (2): p.e000212

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 09 Jan 2018

Alice Kamau, Centre for Geographic Medicine Research-Coast, Kilifi, Kenya

We are grateful for this review and the helpful comments and suggestions that have been made. We have included a point-by-point response (in bold) to the issues raised.

Q1) The biggest single problem with this paper is that the indoor and outdoor biting rate estimates come from stationary, fully exposed human volunteers exhibiting artificial experimental behaviours, without adjusting them for normal human behaviours that mean most of us are indoors asleep during the peak biting hours of nocturnal African malaria vector mosquitoes. This is an understandable and common mistake, but a very important one. Like *Anopheles funestus* in most locations across Africa, the 55-45 distribution of biting location preference for this population is essentially indiscriminate, so it is the behaviour of humans that determines where exposure actually occurs. So unless everyone in coastal Kenya sleeps half indoors and half outdoors throughout the night, simply comparing indoor versus outdoor HLCs is misrepresentative and greatly exaggerates the contribution of outdoor biting to transmission by this species. Once adjusted for human behaviour patterns, >90% of human biting exposure to this key vector species is consistently estimated to occur indoors in the absence of some protective measures at locations scattered all across Africa [1]. Unless human behaviour on the coast of Kenya is far more exophilic (everyone sleeps outdoors?) than all the other human populations we have data for, there is nothing in the data presented that is unusual or that convince me this vector population behaves differently from *Anopheles funestus* elsewhere. The logical conclusion of this paper (albeit with some additional data and analyses to support it) is that, unsurprisingly, there is little difference in the effectiveness of nets across landscapes dominated by the same vector that primarily encounters people indoors at night while they are asleep and can use a net.

A1: We have made adjustments to the indoor and outdoor biting rate by human behavior as follows in the following sections:

Methods section

“To determine the human-mosquito contact, we administered questionnaires to 304 randomly selected households in the six selected areas between September and October 2016. We asked the household head time when each household member went to sleep and the time they woke up. Data on human behaviour was used to make adjustments to the indoor and outdoor biting rate.”

Statistical analysis section

“Questionnaire data about the time household members went to sleep and at what time they woke up were combined with human landing catches measurements of hourly rates for indoor and outdoor biting. We estimated the proportion of human exposure to mosquito bites occurring indoors (π_s) by taking into consideration the movement pattern of people using the following method [1]: by weighting the mean indoor and outdoor biting rates throughout the night by the proportion of humans reporting to have gone to sleep at each hour of the night as follows;

$$\pi_s = \frac{\sum (B_{i,t} S_t)}{\sum (B_{i,t} S_t + B_{o,t} (1 - S_t))} \quad (1)$$

Where:

= an estimate of human exposure to bites which occurs when residents are both indoors and sleeping

S_t = the proportion of humans indoors reporting to have gone to sleep at each hour of the night (t)

$B_{i,t}$ = mean indoor biting rate at each hour of the night (t)

$B_{o,t}$ = mean outdoor biting rates at each hour of the night (t)

$(1-S_t)$ = proportion of humans not yet asleep at each hour of the night.”

Result section

“Seventy three percent of children <5 years were reported to be asleep between 6 pm and 9 pm, these rose monotonically over the course of the night reaching 100% by 10 pm (Table 4 & Figure 6). Children aged between 6-14 years spent more time awake, only 45% were asleep before 9 pm (Figure 6 & Supplementary Table 3). Human landing catches are not sufficient in themselves to survey normal human exposure to mosquito bite. The timing of human activity and sleeping behaviour in particular modulates the effect of human-mosquito contact and the effectiveness of ITN. We quantified the interaction between mosquitoes and humans to evaluate whether outdoor vector biting is a potential explanation for the variation in ITN effectiveness. The peak biting activity for each mosquito vector is illustrated in Figure 7. Clearly higher indoor biting activity was observed for the *An. funestus* group. The overall propensity to feed at times when most people are indoor was high (Figure 8): the vast majority of the *Anopheles* mosquitoes were caught at times when most people are indoors (Figure 7). Estimates for the proportion of human-mosquito contact between the first and last hour when most humans were indoors was consistently high, ranging from 0.83 to 1.00. Therefore, the estimated proportion of exposure to *Anopheles* mosquito bites that occurred indoor was high.”

Discussion section

It is possible that a higher proportion of mosquitoes caught outdoors represents a behavioral response to unsuccessful feeding attempts made indoors during the night, and therefore it may simply be a marker of successful ITN use. This avoidance behavior may exert a cost on the vector, and so ITNs may in fact still be protective in areas where outdoor biting is observed, as has been suggested previously [2]. Furthermore, outdoor biting exposure and the probability of successful feeding outdoors cannot be directly inferred from the human landing catches, since the landing catches are not in themselves sufficient to survey pattern of normal human exposure to mosquito bite. Once adjusted for human behaviour, most human-vector interaction in this study occurred indoors (Figure 8 & Supplementary Table 3). Outdoor biting is currently not a major factor influencing residual malaria transmission since 95% of the population are indoors at the peak biting period for malaria vector mosquitoes. Human behaviour is the primary driver of when and where exposure occurs and is far more variable than the mosquito behaviour that matter within a single vector species [3].

