



RESEARCH ARTICLE

REVISED **The ability of *Anopheles funestus* and *A. arabiensis* to penetrate LLINs and its effect on their mortality [version 3; peer review: 2 approved]**

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Abstract

Background: Variation in mosquito body size and the ability to penetrate long-lasting insecticide-treated nets (LLINs) remains unknown. This study evaluated the ability of *Anopheles funestus* and *A. arabiensis* to penetrate commercially available treated and untreated bednets and how this behaviour affects mosquito mortality.

Methods: Three types of LLINs; DawaPlus 2.0, PermaNet 2.0, Olyset 2.0, and untreated (Safi Net) were tested inside a semi-field system. One hundred 3–5-day-old and non-starved female *A. funestus* and *A. arabiensis* were released in a chamber with a sleeping adult volunteer under a treated or untreated bednet. Mosquitoes that penetrated inside the nets were collected every two hours using a mouth aspirator. Live mosquitoes were put in paper cups, fed on glucose *ad libitum* and their mortality rate was monitored for 48 h.

Results: The ability of *A. funestus* to penetrate treated and untreated bednets was significantly higher than for *A. arabiensis* for all three LLIN net types ($P < 0.001$). For both species the penetration rate was higher for untreated bednets than treated ones except for the Olyset net. Regardless of the assessed mosquito species, all the mosquitoes that penetrated the net, successfully blood-fed on the sleeping volunteer. Compared to *A. arabiensis*, significant mortality was recorded for *A. funestus* that were caught inside Olyset nets within 48 hrs of monitoring ($P < 0.001$).

Conclusions:

These findings demonstrate the ability of *A. funestus* and *A. arabiensis* mosquitoes to penetrate the human-occupied treated and untreated bednets. Despite this ability, mosquitoes that penetrated the bednet succumbed to death within two days.

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Any reports and responses or comments on the article can be found at the end of the article.

Keywords

Long -lasting insecticidal nets, Malaria vectors, *Anopheles funestus*, *Anopheles arabiensis*, Malaria, Tanzania



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REVISED Amendments from Version 2

The difference between version 2 and previous version 1, is that the current version has modified Figure 2 that clearly display different net types along number of mosquitoes that penetrate net. In addition, the revised version as key information, that the mosquitoes that were used for the experiment were not starved prior release to avoid influencing its host seeking behavior.

Any further responses from the reviewers can be found at the end of the article

Introduction

Amongst vector control strategies to combat malaria, the use of long-lasting insecticide-treated nets (LLINs) has been key in sub-Saharan Africa, which bears the brunt of the disease and accounts for nearly 95% of all associated cases and deaths. In the last two decades, LLINs contributed to nearly 70% of the progress made¹. In addition, a recent study demonstrated the contribution of LLINs in the reduction of disease incidence and malaria-related child mortality from all causes by 17% compared to no nets². LLINs not only provide a physical barrier against mosquitoes that attempt to bite its sleeping occupant, it also diverges mosquitoes attempting to enter the house, kills mosquitoes that make physical contact with the mesh, and reduces mosquito survival³.

Similar to other African countries, Tanzania relies heavily on LLINs as the standard of care against malaria transmission. The use of such effective tools in Tanzania goes back 20 years, a period during which its access, coverage and proper usage was maintained and implemented through under-five catch-up campaign⁴, universal coverage campaign, and keep-up campaign, that encompass a piloted school net programmes⁵, with special emphasis on vulnerable populations⁶. These initiatives resulted in significant reductions of malaria, exemplified by the recent empirical evidence of near-elimination of the historically dominant malaria vector *Anopheles gambiae* in Kilombero valley⁷; a similar trend has been documented in other part of East Africa⁸. More recently, in the Kilombero Valley and in other parts of Africa, there is growing evidence that on-going residual malaria transmission is mediated by *A. funestus* and *A. arabiensis*⁹. While the latter species represents the most abundant malaria vector, the former predominates most of the transmission^{9,10}.

The constant use of LLINs and continuous interaction of LLINs and targeted malaria vectors have brought major threats such as resistance to insecticides that are used for impregnation¹¹ as well as emergence of behavioural avoidance strategies of malaria vectors^{10,12}. For instance, *A. arabiensis* has adapted to avoid contacting treated nets by biting and resting outdoors, feeding on cattle instead of human^{8,10,12}. *Anopheles funestus*, on the other hand, is highly anthropophilic¹⁰, has high vectorial competence for *Plasmodium* spp., is highly resistant to pyrethroids throughout Africa and has adapted biting times that avoid interventions such as LLINs and maintains malaria transmission during the drier parts of the year⁹. Previous studies have documented the resistance^{13,14} and

behavioural avoidance^{12,15} of *A. arabiensis* and *A. funestus* of the insecticides used in LLINs. However, the possibility of these major malaria vectors to evade and penetrate the mesh of LLINs and reach and feed on their designated host is yet to be established.

