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Assessments of *Anopheles arabiensis* behaviour around bed nets using partial adhesive treatments

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Understanding the behaviour of malaria vectors could lead to improved designs of partially treated insecticidal nets and low-cost nets with less insecticide content. The behaviour of *Anopheles arabiensis* around cow baited bed nets with partial adhesive treatments were assessed in experimental huts. The study was conducted in Moshi Tanzania, using a Latin Square design with five arms: no bed-net, intact untreated bed-net, roof, sides and whole adhesive treated bed-nets. The data analyses were done using generalized linear mixed effects models with proportions of mosquitoes on the bed-net panels, and induced exiting as outcomes and trial arm as the predictor. There were significant reductions in the likelihood of *An. arabiensis* found on the side (adjusted odds ratio, AOR: 0.50; 95% CI 0.26–0.98; *p* value = 0.044) and roof (AOR: 0.18; 0.07–0.43; *p* value < 0.001) of adhesive treated bed-nets compared to the whole adhesive treated bed-net. The likelihood of *An. arabiensis* exiting was significantly higher in the intact untreated net trial arm compared to the no net trial arm (AOR: 2.12; 1.19–3.79; *p* value = 0.010). Host seeking *An. arabiensis* is not persistent and untreated bed-nets induced exiting, implying reduced efficacy of partially treated insecticidal nets.

Keywords Adhesive treatments, Exophily, Persistence, Vector control, Vector biology, Indoor resting, Blood feeding, Hybrid nets

Vector control using insecticide treated bed nets (ITNs) and indoor residual spraying (IRS) are the main malaria control strategy and contributed to approximately 78% of the decline in malaria cases between 2000 and 2015[1,2]. ITN intervention exploits the host seeking behaviour of female mosquitoes by acting as a physical barrier between the host and the vector and the insecticide component could act by killing, sterilizing or repelling the vector that contacts the net[3,4]. IRS intervention exploits the resting behaviour of the malaria vector as it is likely to rest on indoor surfaces after taking a blood meal[5]. As such, IRS is used to treat potential malaria vector resting surfaces such as internal walls, eaves and ceilings of all housing including domestic animal shelters[5].

Insecticide resistance has the potential to reduce the impact of ITNs and IRS in the fight against malaria[6]. With widespread resistance of malaria vectors to pyrethroids[7], the main active ingredient (AI) in ITNs, there has been a drive to develop ITNs with AIs that have different modes of action[8–11]. Similarly, there are reports of wide spread resistance to insecticides (carbamates, organophosphates and organochlorines) used in IRS[7] hence the drive to develop products with AIs that have different modes of actions[12–17]. Development of ITNs and IRS products with AIs that have different modes of action to the conventional insecticides used for malaria vector control, offers opportunities to manage insecticide resistance via rotation. Furthermore, an increased awareness to detrimental effects to non-target organisms due to increased use of insecticides and disposal practices and call to reduce costs, has catalysed innovation of tools with less insecticides for vector control such as selective spraying for IRS[18] or weighing the use of durable untreated nets[19] to reflect a shift to environmentally safe and cost-effective options; and could contribute to sustaining gains so far made in malaria control.

The malaria burden continuously declined between 2000 and 2015, but progress has stalled[20]. To accelerate progress in reducing the burden of malaria, deployment of both ITNs and IRS has been explored in randomized

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control trials (RCTs) and observational studies[21]. However, these studies have produced contradictory evidence[21]. For example, a RCT in Tanzania showed that deploying ITNs and IRS together reduced malaria prevalence compared to deploying the ITN only[22]. On the contrary, in Benin and Gambia deploying ITNs and IRS did not significantly reduce clinical malaria compared to deploying ITNs only[22,23].

The World Health Organization (WHO) does not recommend co-deployment of ITNs and IRS for prevention and control of malaria in areas where there is ongoing malaria transmission, as evidenced from a systematic review[20,24]. However, co-deployment of ITNs and IRS may be considered for insecticide resistance prevention, mitigation or management[20].