Q2) The most important data clearly missing from the characterization of the study scenario are (a) sporozoite rates (mentioned in the methods but not the results) and EIR estimates, to confirm that *Anopheles funestus* group mosquitoes are the most important vectors of malaria in this area, (b) quantitative estimates of where and when humans are exposed to these two major vector taxa (not species unless PCR data are added) that weight the biting estimates by surveys of human behaviour [2-5]. These are increasingly common calculations applied to data from all over the

tropics [6-13], and vitally important to conduct before making any quantitative statements about proportional contributions of outdoor biting exposure.

A2: We have included data obtained from the ELISA-CSP and molecular analysis. We have also added data on sporozoite rate as shown below in the result section.

Result section

“Over 26 nights, 415 female *Anopheles* mosquitoes were collected by both methods (i.e. 272 by HLC and 143 by CDC-LT), representing a mean of 16 mosquitoes per night. 66% of mosquitoes were collected using HLC. Of the 415 mosquitoes morphologically identified, 311 (75%) were *An. funestus* group, 84 (20%) were *An. gambiae* s.l. and 20 (5%) were other *Anopheles* i.e. *An. protoriensis*, *An. coustani*, *An. moucheti* and *An. squamosus* (Table 2). The *An. funestus* group was significantly greater than *An. gambiae* s.l. ($p < 0.001$). Out of the 84 amplified samples of *An. gambiae* s.l., 68 (81%) were *An. Arabiensis* and 16 (19%) were *An. gambiae* s.s. The proportion of *Anopheles* mosquitoes caught outdoors (60%; 95% CI: 55%, 65%) was significantly greater than the proportion caught indoors ($p < 0.001$). There were more *Anopheles* mosquitoes collected outdoors in all geographical areas except area 6, where most of the mosquitoes were collected indoor (Table 2). The frequencies of vectors collected in each geographical area are summarized in Supplementary Table 2. *An. funestus* group was the most prevalent vector in all areas. Of the 272 mosquitoes collected by HLC, 3.3% (9/272) tested positive for *P. falciparum* sporozoites. Higher sporozoite rate was observed among the *An. funestus* group (7/9). The rate of indoor and outdoor biting estimated by HLC was 19.8 and 25.5 bites per person per night, respectively.”

Q3) There is no evidence of any “shift” in behaviours over time presented here, so the term “undermines” is unjustified and seems to create an argument that hasn’t been made. Most behaviours that enable residual malaria transmission despite LLIN use are pre-existing, although plastic, and often it’s just the vector population composition that shifts 14, so the term “limits” is more appropriate.

A3: We have revised as proposed above in the abstract section.

Conclusion

“Our data therefore do not support the hypothesis that outdoor biting limits the effectiveness of ITNs in our study area.”

Q4) While indeed there is no evidence here that outdoor transmission contributes to ongoing transmission, there is also no evidence that it does not. Such outdoor fractions of transmission can only be expected to become epidemiologically detectable once larger quantities of indoor transmission (which I’m convinced is the case here as explained above) have been tackled. So the phraseology of conclusions needs to be tempered using words like “yet”, and explain how these currently minor fractions of transmission may emerge as important contributors to sustained endemicity once further progress has been made with indoor control [14,15].

A4: We have revised as proposed above in the discussion section.

Discussion section

“In summary, our data do not support the hypothesis that outdoor biting limits the

effectiveness of ITNs in our study area. The outdoor biting observed may therefore have been the result of high levels of ITN use leading to unsuccessful attempts at indoor feeding. However, it remains possible that continued selection pressures might lead to the emergence of populations of mosquitoes that are better adapted to outdoor feeding in the future. Outdoor feeding is becoming more common in parts of Africa [4] and may represent evolutionary change in some areas, with a potential to undermine ITN effectiveness. The outdoor fractions of transmission can be expected to be epidemiologically detectable once indoor transmission has been tackled. Therefore, malaria control programs require monitoring to assess the impact of ITNs on vector populations and vector behavioral change as well as monitoring ITN effectiveness as vectors evolve [5-9]. Continuous monitoring of vector bionomics, and malaria transmission dynamics are essential for predicting disease outbreaks and guiding vector control in the region. Furthermore, capacity needs to be built in interpreting and applying these data to malaria control policy.”

Q5) In any case, LLINs clearly fall a long way short of being 100% efficacious with 22% personal protection estimated here, so there clearly are considerable limitations to this technology that need explanation. To get a better handle on whether outdoor exposure does contribute to residual transmission, in our experience it's necessary to test as a function of individual human behavioural profiles weighted by activity patterns for the most dominant local vectors [13]. Indeed human behaviour is the primary driver of where and when exposure occurs [1] and is far more variable than the mosquito behaviours that matter within a single vector species [15].