LLIN effectiveness against malaria vectors depends on the physical integrity (holes), insecticide content, bio-efficacy, use, and attrition over time¹⁶. However, the mesh size of the net fabric that prevents mosquitoes from penetrating it may have an important contribution to the overall protection offered by the net. Many studies have focused on monitoring of the physical integrity^{17,18}, insecticide activity, bio-efficacy, usage and net loss¹⁹ as recommended by the World Health Organization (WHO) guidelines²⁰, leaving out the interaction between the mesh size of the net and malaria vector behaviour. A few studies have described net penetration and the interaction of mosquitoes with different simulated net hole sizes²¹, or the wide mesh sizes of the net for *Culex* spp. mosquitoes²². For nets to be effective in preventing mosquitoes from entrance or penetration, WHO recommend that the mesh size of the net fabric should be 156 or 196 holes per square inch or between 1.2–1.5 mm²³. Nevertheless, the mosquitoes with small body sizes and their ability to penetrate the mesh of commercially available LLINs remains unknown. We hypothesized that mosquitoes with a small body size, such as *Anopheles funestus*, and possibly the generally larger *A. arabiensis* can penetrate commercially available bednets (1.2–1.5 mm mesh treated or untreated) and access and feed on the host before they die, which might be one of the causes for residual malaria transmission. This study aimed to evaluate the ability of *A. funestus* and *A. arabiensis* to pass through the mesh of commercially available treated or untreated bed nets and how passage impacts their survival post penetration.

Methods

Study area and site

Experiments were carried out inside a semi-field system (SFS) of the Ifakara Health Institute located at Kining'ina village (8.11417 S, 36.67484 E), Kilombero Valley, southern Tanzania. The Kilombero Valley has seen a notable scale-up of LLINs over the past 20 years, that led to undetectable levels of the major malaria vector *A. gambiae*⁷. Despite this success, most malaria transmission within this area today is mediated by *A. funestus*, a species that feeds almost exclusively on humans (anthropophilic) and rests indoor after feeding (endophilic) and is strongly resistant to the commonly used pyrethroid insecticides, both metabolically and physiologically^{9,24}.

Adult collection and establishment of *A. funestus* colony

Field collection of adult *A. funestus* to establish a first generation (F1) in the semi-field insectary was undertaken between January and June 2021. Adult *A. funestus* were sampled and collected from 10 houses selected randomly in Ikwambi (7.98033°S, 36.81701°E) and Sululu (8.00324°S, 36.83118°E) villages, using CDC light traps and Prokopack aspirators. One CDC light trap, modified with a long collection

bag or cups to prevent trapped mosquitoes from drying out because of the CDC fan, was used to sample *A. funestus* in each household. The traps were positioned near the bednet's occupant in the evening and retrieved in the morning for mosquito collection. Sampling of indoor resting mosquitoes using the Prokopack aspirator was done every day in the morning starting at 6 a.m. and finishing around 7 a.m. Mosquitoes were morphologically identified to species level in the field using the Gillies and Coetzee identification key^{25,26}. Afterwards, *A. funestus* were kept in a small cage with 10% glucose to feed on and transported to the insectary in the semi-field at Kining'ina. Upon arrival, mosquitoes were blood-fed on a chicken and allowed to lay eggs individually in a paper cup for the establishment of an *A. funestus* colony.

In the insectary, larvae resulting from laid eggs, were held in rearing basins of 32 cm diameter and 5-L holding capacity and fed on ground fish food, Tetramin®, until they developed into pupae²⁷. Pupae were collected every day and transferred to 0.5-L plastic cups placed inside the 0.3×0.3×0.3 m cage covered with netting prior to adult emergence. Emerging adults were morphologically identified as *A. funestus* using identification keys by Gilles and Coetzee²⁸. A subsample of 100 *A. funestus* was confirmed to species level using PCR²⁹. *A. arabiensis* were obtained from the Kining'ina insectary colony in which larvae are fed on ground fish, Tetramin® (Tetra, Melle, Germany), and adults are maintained in the same standard rearing conditions of 12 h:12 h

photoperiod, 25–27 °C ambient temperature and (70–85%) relative humidity as *A. funestus* colony.