Understanding the behaviour of malaria vectors in the vicinity of control tools such as ITNs and IRS could allow optimal co-deployment of ITNs and IRS. A number of methods have been used to study the behaviour of mosquitoes around bed nets including the use of infra-red video tracking and adhesives among others[25,26]. Using adhesive on bed nets to study mosquito behaviour in experimental huts is ideal as it may represent a theoretical potent insecticide that immediately kills all mosquitoes that come in contact or highlights the key contact net panels where most mosquitoes contact first. This may enhance the understanding of the behaviour of host-seeking malaria vectors that come in contact with ITNs, and in houses where ITNs and IRS are co-deployed or selectively applied with the view of optimizing insecticide use and deployment of the interventions.

Behavioural studies focussing on host seeking for a blood meal suggest that host seeking *Anopheles gambiae* and *A arabiensis* make multiple brief contacts on the roof compared with side panels of ITNs[25–28]. This has opened possibilities for designing nets to optimize insecticide use while controlling resistance. If host seeking malaria vectors are persistent, those that first contact the roof panel could also move on to contact with the side panel of a partially treated insecticidal net and vice versa; resulting in mortality equivalent to a whole treated ITN. A previous study showed that nets partially treated with a pyrethroid on the roof or side were as efficacious as fully treated nets against *An. arabiensis*[29]. In contrast, another study showed that the efficacy of a dual AI (pyrethroid and chlorfenapyr) roof treated net against *An. arabiensis* had reduced efficacy compared to a whole dual AI treated net in cow baited experimental huts[30]. Such partially treated insecticidal nets could be co-deployed with IRS to manage resistance. There is need to investigate the behaviour of malaria vectors around ITNs in experimental huts to inform strategies on co-deployment of IRS and ITNs or development of selective treated ITNs. In this study the behaviour of *An. arabiensis* around cow baited partially treated adhesive bed nets in experimental huts was investigated. It included persistence, panel of a bed net which more mosquitoes contact, and associations between induced exophily, and blood feeding.

Results

A total of 836 female *An. arabiensis* were collected over 25 trap nights in all trial arms. Of these, 214 (25.6%; 95% CI 22.7–28.7%) were from the no net; 121 (14.5%; 95% CI 12.1–17.0%) from the untreated, 140 (16.7%; 95% CI 14.3–19.4%) from the roof panel with adhesive, 144 (17.4%; 95% CI 14.7–20.0%) from the side panel with adhesive and 217 (26.0%; 95% CI: 23.0–29.1%) from whole adhesive treated net trial arms.

In the trial arms with adhesive coated nets, the highest proportion of *An. arabiensis* caught on the nets was recorded in the whole adhesive treated net arm compared to the side panel and roof panel adhesive treated net arms (Fig. 1). There was no persistence shown as there were significant reductions (p value > 0.05) in the likelihood of *An. arabiensis* being caught on either the side panel or roof panel adhesive treated nets compared to the whole adhesive treated net (Table 1).

The predicted mean numbers of *An. arabiensis* that were caught on the roof and side panels of the whole adhesive treated net were 0.64 (95% CI 0.29–0.99) and 1.32 (95% CI 0.77–1.87) respectively. These results showed that a significant majority of *An. arabiensis* first contacted the bed net on the side panel compared to the roof panel of the whole adhesive treated net (Table 2).