A5: We have made adjustments to the indoor and outdoor biting rate by human behavior as shown above, and accordingly revised the discussion as above.

Q6) In any case, for many of the surveyed locations, very few mosquitoes were caught (Supplementary Table 2) and CDC light traps catches indoors and outdoors are not comparable, so reporting these data as indicators of the degree of exophagy or endophagy is going too far and overstretching very little entomological data.

A6: We have made revision in the result and discussion section as shown above.

Q7) The fact that these are not differentiated to species (again, though this is mentioned in the methods but no results are presented) also means that areas with apparently different mosquito behaviours are probably areas that simply have different relative abundances of primary vector, secondary vector and non-vector species within the *Anopheles funestus* group and within the *Anopheles gambiae* complex. For example, greater outdoor feeding at dawn and dusk is a known characteristic of *Anopheles rivulorum* and *Anopheles parensis*, originally discovered in this region on the basis of their distinctive behaviours and much weaker vectorial capacities.

A7: We have addressed the above comment as follows:

We have included Figure 7 which illustrates hourly biting pattern of *Anopheles* mosquitoes occurring both indoors (solid lines) and outdoors (dashed lines). The grey area represents the proportion of the children <5 years asleep at each hour of the night.

We do not have molecular data for the *An. funestus* group. We have included this as a limitation in the discussion section as shown below.

Discussion section

“Lack of explicit molecular data for distinguishing sibling species and molecular forms within the *An. funestus* group introduces ambiguity into the interpretation of the results of the study.”

Q8) The term “species” is used very loosely and interchangeably with other taxonomic classification levels, resulting in some misleading over-interpretation. While *Anopheles gambiae* sensu lato is indeed a complex, *Anopheles funestus* sensu lato is a group (not a complex, as stated in the introduction) and neither can be described as a species, unless one is talking about unambiguously identified individual specimens of the nominate species, which are by far the most efficient species within each taxon.

A8: We have made revisions accordingly.

Q9) All of these most important limitations seem to be missing from the paragraph opening with the sentence “Our study has some limitations”.

A9: We have updated the manuscript with the adjustments to the indoor and outdoor biting rate by human behaviour as shown above. We have also updated the limitations of our study as shown under the discussion section.

Discussion section

“Our study has a number of limitations. Data on ITN use may have been incorrectly reported, as we did not require each resident to be present during the survey. We attempted to minimize this by instructing data collecting teams to interview only residents of the same homestead regarding ITN ownership and usage. There may have been some misclassification as we did not ascertain ITN use during hospital presentation but instead used the yearly ITN data collected by the annual survey. The results may also be confounded by other unmeasured factors (e.g., variation in the quality and type of ITN, urbanization, socio-economic status and mother’s education). It is likely that we underestimated the protection afforded by the use of high-quality ITN because we included all ITNs, regardless of quality, physical integrity or bioefficacy of the insecticidal compounds. The vast majority of ITNs in the area are long-lasting insecticidal nets, hence we do not expect substantial variation in insecticidal efficacy. The accuracy of the mosquito survey is limited by the practical challenges of maintaining consistently sensitive human landing catches throughout the night. Lack of explicit molecular data for distinguishing sibling species and molecular forms within the *An. funestus* group introduces ambiguity into the interpretation of the results of the study. In this study, we examined variations in the personal protection afforded by ITNs and did not examine variation in community level effect. The size of our study limits power: with a sample size of 415, and the proportion of mosquitoes biting outdoors at 67% in low ITN effectiveness areas we therefore had >90% power to detect a reduction to 27% or lower in high ITN effectiveness areas. Our study was therefore powered to detect only a large difference in the proportion of vectors caught outdoors. However, we reasoned that reductions of ITN effectiveness to less than half of the previously documented efficacy of 50% would require a doubling of the proportion of mosquitoes feeding outdoors. Hence our study was powered to detect large variations in the frequency of outdoor biting. In addition, the accuracy of mosquito sampling data is limited as only one month of sampling was

conducted in this study, we recommend sampling for a longer duration of time.”

Q10) What is called “effectiveness” here refers only to the relatively minor personal protection effect of bednets, and does not capture any variations in community-level impact. All fine but please explain this study limitation clearly.

A10: We have made revision in the discussion section indicating this limitation as follows.

Discussion section

In this study, we examined variations in the personal protection afforded by ITNs and did not examine variation in community level effect.

Q11) Correspondingly, doesn't capture how big a change this transmission picture is relative to the same setting 10 to 15 years ago when nominate *Anopheles gambiae* were still quite abundant. The explanations about the relative abundance of vector taxa (not species) is accurate but rather static and lacking in long term context, demonstrating the much bigger overall impact on vector populations and endemicity. This is a pity when this contemporary study has been conducted in an area with so much historical entomological literature, so please enrich the narrative.