Semi-field system experiments

The experiment was conducted inside two chambers of the four chambers-cage constructed inside the SFS measuring 3×3×2 m using a double layer of fiber glass nets on the outside, and lined with small-mesh fabric in the inside to prevent mosquito escape (Figure 1a, b and c). Of the two chambers, one was used treated net and the other for untreated net set ups (Figure 1d). Three types of insecticide-treated nets were tested, namely DawaPlus 2.0 (Tana Netting FZ LLC, Dubai), PermaNet 2.0 (Vestergaard S.A, Switzerland), Olyset 2.0 (Sumitomo Chemical Co., Ltd, Japan), besides an untreated SafiNet (A to Z Textile Mills Limited Tanzania). DawaPlus® 2.0 and PermaNet 2.0 consist of 100% polyester, with deltamethrin coated on 100 deniers at 80 mg/m² and 55 mg/m², respectively. Both nets have a mesh size of 160 per square inch³⁰. OlysetNet® is made of 100% polyethylene, with permethrin incorporated in 150 deniers at 50 mg/m² with a mesh size of 160 holes per square inch, which is characteristically large (4×4 mm)³⁰. The SafiNet that was used has the control, of 100% polyester, and has 75 deniers and mesh size of 156 holes per square inch.

Each test chamber was fitted with a mattress on the floor to make a bed for a sleeping adult volunteer protected with a treated or untreated net in the treatment or control chambers, respectively (Figure 1c and d). Experiments started at

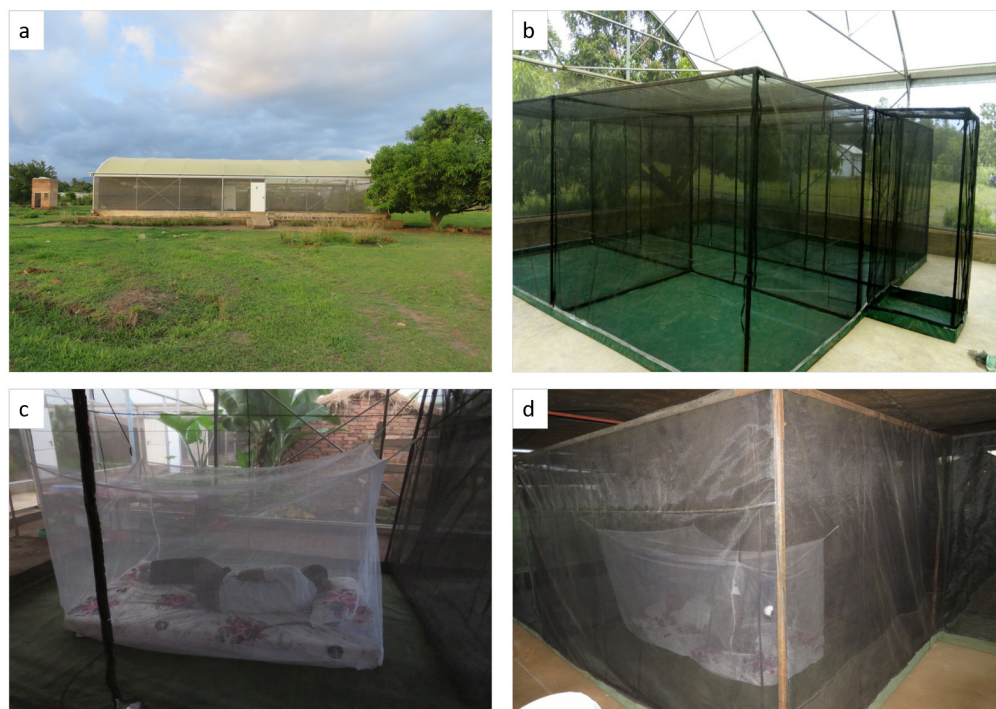


Figure 1. (a) Semi field system in Kining'ina, (b) a 3×3×2 m cages that were constructed inside one of the chambers of the Semi-field system, (c) A sleeping volunteer inside the constructed cage, sleeping under bed net and (d) Orientation view of a sleeping volunteer in a cage.

6.00 pm by releasing 100 non-starved female *A. funestus* or *A. arabiensis* in the presence of a volunteer. The mosquitoes were not starved prior release to avoid influencing its host-seeking behavior. In both chambers, all mosquitoes were to be released in less than one minute. Every two hours the volunteer woke up to collect mosquitoes that had penetrated the net using a mouth aspirator and put these in a labelled paper cup. In the morning, all dead and live mosquitoes were collected from inside and outside the net. Live mosquitoes from inside and outside the net were put in paper cups, fed *ad libitum* on 10% glucose solution, and monitored for mortality for 48-hours consecutive on 24-hours cycle and delayed mortality³¹. Six replicates were performed for each net type.

Statistical analysis

A generalized linear mixed effects models (GLMM) following binomial distribution with a logit function built on the *lme4* package³², was used to determine whether *A. funestus* and *A. arabiensis* successfully penetrated the treated or untreated bednets in the semi-field. The number of *A. funestus* or *A. arabiensis* caught inside the net denoted as success, and those caught outside the nets denoted as failure were modeled as a response variable, and replicates as a random variable. The net status (treated or untreated net), species (*A. funestus* or *A. arabiensis*) and collection site (inside the net or outside nets) were modeled as fixed variables. Model coefficients were exponentiated to obtain the odds ratio, with their respective *P*-values at 95% confidence intervals (CIs).