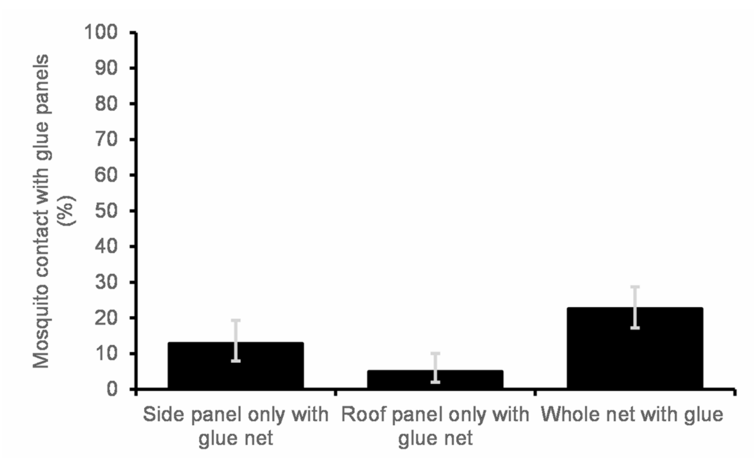

Fig. 1. Percentage of wild *An. arabiensis* that had contact with glue treated net panels in experimental huts. Error bars represent 95% Confidence Interval.

Exposure	AOR (95% CI)	*p* value, Z-value
Net with glue on sides and roof	1	
Net with glue on sides	0.50 (0.26–0.98)	**0.044, − 2.01**
Net with glue on roof	0.18 (0.07–0.43)	**<0.001, − 3.79**

Table 1. Measures of association between trial arms and contact with adhesive treated net panels for wild free flying *An. arabiensis. AOR* is adjusted odds ratio, *CI* is confidence interval, *p* value in bold is significant.

Trial arm	Density (95% CI)	Density ratio (95% CI)	*p* value, Z-value
Roof	0.64 (0.29–0.99)	1	–
Side	1.32 (0.77–1.87)	2.06 (1.04–4.08)	**0.037, 2.08**

Table 2. Measures of effect between *An. arabiensis* density and landing position on a bed net. *CI* is confidence interval, *p* value in bold is significant.

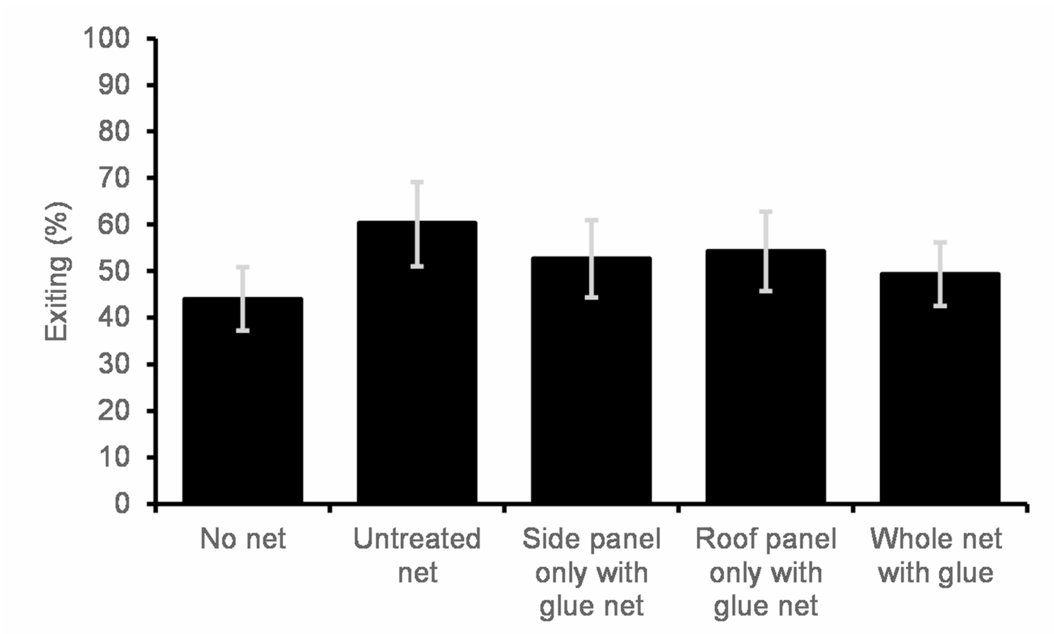

Fig. 2. Percentage of *An. arabiensis* that exited experimental huts in each trial arm. Error bars represent 95% Confidence Interval.