A11: We have added points as follows in the discussion section;

“Malaria transmission has reduced dramatically over the last 15 years in Kilifi, evidenced by falling rates of clinical malaria cases in hospital [10, 11] in the community [12] and falling community prevalence of asymptomatic infection [13]. A recent resurgence has been noted with increasing cases among older children, and increasing prevalence of infection more widely around the coast [14]. The reductions have been temporally associated with marked reductions in the prevalence of the abundance of vectors [15] and with a pronounced shift away from *Anopheles gambiae s.s.*, which was previously the dominant vector, and a shift away from *Anopheles arabiensis.*”

Q12) While I agree with the closing statement about enhancing entomological surveillance, in my experience many groups are under-interpreting or misinterpreting the data they already have, so perhaps that capacity limitation merits some emphasis as a priority.

A12: We have added a statement in the summary section as shown below.

“Furthermore, capacity needs to be built in interpreting and applying these data to malaria control policy.”

References

1. Seyoum A, Sikaala CH, Chanda J, Chinula D, Ntamatungiro AJ, Hawela M, Miller JM, Russell TL, Briet OJ, Killeen GF: **Human exposure to anopheline mosquitoes occurs primarily indoors, even for users of insecticide-treated nets in Luangwa Valley, South-east Zambia.** *Parasit Vectors* 2012, **5**:101.
2. Govella NJ, Okumu FO, Killeen GF: **Insecticide-treated nets can reduce malaria transmission by mosquitoes which feed outdoors.** *Am J Trop Med Hyg* 2010, **82**:415-419.
3. Huho B, Briet O, Seyoum A, Sikaala C, Bayoh N, Gimnig J, Okumu F, Diallo D, Abdulla

- S, Smith T, Killeen G: **Consistently high estimates for the proportion of human exposure to malaria vector populations occurring indoors in rural Africa.** *Int J Epidemiol* 2013, **42**: 235-247.
4. Githeko AK, Adungo NI, Karanja DM, Hawley WA, Vulule JM, Seroney IK, Ofulla AV, Atieli FK, Ondijo SO, Genga IO, et al: **Some observations on the biting behavior of *Anopheles gambiae* s.s., *Anopheles arabiensis*, and *Anopheles funestus* and their implications for malaria control.** *Exp Parasitol* 1996, **82**:306-315.
 5. Mutuku FM, King CH, Mungai P, Mbogo C, Mwangangi J, Muchiri EM, Walker ED, Kitron U: **Impact of insecticide-treated bed nets on malaria transmission indices on the south coast of Kenya.** *Malar J* 2011, **10**:356.
 6. Bayoh MN, Mathias DK, Odiere MR, Mutuku FM, Kamau L, Gimnig JE, Vulule JM, Hawley WA, Hamel MJ, Walker ED: ***Anopheles gambiae*: historical population decline associated with regional distribution of insecticide-treated bed nets in western Nyanza Province, Kenya.** *Malar J* 2010, **9**:62.
 7. Gimnig JE, Kolczak MS, Hightower AW, Vulule JM, Schoute E, Kamau L, Phillips-Howard PA, ter Kuile FO, Nahlen BL, Hawley WA: **Effect of permethrin-treated bed nets on the spatial distribution of malaria vectors in western Kenya.** *Am J Trop Med Hyg* 2003, **68**:115-120.
 8. Russell TL, Govella NJ, Azizi S, Drakeley CJ, Kachur SP, Killeen GF: **Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania.** *Malar J* 2011, **10**:80.
 9. Killeen GF, Kihonda J, Lyimo E, Oketch FR, Kotas ME, Mathenge E, Schellenberg JA, Lengeler C, Smith TA, Drakeley CJ: **Quantifying behavioural interactions between humans and mosquitoes: evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania.** *BMC Infect Dis* 2006, **6**:161.
 10. Mogeni P, Williams TN, Fegan G, Nyundo C, Bauni E, Mwai K, Omedo I, Njuguna P, Newton CR, Osier F, et al: **Age, Spatial, and Temporal Variations in Hospital Admissions with Malaria in Kilifi County, Kenya: A 25-Year Longitudinal Observational Study.** *PLoS Med* 2016, **13**:e1002047.
 11. O'Meara WP, Bejon P, Mwangi TW, Okiro EA, Peshu N, Snow RW, Newton CR, Marsh K: **Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya.** *Lancet* 2008, **372**:1555-1562.
 12. Mwangi TW, Ross A, Snow RW, Marsh K: **Case definitions of clinical malaria under different transmission conditions in Kilifi District, Kenya.** *J Infect Dis* 2005, **191**:1932-1939.
 13. Mogeni P, Williams TN, Omedo I, Kimani D, Ngoi JM, Mwacharo J, Morter R, Nyundo C, Wambua J, Nyangweso G: **Detecting Malaria Hotspots: a comparison between RDT, Microscopy and Polymerase Chain Reaction.** *The Journal of Infectious Diseases* 2017.
 14. Snow RW, Kibuchi E, Karuri SW, Sang G, Gitonga CW, Mwandawiro C, Bejon P, Noor AM: **Changing Malaria Prevalence on the Kenyan Coast since 1974: Climate, Drugs and Vector Control.** *PLoS One* 2015, **10**:e0128792.
 15. Mwangangi JM, Mbogo CM, Orindi BO, Muturi EJ, Midega JT, Nzovu J, Gatakaa H, Githure J, Borgemeister C, Keating J, Beier JC: **Shifts in malaria vector species composition and transmission dynamics along the Kenyan coast over the past 20 years.** *Malar J* 2013, **12**:13.