Results

Overall, the ability of *A. funestus* to penetrate treated and untreated netting was higher than that of *A. arabiensis* for all four net types (Figure 2, Table 1). Both species more easily penetrated untreated than treated nets with the exception of the Olyset net, which was penetrated by significantly higher number of mosquitoes compared to the untreated net ($p < 0.001$) (Figure 2, Table 1). The mean number (\pm S.E) of

A. arabiensis was less than one per night across all net types, whereas for *A. funestus* this was up (7.5 ± 0.31) and (3.9 ± 0.34) mosquitoes in a treated and untreated nets respectively. Regardless of the assessed mosquito species, almost all mosquitoes that penetrated the net, successfully blood-fed on the sleeping volunteer (Table 2). Moreover, *A. funestus* showed significantly higher ability of penetrating DawaPlus, Olyset, and PermaNet nets (OR 0.02 [0.016, 0.288], $p < 0.001$), (OR 0.03 [0.027, 0.042], $p < 0.001$), and (OR 0.016 [0.011, 0.022], $p < 0.001$) respectively, when compared to *A. arabiensis* (Table 1). The 48-h mean mortality was similar for both species that penetrated treated DawaPlus 2.0 and PermaNet 2.0 nets (Table 3). In comparison to *A. arabiensis*, significant mortality was recorded for *A. funestus* that were caught inside Olyset Nets within 48 hrs of monitoring ($p < 0.001$).

Discussion

This study documented the ability of *A. funestus* and *A. arabiensis* mosquitoes to penetrate commercially available treated and untreated nets. Worryingly, *A. funestus* showed a marked ability of penetrating all nets types compared to *A. arabiensis*. Among other factors^{9,12} this ability of *A. funestus* to penetrate intact nets, might be an additional risk for sustained malaria transmission across Africa^{9,11}. This phenomenon signals an alarming situation for malaria mosquitoes control efforts that heavily relies on the use of LLINs, which its ability to prevent mosquito bites that exceeds 80% come from physical barrier and integrity^{11,33}. Although the numbers of both mosquito species that penetrated the nets was relatively low, the fact that the majority that did successfully blood fed on the volunteers, its effect on malaria transmission is likely to be noticeable if these numbers are magnified across mosquito populations under real life settings.

Although wing length for both mosquito species were not measured in the current study, estimates of mosquito body sizes of F1-laboratory reared mosquitoes were extracted

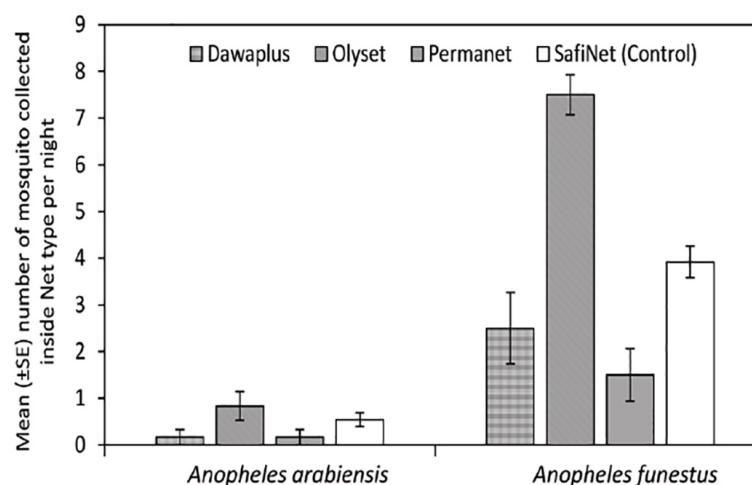


Figure 2. Mean number of *Anopheles arabiensis* and *Anopheles funestus* collected inside different net types.

Table 1. Penetration rate of *A. funestus* caught outside the net with the model output showing the ability of mosquitoes to penetrates (inside the net) in test chambers.

Net type	Reference item [1*]	Odd ratio	95%CI	P value
DawaPlus Net 2.0	Safi Net	NA	NA	NA
<i>A. funestus</i>	<i>A. arabiensis</i>	1.03	[0.68, 1.48]	0.580
Collected (Inside a net)	Outside a net	0.02	[0.02, 0.29]	<0.001
Olyset Net 2.0	Safi Net	NA	NA	NA
<i>A. funestus</i>	<i>A. arabiensis</i>	1.04	[0.93, 1.17]	0.450
Collected (Inside a net)	Outside a net	0.03	[0.03, 0.04]	<0.001
PermaNet 2.0	Safi Net	NA	NA	NA
<i>A. funestus</i>	<i>A. arabiensis</i>	1.02	[0.92, 1.15]	0.669
Collected (Inside a net)	Outside a net	0.016	[0.01, 0.02]	<0.001

CI = confidence interval, NA = not applicable because it's a reference with value [1*]

Table 2. Mean (\pm SE) number of blood fed mosquitoes that were caught inside treated and untreated nets.