A total of 428 *An. arabiensis* exited the experimental hut of which 94, 73, 78, 76, and 107 were in the no net, untreated net, side adhesive treated net, roof adhesive treated net, and whole adhesive treated net trial arms respectively. The highest percentage of induced exophily was shown in the untreated net trial arm (Fig. 2). The likelihood of *An. arabiensis* exiting was significantly higher in the untreated net trial arm compared the no net trial arm (AOR: 2.12; 95% CI 1.19–3.79; p value = 0.010; $Z = 2.57$). There was no significant difference in the likelihood of exiting between the no net trial arm and the other trial arms (Table 3). Further analysis in the no net trial arm showed that blood feeding success significantly reduced the likelihood of *An. arabiensis* exiting the huts (AOR: 0.10; 95% CI 0.02–0.41; p value = 0.001; $Z = -3.22$).

The association between blood fed status and exiting in the No net trial arm is summarized in Table 4 below.

Discussion

This study assessed persistence, point of first contact on bed nets, and associations between induced exophily, and blood feeding of *An. arabiensis* in East African style experimental huts. These are aspects of mosquito behaviour that could be exploited for vector control and inform rational strategies for management of insecticide resistance; including hybrid nets, selective treatment of ITNs and co-deployment of ITNs and IRS. The results in this study showed that *An. arabiensis* is not persistent on cow baited bed nets, i.e. mosquitoes that first have contact with the roof panel are unlikely to go on and contact the side panel. Additionally, the results showed that the number of *An. arabiensis* mosquitoes contacting the side panels were twice those contacting the roof panel, but less

Exposure	Number of mosquitoes that exited	AOR (95% CI)	*p* value, Z—value
No net	94	1	
Untreated net	73	2.12 (1.19–3.79)	**0.010, 2.57**
Net with glue on sides	78	1.44 (0.84–2.46)	0.180, 1.34
Net with glue on roof	76	1.53 (0.89–2.64)	0.123, 1.54
Net with glue on sides and roof	107	1.28 (0.78–2.12)	0.327, 0.98

Table 3. Association between trial arms and exiting for wild free flying *An. arabiensis. AOR* is adjusted odds ratio, *CI* is confidence interval, *p* value in bold is significant.

Blood fed status	Number of mosquitoes exiting	AOR (95% CI)	p-value, Z-value
Unfed	55	1	–
Fed	39	0.10 (0.02–0.41)	**0.001, – 3.22**

Table 4. Association between blood fed status and exiting in the No net trial arm. *CI* is confidence interval, *p* value in bold is significant.

than whole adhesive net. The implication is that bed nets with insecticides restricted to either the roof or side panel may not be as efficacious as a whole insecticide treated bed nets. This assertion is supported by a previous study by Mbewe et al. (2022) and a systematic review by Lissenden et al. (2025), which reported a significantly lower mortality of *An. arabiensis* in a trial arm with insecticide treated roof bed net panels compared to whole insecticide treated bed net[30,31]. Furthermore, a meta-analysis of studies conducted at nine facilities showed that bed nets with insecticides restricted to either the roof or side panels were not equivalent or superior to whole insecticide treated bed nets in terms of mortality and blood feeding inhibition[31]. Therefore, studies on different designs of bed nets with restricted applied insecticides are recommended. Such designs could include bed nets with strip(s) of insecticide treated netting running across from one side panel to another through the roof panel.

It is possible that the lack of persistence could be unique to *An. arabiensis*, the bait (cow) and experimental hut design used in this study. More so that other studies have implicated odour plumes from the host, indoor air currents and cross-draughts to influence *An. gambiae* activity at either the roof or side panels of untreated bed nets[32,33]. However, a meta-analysis of nine studies that used more than one *Anopheles* species, human and animal baits, and a variety of experimental hut designs showed that partially treated insecticidal nets were less efficacious in terms of mortality compared to whole treated insecticidal nets[31]. Therefore, the results observed in this study could imply that the lack of persistence observe by *An. arabiensis* applies to other *Anopheles* mosquitoes.