Competing Interests: No competing interests were disclosed.

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The authors present here the study on the variation of the effectiveness of insecticide-treated bed nets against malaria and the outdoor biting by vectors in Kilifi, Kenya.

The manuscript reported the geographical heterogeneity of malaria prevalence according several parameters mainly including the ITN effectiveness and the feeding behaviour of *Anopheles* vectors. The design and method of the study are well presented in the section “Methods” as well as the statistical analysis. Clinical surveillance was analyzed in the study between January 2009 and December 2014 that covers a long period. Thus it will be interesting if authors add in their explanatory factors the dry and wet season. It will be also important to explain the discrepancy between the date of clinical surveillance data collection (January 2009 and December 2014) and the mosquito collection (July and August 2016). We have any informations if the level of ITN use varied or is the same during both periods.

Additionally the main part of the subject underlines the effectiveness of the ITNs. However, authors should describe at first that the effectiveness of ITNs is monitoring taking into account the physical integrity of nets, bioefficacy and the insecticidal compounds even though they focused more their study on feeding place and malaria prevalence. It will be also more appropriate if authors interpreted their result according to level of ITNs use according to areas and discuss though their outcomes the effectiveness of ITN. For instance in the abstract the expression of “*high and low effectiveness*” in the part of method is a hasty affirmation.

In the section of “Results” I think that the Supplementary Table 1 has to be presented in the main manuscript as it present malaria prevalence according to area and the level of ITNs use. Moreover the presentation of results must be more detailed and the effect of each risk factors cited in the part of “Statistical analysis” must be presented. I don’t understand why authors said “*ITN use was consistently >50% in all geographical areas*”, meaning that here we have no information about the difference of level use between areas. The authors have summarized too much the description of the results in this part. Authors presented in the part “Mosquito processing” laboratory works such ELISA-CSP and molecular analysis, however the results of these analysis have not been presented in this study. Regarding the result on vector abundance, authors have to present the results according to absolute densities and less on the proportion of species in the place of mosquito collection.

The relevance of the study will be more remarkable if authors greatly discuss in deep their outcomes by comparing with other studies. Additionally, the review of the literature has to be strengthened, “33 off the 43 references are more than 5 years old and some newer papers are missing.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Entomology, immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 06 Jan 2018

Alice Kamau, Centre for Geographic Medicine Research-Coast, Kilifi, Kenya

We are grateful for this review and the helpful comments and suggestions that have been made. We have included a point-by-point response (in bold) to the issues raised.

Q1) The manuscript reported the geographical heterogeneity of malaria prevalence according several parameters mainly including the ITN effectiveness and the feeding behaviour of Anopheles vectors. The design and method of the study are well presented in the section "Methods" as well as the statistical analysis. Clinical surveillance was analyzed in the study between January 2009 and December 2014 that covers a long period. Thus it will be interesting if authors add in their explanatory factors the dry and wet season. It will be also important to explain the discrepancy between the date of clinical surveillance data collection (January 2009 and December 2014) and the mosquito collection (July and August 2016). We have any information if the level of ITN use varied or is the same during both periods.

**A1: We have made revision to address all the questions above as follows:
We have updated the clinical surveillance data to December 2016 and updated the manuscript accordingly. We have also included season as a covariate in both univariable and multivariable analysis, Supplementary Table 1.**

Changes are as follows;

Method section

“The clinical surveillance study was conducted between January 2009 and December 2016 within a 6km radius of Pingilikani dispensary in Kilifi County on the Kenyan Coast (Figure 1): within the Kilifi Health and Demographic Surveillance System (KHDSS).”

Statistical analysis section

“The outcome of interest was presence of malaria by microscopy on presentation to the dispensary. The potential risk factors included: ITN use, age of the child, year of presentation to the dispensary, season (the wet season comprised of April, May, June, October and November) and the geographical area, as defined by the 2.5x2.5 km regular polygons.”

Q2) Additionally the main part of the subject underlines the effectiveness of the ITNs. However, authors should describe at first that the effectiveness of ITNs is monitoring taking into account the physical integrity of nets, bioefficacy and the insecticidal compounds even though they focused more their study on feeding place and malaria prevalence. It will be also more appropriate if authors interpreted their result according to level of ITNs use according to areas and discuss though their outcomes the effectiveness of ITN. For instance, in the abstract the expression of “high and low effectiveness” in the part of method is a hasty affirmation.