Species	DawaPlus Net		Olyset Net		PermaNet		SafiNet	
Mean (\pm S.E)	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed
<i>A. arabiensis</i>	0.17 \pm 0.17	0	0.83 \pm 0.31	0	0.17 \pm 0.16	0	0.50 \pm 0.13	0.04 \pm 0.04
<i>A. funestus</i>	2.50 \pm 0.76	0	6.67 \pm 0.31	0.83 \pm 0.54	1.50 \pm 0.56	0	3.88 \pm 0.34	0.04 \pm 0.04

Table 3. 48-hours mortality of mosquitoes penetrated the treated and untreated nets. Model output to show the effects of net status (Treated or Untreated) on the survival of *A. funestus* and *A. arabiensis* in the test chambers.

Net type	Reference item [1*]	Odd ratio (at 48-hours)	95%CI	P value
DawaPlus 2.0				
Net status (Treated)	Safi Net (Untreated)	12.18	[8.23, 18.1]	<0.001
<i>A. funestus</i>	<i>A. arabiensis</i>	1.02	[0.79, 1.31]	0.897
Olyset 2.0				
Net status (Treated)	Safi Net (Untreated)	12.20	[8.26, 17.9]	<0.001
<i>A. funestus</i>	<i>A. arabiensis</i>	1.89	[1.46, 2.45]	<0.001
PermaNet 2.0				
Net status (Treated)	Safi Net (Untreated)	7.78	[5.56, 10.9]	<0.001
<i>A. funestus</i>	<i>A. arabiensis</i>	1.12	[0.87, 1.44]	0.373

CI = confidence interval

from recent studies that used the same colonies and/or collected adults for F1 from the same village^{34,35}. Compared to *A. arabiensis* with relatively large body size (2.6 – 3.6 mm), the marked ability of *A. funestus* to penetrate all net types

might be attributed to its small body size (2.6 – 2.7 mm) relative to the mesh holes size of the net^{7,35}. Olyset mesh size of 4 mm x 4 mm that ensure better ventilation to the bednet occupant, might also be the reason for the recorded

significantly high number of mosquitoes penetrating this net type compared to other nets. Under field settings, the penetration and successful feeding by wild and resistant *A. funestus* population on bednet occupant(s), especially in a situation where nets are not treated and/or its fabric integrity is compromised, might accelerate and exacerbate malaria transmission in communities³⁶.

Treated nets resulted high mortality in *A. arabiensis* and *A. funestus*, which highlights the importance of incorporated insecticides in LLINs over untreated nets. For both species, only a small proportion that were collected outside the net died, probably from short-contact with a treated net or from insecticide vapours^{37,38}. Almost all *A. arabiensis* that penetrated treated nets, although in low numbers, died within 48 h of monitoring. This might be due to full susceptibility of the used mosquitoes from the laboratory to the insecticides in the nets. On the other hand, *A. funestus* that penetrated the Olyset net and successful fed died within 48 h of monitoring. Although previous reports have confirmed resistance of *A. funestus* to permethrin, a pyrethroid used in the LLINs used in this study^{9,24}, the observed increase in mortality might have been due to prolonged exposure to insecticide as mosquito that were trapped inside the net. Majority of *A. funestus* and *A. arabiensis* that penetrated the net were blood-fed, and died within 48 h of monitoring. The recorded delayed mortality (*i.e.* dying within 48 h and not 24 h) might have been due to increased insecticide tolerance as the results acquired blood meals³⁹.

While debate on the impact of insecticide resistance in reducing protective effectiveness of LLINs has remained inconclusive^{40,41} findings from this study highlights the benefits of incorporated insecticides in causing mortality even to resistant mosquitoes, such as *A. funestus*, that penetrated the nets. These findings point out the importance of considering future modification of mesh sizes that offer a complete bite protection by preventing mosquitoes to penetrate the net without compromising ventilation. Because only new nets were tested in the current study, further studies might be warranted to assess the penetration rate and associated consequences on their fitness as the nets get old. In addition, these findings can be coupled with community perception on mosquito penetration to different nets in question.

Nevertheless, the current study had number of limitations. First, no direct wing length measurement that were taken for mosquitoes used in this study, instead estimate of mosquito body sizes were drawn from recent studies that measured the body of the same mosquito colonies used. Second, no testing of insecticide resistance that was carried out to confirm the resistance within *A. funestus*. The claimed resistance within *A. funestus* was based on the previous studies conducted in the similar location¹³. Third, no attempts were made to establish if the recorded penetration was a mosquito learned behaviour to maximize its feeding success. A recent study documented learning behaviour in *Aedes* and *Culex* mosquitoes that enables them avoid lethal contact via

detection of insecticide smell⁴². Fourth, the landing and penetration activities of *A. funestus* on the nets were not recorded. Of importance, this can be achieved by setting up camera traps that are already used in mosquito behaviour and ecological studies⁴³. This will provide information on which part of the net do mosquitoes prefer to penetrate most, with potential to guide the customization of mesh size and insecticide impregnation at specific areas of the net.