Exiting of *An. arabiensis* from the huts was highest in the untreated net compared to the no net and adhesive treated trial arms. It was further observed that the likelihood of exiting was significantly higher in the untreated net arm compared to the no net arm. The observation suggests that the untreated net induced exiting. On the other hand, the likelihood of exiting in the adhesive treated net arms was not significantly different to the no net arm. This could have been due to the adhesive treated nets' ability to retain the mosquitoes. Furthermore, mosquitoes that were blood fed in the no net arm were more likely to rest indoors than blood unfed. It is possible that the observed higher exiting in the untreated net arm was due to unsuccessful blood feeding attempt by host seeking *An. arabiensis,* attributed to the net barrier effect. With host seeking mosquitoes unlikely to rest indoors in the presence of bed net, the implications are that, where ITNs (more so with excito repellent insecticides like pyrethroids) are deployed and usage is high, IRS may not have an additive killing effect. This assertion seems to be supported by a meta-analysis that reported no additive impact of IRS on malaria incidence in communities using pyrethroid-like ITNs[34].

The current study's observations seem to reinforce the WHO guideline of not recommending co-deployment of ITNs and IRS for prevention and control of malaria in children and adults in areas with ongoing transmission[20]. Furthermore, the induced exiting of *An. arabiensis* in the presence of a bed net raises a number of questions. Where next do the exiting blood unfed mosquitoes make an attempt to take a blood meal? Could these blood unfed exiting mosquitoes be responsible for outdoor malaria transmission? More studies are required to answer these questions and understand the malaria transmission dynamics especially that the core vector control interventions ITNs and IRS target indoor resting and biting mosquitoes. Such studies could help in developing further guidelines and strategies for malaria vector control.

An aspect that has not been looked at in this study is whether a torn net would still induce *An. arabiensis* to exit the huts. With a higher chance of blood feeding on a host under a torn bed net, it is possible that mosquitoes could rest indoors; and if indoor resting surfaces are sprayed with insecticides, perhaps there could be an additive impact on mosquito mortality. In such a case, co-deployment of ITNs and IRS could be synergetic to impact malaria transmission, especially that overtime, ITNs get damaged and develop holes[35–38]. Therefore, there is need to investigate whether IRS could have an additive effect on mosquito mortality in the presence of a torn net.

Conclusions

This study found that more *An. arabiensis* contacted the side than the roof panel of a bed net; and those that contact the roof panel do not go on to contact the side panel and vice versa. Therefore, studies on different