A2: We have included the above comment as a limitation to our study as we did not have data on the physical integrity of nets or the bioefficacy of the insecticidal compounds. We’ve also revised the abstract session. We’ve revised the abstract session to indicate “varying ITN effectiveness” rather than “high and low ITN effectiveness”

Abstract section

“We linked homestead level geospatial data to clinical surveillance data at a primary healthcare facility in Kilifi County in order to map geographical heterogeneity in ITN effectiveness and observed vector feeding behaviour using landing catches and CDC light traps in six selected areas of varying ITN effectiveness.”

Discussion section

“It is likely that we underestimated the protection afforded by the use of high-quality ITN because we included all ITNs, regardless of quality, physical integrity or bioefficacy of the insecticidal compounds.”

Q3) In the section of “Results” I think that the Supplementary Table 1 has to be presented in the main manuscript as it present malaria prevalence according to area and the level of ITNs use. Moreover, the presentation of results must be more detailed and the effect of each risk factors cited in the part of “Statistical analysis” must be presented. I don’t understand why authors said “ITN use was consistently >50% in all geographical areas”, meaning that here we have no information about the difference of level use between areas. The authors have summarized too much the description of the results in this part. Authors presented in the part “Mosquito processing” laboratory works such ELISA-CSP and molecular analysis, however the results of these analysis have not been presented in this study. Regarding the result on vector abundance, authors have to present the results according to absolute densities and less on the proportion of species in the place of mosquito collection.

A3: We have made revision to address all the questions above as follows:

We have included two tables: (i) a descriptive table in the main text that indicates the prevalence of malaria and ITN use; and (ii) a table showing the full result of the univariable and multivariable analysis. We have included data obtained from the ELISA-CSP and molecular analysis. We've made revisions as shown below.

Result section

We've have included a descriptive table in the main text that indicates the prevalence of malaria and ITN use i.e. Table 1.

The full result of the univariable and multivariable analysis are shown in the Supplementary Table 1.

“Over 26 nights, 415 female *Anopheles* mosquitoes were collected by both methods (i.e. 272 by HLC and 143 by CDC-LT), representing a mean of 16 mosquitoes per night. 66% of mosquitoes were collected using HLC. Of the 415 mosquitoes morphologically identified, 311 (75%) were *An. funestus* group, 84 (20%) were *An. gambiae* s.l. and 20 (5%) were other *Anopheles* i.e. *An. protoriensis*, *An. coustani*, *An. moucheti* and *An. squamosus* (Table 2). The *An. funestus* group was significantly greater than *An. gambiae* s.l. ($p < 0.001$). Out of the 84 amplified samples of *An. gambiae* s.l., 68 (81%) were *An. Arabiensis* and 16 (19%) were *An. gambiae* s.s. The proportion of *Anopheles* mosquitoes caught outdoors (60%; 95% CI: 55%, 65%) was significantly greater than the proportion caught indoors ($p < 0.001$). There were more *Anopheles* mosquitoes collected outdoors in all geographical areas except area 6, where most of the mosquitoes were collected indoor (Table 2). The frequencies of vectors collected in each geographical area are summarized in Supplementary Table 2. *An. funestus* group was the most prevalent vector in all areas. Of the 272 mosquitoes collected by HLC, 3.3% (9/272) tested positive for *P. falciparum* sporozoites. Higher sporozoite rate was observed among the *An. funestus* group (7/9). The rate of indoor and outdoor biting estimated by HLC was 19.8 and 25.5 bites per person per night, respectively.”

Q4) The relevance of the study will be more remarkable if authors greatly discuss in deep their outcomes by comparing with other studies. Additionally, the review of the literature has to be strengthened, “33 off the 43 references are more than 5 years old and some newer papers are missing.

A4: We have added 10 more recent reference as shown under the reference section. We have discussed our outcomes by comparing with other studies under the discussion section

Reference section

1. Bradley J, Lines J, Fuseini G, Schwabe C, Monti F, Slotman M, Vargas D, Garcia G, Hergott D, Kleinschmidt I: **Outdoor biting by *Anopheles* mosquitoes on Bioko Island does not currently impact on malaria control.** *Malar J* 2015, **14**:170.
2. Moiroux N, Damien GB, Egrot M, Djenontin A, Chandre F, Corbel V, Killeen GF, Pennetier C: **Human exposure to early morning *Anopheles funestus* biting behavior and personal protection provided by long-lasting insecticidal nets.** *PLoS One* 2014, **9**: e104967.