Conclusion

A. funestus and *A. arabiensis* have the ability to penetrate treated and untreated bednets and contact their preferred host due to their small size relative to net mesh hole sizes. This study highlights the limitation of current net-mesh size in offering biting proof against *A. funestus* and *A. arabiensis*, the dominant malaria vector, and counterbalance importance of impregnated insecticides on mosquito mortality. Owing to its functional value on the overall bednet protective effect, future studies on the WHO recommended new generation bednets should also consider assessing its penetration by targeted mosquitoes species and effect on its fitness.

Ethical approval

The Institutional Review Board of the Ifakara Health Institute (IHI/IRB/EXT/No: 24-2021) and the Medical Research Coordination Committee of the National Institute for Medical Research in Tanzania (NIMR/HQ/R.8a/Vol.IX/3477) approved the study.

Data availability statement

Underlying data

Figshare: Underlying data for “The ability of *Anopheles funestus* and *A. arabiensis* to penetrate LLINs and its effect on their mortality” <https://doi.org/10.6084/m9.figshare.20621664>⁴⁴

This project contains the following underlying data: Data file 1 (Mosquito penetrating different bed nets types and its feeding success and mortality).

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Authors’ contributions

DWL and FCM formulated the idea and conducted the experiment. FCM, AJL and DWL designed the study protocol, contributed to the study design, analysis and interpretation. FCM, LLM, DWL performed data analysis, interpreted the results and FCM drafted the manuscript. AJL, DWL and LLM provided comments upon the manuscript. All authors read and approved the final manuscript.