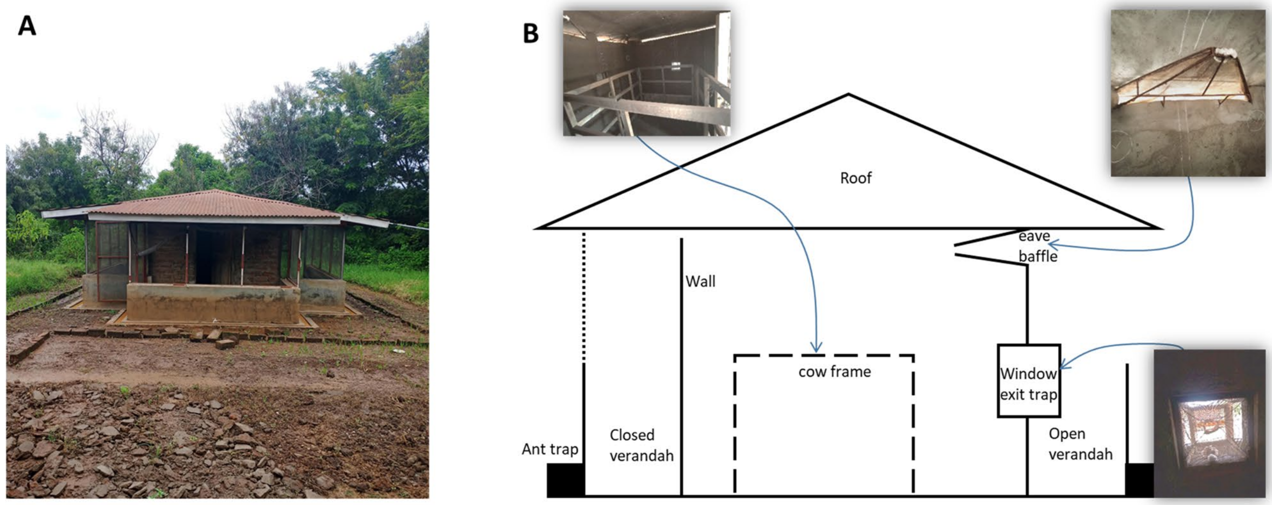

Fig. 3. East African style experimental hut (**A**) and schematic diagram of its set up in this study (**B**).

designs of bed nets that do not only restrict treatments to particular panels to reduce loading of insecticides are recommended. Additionally, host seeking mosquitoes are unlikely to rest indoors in the presence of an intact bed net. Therefore, co-deploying of intact bed nets and IRS may not be appropriate, as the latter requires mosquitoes to rest indoors to be effective. However, further studies on co-deployment of damaged bed nets and IRS are recommended.

Methods
Study site
The study was conducted at the Kilimanjaro Christian Medical University College Pan African Malaria Vector Research Consortium (KCMUco-PAMVERC) Pasua field site (S03° 22.764′, E37° 20.793′)[39] in Lower Moshi, Tanzania from June to October 2022. The site has seven East African style experimental huts[40] next to the Lower Moshi rice irrigation scheme. The rice plant periods: June to September and November to January coincide with the peak density of the major Anopheline species, *An. arabiensis* in the area[29,39]. This strain of *An. arabiensis* is partly zoophilic, feeding on cattle and humans[41,42]; and exhibits moderate pyrethroid resistance driven by overexpression of P450 mixed function oxidases[43,44].

Study design
A behavioural experiment was conducted in the experimental huts (Fig. 3) focusing on the behaviour of *An. arabiensis* in huts with intact cow baited glue treated bed nets. The experiment used wild free flying mosquitoes that entered the huts. Due to the partly zoophilic behaviour of the local *An. arabiensis*, young cows were used as bait instead of human volunteers[41,42]. Wooden enclosures (cow frames) of dimensions 140 cm width, 120 cm height and 180 cm length were made to hold the cows within the huts (Fig. 3). The cows were put in the huts at 18:00 until mosquito collections at 06:00 the following morning. The untreated bed nets (Safi net: A to Z Textile Mills, Arusha Tanzania) used in the experiment were locally sourced and prepared at the KCMUCo-PAMVERC Whole net store in Moshi.

Net preparation and trial arms: The net panels were coated with Tangle-Trap® sticky coating brush adhesive (The Tanglefoot Company, Grand Rapids USA). The present experiment hut trial had five arms where four arms had nets while in one arm there was no net. Four nets were assigned unique codes 178B–179B and 181B–182B. Therefore, the treatment arms were as follows:

i. 178B: Roof coated with adhesive, Sides coated with adhesive
ii. 179B: Roof without adhesive, Sides coated with adhesive
iii. 181B: Roof coated with adhesive, Sides without adhesive
iv. 182B: Roof without adhesive, Sides without adhesive
v. No net

Net preparations
Net 178B was prepared by cutting the stitching of all the panels using scissors to end up with five panels comprising of one roof panel, two short-side panels and two long-side panels. Each net panel was placed on a horizontal surface and adhesive coats applied. Both sides of each net panel were covered with non-stick baking paper and roll up. The ends of the cylinders that formed were fastened with rubber bands and label as 178B-R for the roof panel, 178B-SS1 for the first short-side panel, 178B-SS2 for the second short-side panel, 178B-LS1 for the first-long side panel and 178B-LS2 for the second-long side panel. Net 179B was prepared similarly to net 178B except for coating the roof panel with adhesive. These panels were labelled as 179B-SS1 for the first short-