3. Killeen GF, Marshall JM, Kiware SS, South AB, Tusting LS, Chaki PP, Govella NJ: **Measuring, manipulating and exploiting behaviours of adult mosquitoes to optimise malaria vector control impact.** *BMJ Glob Health* 2017, **2**:e000212.
4. Kamau A, Nyaga V, Bauni E, Tsofa B, Noor AM, Bejon P, Scott JAG, Hammit LL: **Trends in bednet ownership and usage, and the effect of bednets on malaria hospitalization in the Kilifi Health and Demographic Surveillance System (KHDSS): 2008-2015.** *BMC Infect Dis* 2017, **17**:720.
5. Royston P, Sauerbrei W: **Building multivariable regression models with continuous covariates in clinical epidemiology--with an emphasis on fractional polynomials.** *Methods Inf Med* 2005, **44**:561-571.
6. Sauerbrei W, Meier-Hirmer C, Benner A, Royston P: **Multivariable regression model building by using fractional polynomials: description of SAS, STATA and R programs.** *Computational Statistics & Data Analysis* 2006, **50**:3464-3485.
7. Kezdi G: **Robust Standard Error Estimation in Fixed-Effects Panel Models.** 2003.
8. Seyoum A, Sikaala CH, Chanda J, Chinula D, Ntamatungiro AJ, Hawela M, Miller JM, Russell TL, Briet OJ, Killeen GF: **Human exposure to anopheline mosquitoes occurs primarily indoors, even for users of insecticide-treated nets in Luangwa Valley, South-east Zambia.** *Parasit Vectors* 2012, **5**:101.
9. Lengeler C: **Insecticide-treated bed nets and curtains for preventing malaria.** *Cochrane Database Syst Rev* 2004:CD000363.
10. Moiroux N, Gomez MB, Pennetier C, Elanga E, Djenontin A, Chandre F, Djegbe I, Guis H, Corbel V: **Changes in Anopheles funestus biting behavior following universal coverage of long-lasting insecticidal nets in Benin.** *J Infect Dis* 2012, **206**:1622-1629.
11. Mwangi TW, Ross A, Snow RW, Marsh K: **Case definitions of clinical malaria under different transmission conditions in Kilifi District, Kenya.** *J Infect Dis* 2005, **191**: 1932-1939.
12. Mogeni P, Williams TN, Omedo I, Kimani D, Ngoi JM, Mwacharo J, Morter R, Nyundo C, Wambua J, Nyangweso G: **Detecting Malaria Hotspots: a comparison between RDT, Microscopy and Polymerase Chain Reaction.** *The Journal of Infectious Diseases* 2017.
13. Kapesa A, Kweka EJ, Atieli H, Kamugisha E, Zhou G, Githeko AK, Yan G: **Why some sites are responding better to anti-malarial interventions? A case study from western Kenya.** *Malar J* 2017, **16**:498.
14. Yohannes M, Boelee E: **Early biting rhythm in the Afro-tropical vector of malaria, Anopheles arabiensis, and challenges for its control in Ethiopia.** *Med Vet Entomol* 2012, **26**:103-105
15. Huho B, Briet O, Seyoum A, Sikaala C, Bayoh N, Gimnig J, Okumu F, Diallo D, Abdulla S, Smith T, Killeen G: **Consistently high estimates for the proportion of human exposure to malaria vector populations occurring indoors in rural Africa.** *Int J Epidemiol* 2013, **42**:235-247.

Competing Interests: No competing interests were disclosed.

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Heiko Becher

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This report refers to statistical methods. Other relevant issues (“... and does it cite the current literature?”) are not considered.

The authors investigate the relation between ITN use and malaria prevalence, and secondly spatial variation in the effectiveness of ITN.

Overall, the methods are too briefly described and make a thorough evaluation difficult. Some remarks may help to update the manuscript.

- The authors collected data from 20827 visits of 4992 children, i.e. about 5 visits for each child on average, from 21 areas. For each visit, parasitemia was assessed. The probability of parasitemia was modeled with a logistic regression model with ITN use, age, year and area as covariables, plus interaction terms. To account for correlated observations, a robust estimate of the standard errors was employed, although the exact method used is not given (reference should be provided). I wonder why season (rainy / dry) was not considered. The full result of the model is not given, and I wonder whether the large number of interaction terms in the model gave in a meaningful result. The Supplementary Table 1 gives the ORs for ITN use by area which is difficult to follow since (i) the numbering of the areas does not give information on spatial distribution (ii) it is not easy to see from the table whether malaria prevalence and ITN use differs between areas (iii) the effect of the other covariables is unknown (is there some confounding? What is the effect of age? Was a full fractional polynomial procedure used?).
- The Kulldorf statistic was used, if I understand correctly, to identify clusters of high or low ITN effectiveness without taking malaria prevalence and ITN use into account. Is that true? This seems not correct to me but maybe I misunderstood the procedure.
- The proportion of vectors biting outdoors was compared for the areas. This would mean ignoring the absolute biting frequency which differs largely between areas.

Overall, the authors have carefully interpreted the results.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 06 Jan 2018

Alice Kamau, Centre for Geographic Medicine Research-Coast, Kilifi, Kenya

We are grateful for this review and the helpful comments and suggestions that have been made. We have included a point-by-point response (in bold) to the issues raised.