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References

1. Bhatt S, Weiss D, Cameron E, *et al.*: **The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015.** *Nature*. 2015; 526(7572): 207–11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
2. Pryce J, Richardson M, Lengeler C: **Insecticide-treated nets for preventing malaria.** *Cochrane Database Syst Rev*. 2018; 11(11): CD000363.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
3. Hawley WA, Phillips-Howard PA, ter Kuile FO, *et al.*: **Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in western Kenya.** *Am J Trop Med Hyg*. 2003; 68(4 Suppl): 121–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
4. Magesa SM, Lengeler C, DeSavigny D, *et al.*: **Creating an “enabling environment” for taking insecticide treated nets to national scale: the Tanzanian experience.** *Malar J*. 2005; 4(1): 34.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
5. Renggli S, Mandike R, Kramer K, *et al.*: **Design, implementation and evaluation of a national campaign to deliver 18 million free long-lasting insecticidal nets to uncovered sleeping spaces in Tanzania.** *Malar J*. 2013; 12: 85.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
6. Kramer K, Mandike R, Nathan R, *et al.*: **Effectiveness and equity of the Tanzania National Voucher Scheme for mosquito nets over 10 years of implementation.** *Malar J*. 2017; 16(1): 255.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
7. Russell T, Govella N, 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(1): 80.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
8. 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(1): 13.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. Kaindoa EW, Matowo NS, Ngowo HS, *et al.*: **Interventions that effectively target *Anopheles funestus* mosquitoes could significantly improve control of persistent malaria transmission in south-eastern Tanzania.** *PLoS One*. 2017; 12(5): e0177807.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
10. Meza FC, Kreppel KS, Maliti DF, *et al.*: **Mosquito electrocuting traps for directly measuring biting rates and host-preferences of *Anopheles arabiensis* and *Anopheles funestus* outdoors.** *Malar J*. 2019; 18(1): 83.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
11. WHO: **World malaria report 2021.** CC BY-NC-SA 3.0 IGO. Geneva: World Health Organization, 2021.
[Reference Source](#)
12. Kreppel K, Viana M, Main B, *et al.*: **Emergence of behavioural avoidance strategies of malaria vectors in areas of high LLIN coverage in Tanzania.** *Sci Rep*. 2020; 10(1): 14527.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
13. Pinda PG, Eichenberger C, Ngowo HS, *et al.*: **Comparative assessment of insecticide resistance phenotypes in two major malaria vectors, *Anopheles funestus* and *Anopheles arabiensis* in south-eastern Tanzania.** *Malar J*. 2020; 19(1): 408.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
14. McCann RS, Ochomo E, Bayoh MN, *et al.*: **Reemergence of *Anopheles funestus* as a vector of *Plasmodium falciparum* in western Kenya after long-term implementation of insecticide-treated bed nets.** *Am J Trop Med Hyg*. Washington, DC. 2014; 90(4): 597–604.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
15. Sougoufara S, Diédhiou SM, Doucouré S, *et al.*: **Biting by *Anopheles funestus* in broad daylight after use of long-lasting insecticidal nets: a new challenge to malaria elimination.** *Malar J*. 2014; 13(1): 125.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
16. Briet O, Koenker H, Norris L, *et al.*: **Attrition, physical integrity and insecticidal activity of long-lasting insecticidal nets in sub-Saharan Africa and modelling of their impact on vectorial capacity.** *Malar J*. 2020; 19(1): 310.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. Mansiangi P, Umesumbu S, Etewa I, *et al.*: **Comparing the durability of the long-lasting insecticidal nets DawaPlus® 2.0 and DuraNet® in northwest Democratic Republic of Congo.** *Malar J*. 2020; 19(1): 189.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
18. Kilian A, Koenker H, Obi E, *et al.*: **Field durability of the same type of long-lasting insecticidal net varies between regions in Nigeria due to differences in household behaviour and living conditions.** *Malar J*. 2015; 14(1): 123.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
19. Massue DJ, Moore SJ, Mageni ZD, *et al.*: **Durability of Olyset campaign nets distributed between 2009 and 2011 in eight districts of Tanzania.** *Malar J*. 2016; 15(1): 176.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
20. WHO: **Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions.** Geneva: World Health Organization, 2011.
[Reference Source](#)
21. Sutcliffe J, Colborn KL: **Video studies of passage by *Anopheles gambiae* mosquitoes through holes in a simulated bed net: effects of hole size, hole orientation and net environment.** *Malar J*. 2015; 14(1): 199.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
22. Itoh T, Shinjo G, Kurihara T: **Studies on wide mesh netting impregnated with insecticides against Culex mosquitoes.** *J Am Mosq Control Assoc*. 1986; 2(4): 503–6.
[PubMed Abstract](#)
23. Rozendaal JA: **Vector control: methods for use by individuals and communities.** World Health Organization; 1997.
[Reference Source](#)
24. Lwetoijera DW, Harris C, Kiware SS, *et al.*: **Increasing role of *Anopheles funestus* and *Anopheles arabiensis* in malaria transmission in the Kilombero Valley, Tanzania.** *Malar J*. 2014; 13(1): 331.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
25. Coetzee M: **Key to the females of Afrotropical Anopheles mosquitoes (Diptera: Culicidae).** *Malar J*. 2020; 19(1): 70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
26. Gillies M, Coetzee M: **A supplement of the Anopheline of Africa South of the Sahara (Afrotropical region).** Johannesburg South African Medical Research Institute, 1987.
[Reference Source](#)
27. Nambunga IH, Ngowo HS, Mapua SA, *et al.*: **Aquatic habitats of the malaria vector *Anopheles funestus* in rural south-eastern Tanzania.** *Malar J*. 2020; 19(1): 219.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
28. Gillies M, de Meillon B: **The Anopheline of Africa south of the Sahara (Ethiopian zoogeographical region).** Second edition ed: South African Institute of Medical Research, 1968; 343.
[Reference Source](#)
29. Koekemoer LL, Kamau L, Hunt RH, *et al.*: **A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (diptera: culicidae) group.** *Am J Trop Med Hyg*. 2002; 66(6): 804–11.
[PubMed Abstract](#) | [Publisher Full Text](#)
30. WHO: **Report of the thirteenth [13th] WHOPES working group meeting: WHO/HQ, Geneva 28-30 July 2009: Review of Olyset LN, DawaPlus 2.0 LN, Tianjin Yorkool LN.** Geneva: World Health Organization, 2009.
[Reference Source](#)
31. WHO: **Test procedures for insecticide resistance monitoring in malaria vector mosquitoes.** Geneva: World Health Organization, 2018.
32. Pinheiro J, Bates D, DebRoy S, *et al.*: **R Core Team nlme: linear and nonlinear mixed effects models.** R package version 3.1-131. Comput. software], 2017.
33. Lines JD, Myamba J, Curtis CF: **Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania.** *Med Vet Entomol*. 1987; 1(1): 37–51.
[PubMed Abstract](#) | [Publisher Full Text](#)
34. Urio NH, Pinda PG, Ngonzi AJ, *et al.*: **Effects of agricultural pesticides on the susceptibility and fitness of malaria vectors in rural south-eastern Tanzania.** Research Square, 2022.
[Publisher Full Text](#)
35. Ngowo HS, Hape EE, Matthiopoulos J, *et al.*: **Fitness characteristics of the malaria vector *Anopheles funestus* during an attempted laboratory colonization.** *Malar J*. 2021; 20(1): 148.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
36. Tami A, Mubyazi G, Talbert A, *et al.*: **Evaluation of Olyset insecticide-treated nets distributed seven years previously in Tanzania.** *Malar J*. 2004; 3(1): 19.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
37. Kitau J, Oxborough RM, Tungu PK, *et al.*: **Species Shifts in the *Anopheles gambiae* Complex: Do LLINs Successfully Control *Anopheles arabiensis*?** *PLoS One*. 2012; 7(3): e31481.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
38. Okumu F: **The fabric of life: what if mosquito nets were durable and widely available but insecticide-free?** *Malar J*. 2020; 19(1): 260.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
39. Hauser G, Thiévent K, Koella JC: **The ability of *Anopheles gambiae* mosquitoes to bite through a permethrin-treated net and the consequences for their fitness.** *Sci Rep*. 2019; 9(1): 8141.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
40. Kleinschmidt I, Bradley J, Knox TB, *et al.*: **Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: a WHO-coordinated, prospective, international, observational cohort study.** *Lancet Infect Dis*. 2018; 18(6): 640–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
41. Oliver SV, Lyons CL, Brooke BD: **The effect of blood feeding on insecticide**