side panel, 179B-SS2 for the second short-side panel, 179B-LS1 for the first long-side panel and 179B-LS2 for the second long-side panel. The detached roof panel was rolled up and placed in a sealable plastic bag labelled 179B-R. Net 181B was prepared by cutting the stitching that joins the roof panel to the side panels of the net. The roof panel of the net was placed on a horizontal surface and coats of the adhesive applied on it. Both sides of the entire panel were covered with non-stick baking paper and rolled up. Both ends of the cylinder that formed were fastened with rubber bands and labelled 181B-R. Then the line of stitching on the detached side netting of 181B was cut and then rolled up. The netting was put in a sealable plastic bag and labelled 181B. The control net 182B was prepared by cutting the stitching that joins the roof panel to the side panels. Thereafter, one line of stitching was cut down on the detached side netting. The roof and side panels were placed in separate sealable plastic bags labelled 182B-R and 182B-S respectively. All nets prepared, were transferred to Pasua field station where, each roof and side panel were stapled to a wooden roof and side panel frame of the cow frames respectively. Using a permanent marker, each frame was labelled with the treatment arm code.

Hut trial
Five experimental huts were used to compare five trial arms in a 5 × 5 Latin square design. The trial arms were randomly allocated to huts and rotated every sixth day to account for possible location bias of the huts.

Cow frames, blankets and other items used in mosquito collection were also rotated on the 6th day along with the treatment arms to reduce the chance of contamination of these items. The 6th day was also reserved for cleaning and airing the huts to reduce any possible contamination between the treatments. Cows were rotated daily between the huts. This hut trial ran for 25 nights to complete one full Latin square rotation. The number of mosquitoes was recorded according to the location of collection in the experimental hut, i.e. on the net, resting in the room, exit trap and verandah. Two nested surveys were conducted in the adhesive whole treated net and no net trial arms of the hut trial. In the adhesive whole treated net trial arm, data on the point of first contact on the net were collected, while data on associations between induced exophily and blood feeding were collected in the no net arm. In the whole adhesive treated trial arm, mosquito collection on the net was recorded according to the panel i.e. side and roof.

Data analysis
The behaviour of *An. arabiensis* in each trial arm was compared to the control in terms of the outcomes: persistence, point of first contact on the bed net and induced exophily. Persistence is the percent increase in the number of mosquitoes caught on the side panel and roof panel only adhesive treated nets relative to the whole adhesive treated net. Point of first contact on the net is the number of mosquitoes entering the hut found stuck on the roof relative to those found on the side panels of the whole adhesive treated bed net or vice versa in the morning. Induced exophily is percentage of the mosquitoes collected from the exit and verandah traps of treated huts relative to the percentage caught in exit and verandah traps of the control hut.

Stata SE version 16.1 (StataCorp LCC, College Station, TX, USA) to perform statistical analyses. Generalised linear mixed effects models (GLMM) with either a log or logit link function were used to analyse each outcome variable: induced exophily (proportions) and point of first contact on the net (counts); with predicators trial arms, and blood feeding; and random effects due to cows, huts and Latin Square rotation of the trial arm. All graphs were created in Microsoft Excel Office 2019 (Microsoft Corporation, Redmond, WA, USA). Means predicted from the GLMM with the log link are reported. Statistical significance was considered at $\alpha < 0.05$.

Ethics declarations
Informed consent and permission were sought from owners before the cows were used as baits in the trial.

The trial was conducted in conformity with London School of Hygiene and Tropical Medicine's Animal Welfare and Ethical Review Board ethical rules for studies with animal under reference 2020-02. Ethical approval to conduct the study was granted by Tanzania's National Institute for Medical Research reference NIMR/HQ/R.8a/Vol.IX/1656. All methods were performed in accordance with the guidelines and regulations of Tanzania's National Institute for Medical Research.

Data availability
The data generated and analysed during the current study are available from the corresponding author upon reasonable request.

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Author contributions

NJM, MJK, SA, WRM, FWM and JM conceived and designed the study. NJM, KE, BM, MFS and NMP contributed to field work and data collection. NJM analysed the data and drafted the first version of the manuscript, which was revised by all co-authors. All co-authors approved the final version of the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

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