Q1) The authors collected data from 20827 visits of 4992 children, i.e. about 5 visits for each child on average, from 21 areas. For each visit, parasitemia was assessed. The probability of parasitemia was modeled with a logistic regression model with ITN use, age, year and area as covariables, plus interaction terms. To account for correlated observations, a robust estimate of the standard errors was employed, although the exact method used is not given (reference should be provided). I wonder why season (rainy / dry) was not considered. The full result of the model is not given, and I wonder whether the large number of interaction terms in the model gave in a meaningful result. The Supplementary Table 1 gives the ORs for ITN use by area which is difficult to follow since (i) the numbering of the areas does not give information on spatial distribution (ii) it is not easy to see from the table whether malaria prevalence and ITN use differs between areas (iii) the effect of the other covariables is unknown (is there some confounding? What is the effect of age? Was a full fractional polynomial procedure used?).

A1: We have made revisions to address the questions raised above as follows:

Statistical analysis section

Wet vs. dry season was included as a covariate. We have included a reference for the multiple fractional polynomial transformation procedure [1,2]. We used the “mfp” command in STATA to assess the non-linear effect of age. We have also included a reference, which indicates what method was used for the robust standard error [3].

“The outcome of interest was presence of malaria by microscopy on presentation to the dispensary. The potential risk factors included: ITN use, age of the child, year of presentation to the dispensary, season (the wet season comprised of April, May, June, October and November) and the geographical area, as defined by the 2.5x2.5 km regular polygons.

To assess the non-linear effect of age in the regression models, multiple fractional polynomial transformation was used[1]. A list of fractional polynomial (FP) powers (-2, -1, -0.5, 0, 0.5, 1, 2, 3) were investigated for inclusion in the model using an algorithm that combines a backward elimination procedure with a search for an FP function that best predicts the outcome variable as previously described [2].

Given that the hospital malaria episodes were clustered within patients, we allowed for clustering by using a logistic regression model with robust standard errors [3].

Result section

We've have included a descriptive table in the main text indicating the prevalence of malaria and ITN use i.e. Table 1. We have included season as a covariate in both univariable and multivariable analysis, Supplementary Table 1. The full result of the univariable and multivariable analysis are shown in the Supplementary Table 1. We found the interaction terms to be significant and therefore retained them in the model.

Q2) The Kulldorf statistic was used, if I understand correctly, to identify clusters of high or low ITN effectiveness without taking malaria prevalence and ITN use into account. Is that true? This seems not correct to me but maybe I misunderstood the procedure.

A2: To compute the ITN effectiveness [i.e. $(1 - OR) \times 100$] for each individual homestead, the outcome of interest was presence of malaria and the predictor was ITN use. We have included a description of SaTScan in the statistical analysis section as shown below:

Statistical analysis section

“SaTScan software (version 9.4; <https://www.satscan.org/>), a spatial scan statistic developed by Kulldorf[4], was used to detect potential spatial variations of ITN effectiveness (without smoothing) by identifying statistically significant geographical clustering of ITN effectiveness using the normal model. The space-time parameter of the spatial scan statistic places a cylindrical window on the coordinates grid for the locations studied and moves the center of the cylinder base over the grid so that the sets of geographic units covered by the window are constantly changing. Whenever the cylindrical window includes a new event, SaTScan calculates a likelihood function to test for elevated risk within the cylinder as compared with outside the cylinder. The observed test statistic is obtained by calculating the likelihood ratio maximized over the collection of zones in the alternative hypothesis. The p value for the detection of clusters is calculated by using the Monte Carlo hypothesis testing (where a number of random replications of the dataset under the appropriate null hypothesis are generated, their test statistics computed and then compared with the observed test statistic to obtain the p-value). The null hypothesis is that the risk of malaria inside and outside the scanning window is the same.”

Q3) The proportion of vectors biting outdoors was compared for the areas. This would mean ignoring the absolute biting frequency which differs largely between areas.

A3: We have included the absolute biting frequencies for each area as shown in supplementary Table 2 below:

Result section

“There were more *Anopheles* mosquitoes collected outdoors in all geographical areas except area 6, where most of the mosquitoes were collected indoor (Table 2). The frequencies of vectors collected in each geographical area are summarized in Supplementary Table 2.”

References

1. Royston P, Sauerbrei W: **Building multivariable regression models with continuous covariates in clinical epidemiology--with an emphasis on fractional polynomials.** *Methods Inf Med* 2005, **44**:561-571.
2. Sauerbrei W, Meier-Hirmer C, Benner A, Royston P: **Multivariable regression model building by using fractional polynomials: description of SAS, STATA and R programs.** *Computational Statistics & Data Analysis* 2006, **50**:3464-3485.
3. Kezdi G: **Robust Standard Error Estimation in Fixed-Effects Panel Models.** 2003.

Competing Interests: No competing interests were disclosed.