- resistance intensity and adult longevity in the major malaria vector *Anopheles funestus* (Diptera: Culicidae). *Sci Rep.* 2022; **12**(1): 3877.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
42. Sougoufara S, Yorkston-Dives H, Aklee NM, *et al.*: **Standardised bioassays reveal that mosquitoes learn to avoid compounds used in chemical vector control after a single sub-lethal exposure.** *Sci Rep.* 2022; **12**(1): 2206.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 43. Spitzen J, Takken W: **Keeping track of mosquitoes: a review of tools to track, record and analyse mosquito flight.** *Parasit Vectors.* 2018; **11**(1): 123.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 44. Meza FC, Muyaga LL, Limwagu AJ, *et al.*: **Underlying data for “The ability of *Anopheles funestus* and *A. arabiensis* to penetrate LLINs and its effect on their mortality”.** [DATASET], 2022.
<http://www.doi.org/10.6084/m9.figshare.20621664>

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The authors of this study have provided data for what we would assume has already been published by others. The idea was to find out whether two species of malaria mosquitoes present in Africa, namely *An. funestus* and *An. arabiensis*, are able to penetrate commonly used brands of insecticide treated bed nets (ITNs) and bite occupants. The authors found out that the mosquitoes penetrated the ITNs and bit occupants. These findings are unexpected despite the shortcomings of the study (insecticide resistance in the mosquitoes was not measured, mosquito sizes were not measured, no attempts were made to find out if net penetration was a mosquito learnt behavior and actual mosquito penetration events were not recorded), which the authors discussed. There is at least one more information segment that is missing i.e. it is not known if released mosquitoes were pre-starved, and if yes, for how long. Hunger is an important driver for host seeking and may have triggered the mosquitoes to 'work harder' to reach their blood meal hosts. This issue should be clarified and deliberated upon in the discussion section. Furthermore, a graph showing mortality versus time of collection by the ITN occupant can help to further explain the findings. Lastly, the fill colors of the bars for Dawaplust, Olyset and Permanet nets in figure 2 should be properly differentiated to improve clarity and understanding of the data.

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

Yes

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?

Yes

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.

Reviewer Expertise: Public Health Entomology with a great focus on malaria.

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.

Version 1

Reviewer Report 23 November 2022

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There are a few primary concerns identified in the manuscript, as stated below.

Specific comments:

Methods:

1. Sampling of indoor resting mosquitoes using the Prokopack aspirator was done every day in the morning. There is a need to expand this statement to state the exact timing of the sampling as morning is the period from sunrise to noon. It is that the mosquitoes are likely to escape when it becomes day time. Therefore, timing should be precisely mentioned.
2. For the data analysis section, more details need to be included here overall. More detail should be included for this, and its justification for use of generalized linear mixed model to evaluate the mean numbers indicated in the results. The appropriate test for mean calculation should be included.

Results:

1. Figure 2 which shows the Mean number of *Anopheles arabiensis* and *Anopheles funestus* collected inside different net types is difficult to follow except the control bar, the shading of the different nets has similar patterns. I suggest this to be revised for easy follow-up to the readers of the manuscript.
2. Table 1 is difficult to interpret as it is not clear which is the reference (type of mosquitoes or location of the net?). This also applies to table 2.
3. The statement "Of all mosquitoes that penetrated the net, regardless of the species, >90% successfully blood-fed on the sleeping volunteer (Table 2)" is not supported by the results indicated in the table as the table indicates Mean number of blood fed mosquitoes that were caught inside treated and untreated nets, and not percentage

Discussion:

1. The statement "Although the absolute numbers of both mosquito species that penetrated the nets was relatively low". There is a difference between absolute numbers and mean numbers. Therefore, there is no evidence showing numbers of mosquitoes.

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

Yes

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?

I cannot comment. A qualified statistician is required.

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

No source data required

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: molecular biology, entomology, immunology and genetic epidemiology

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.