PREFERRED RESTING SURFACES OF DOMINANT MALARIA VECTORS INSIDE DIFFERENT HOUSE TYPES IN RURAL SOUTH-EASTERN TANZANIA

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Public Health Research of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Malaria control in Africa relies extensively on indoor residual spraying (IRS) and insecticide-treated nets (ITNs). Indoor residual spraying typically targets mosquitoes resting on walls, and in few cases, roofs and ceilings, using contact insecticides. Unfortunately, little attention is paid to where malaria vectors actually rest indoors, and how such knowledge could be used to improve IRS. This study investigated preferred resting surfaces of two major malaria vectors, *Anopheles funestus* and *Anopheles arabiensis*, inside four common house types in rural south-eastern Tanzania. The assessment was done inside 80 houses in south-eastern Tanzania across four villages. In each house, resting mosquitoes were captured using Prokopack aspirators from walls, undersides of roofs, floors, furniture, utensils, clothes and bed-nets. Overall, only 26% of *An. funestus* and 18% of *An. arabiensis* were found on walls. In grass-thatched houses, 33-55% of *An. funestus* and 43-50% of *An. arabiensis* rested under roofs, while in metal-roofed houses, only 16-20% of *An. funestus* and 8-30% of *An. arabiensis* rested under roofs. Considering all data together, approximately 40% of mosquitoes rested on surfaces not typically targeted by IRS, i.e. floors, furniture, utensils, clothes and bed-nets. These proportions were particularly high in metal-roofed houses (47-53% of *An. funestus*; 60-66% of *An. arabiensis*). While IRS uses contact insecticides to target adult mosquitoes on walls, and occasionally roofs and ceilings, significant proportions of vectors rest on surfaces not usually sprayed. This gap exceeds one-third of malaria mosquitoes in grass-thatched houses, and can reach two-thirds in metal-roofed houses. Where field operations exclude roofs during IRS, the gaps can be much greater. In conclusion, there is need for locally-obtained data on mosquito resting behaviours and how these influence the overall impact and costs of IRS.

Keywords: **Malaria Control, Indoor Residual Spraying, Contact Insecticides, Malaria Vectors, *Anopheles funestus*, *Anopheles arabiensis*, Resting Behaviours.**

DECLARATION

I, Betwel John with registration number NM-AIST/M.400/T.17, hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation titled "Preferred resting surfaces of dominant malaria vectors inside different house types in rural south-eastern Tanzania" is my original work under the guidance of the supervisor listed below. It is being submitted in partial fulfilment of degree of Master of Science in Public Health Research to the Nelson Mandela African Institution of Science and Technology and it has not been submitted to any other learning institution.

-- 26th February 2020 --

Betwel John Date

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CERTIFICATION

I, undersigned have read dissertation titled "Preferred resting surfaces of dominant malaria vectors inside different house types in rural south-eastern Tanzania", submitted by Betwel John in partial fulfillment of the requirements for the degree of Master of Science in Public Health Research of the Nelson Mandela African Institution of Science and Technology, I hereby confirm that it is an authentic work and recommend for its acceptance by the Institute.

-- 26th/Feb/2020

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Supervisor

Date

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DEDICATION

I dedicate this work to my family and friends.

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ABBREVIATIONS

ACT	–	Artemisinin-based Combination Therapy
AIC	–	Akaike Information Criterion
ELISA	–	Enzyme-Linked Immunosorbent Assay
GLMM	–	Generalized linear mixed effects models
IHI	–	Ifakara Health institute
IRS	–	Indoor Residual Spraying
ITNs	–	Insecticide-Treated Nets
LLINs	–	Long-Lasting Insecticide-treated Nets
NBS	–	National Bureau of Statistics
NIMR	–	National Institute for Medical Research
PCR	–	Polymerase Chain Reaction
PMI	–	President's Malaria Initiative
RR	–	Rate Ratio
WHO	–	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1. Background of the problem

Malaria control efforts have yielded significant success in recent decades, resulting in decline in number malaria cases from 251 million in 2010 to 228 million in 2018 (World Health Organization (WHO), 2019b). The most widely used interventions, namely insecticide-treated nets (ITNs), indoor residual spraying (IRS) and artemisinin-based combination therapy (ACT) are credited with 663 million clinical cases of malaria averted between 2000 and 2015 (Bhatt *et al*., 2015). In Tanzania, the impact of these interventions has been demonstrated by multiple investigators (Alba *et al.*, 2014; Bhattarai *et al*., 2007; Mashauri *et al*., 2013; Selemani *et al*., 2016), as well as national surveys, which show significant overall reduction in burden (National Bureau of Statistics (NBS) *et al*., 2018). Despite these gains, there is also evidence that the anti-malaria progress is levelling off and that the gains may be lost (WHO, 2018b). Between 2015–2017, continued utilization of the core interventions led to no significant declines in malaria at global scale (WHO, 2018b).

To rejuvenate the malaria fight, several countries have set ambitious goals in line with the WHO Global Technical Strategy for Malaria Elimination (WHO, 2015b), and more recently, the High Burden to High Impact initiative which targets the ten most malarious countries in Africa, plus India (WHO, 2018a). The new initiatives are expected to be much more aggressive and country-led but involving multiple partners. However, similar to previous efforts, these efforts are primarily reliant on ITNs (now long-lasting insecticide-treated nets (LLINs)), IRS and effective case management (WHO, 2019b). Despite proven effectiveness of the vector control interventions, LLINs and IRS are negatively affected by insecticide resistance (Hemingway *et al*., 2016; Ranson & Lissenden, 2016), increasing outdoor-biting (Finda *et al*., 2019; Monroe *et al*., 2019; Sherrard-Smith *et al*., 2019), high costs and the sub-optimal coverage and usage at community and household level. Resistance is often associated with exposure of vectors to insecticides used in agriculture (Nkya *et al*., 2014) and public health (Protopopoff *et al*., 2008; Stump *et al*., 2004), and the indoor interventions may also induce shifts in vector biting and resting behaviours (Killeen & Chitnis, 2014; Moiroux *et al*., 2012; Russell *et al*., 2010).

1.2. Statement of the problem

IRS is one of the oldest malaria interventions and was the most important component of the initial attempts at global malaria eradication in 1950s and 1960s (Kouznetsov, 1977; Nájera *et al*., 2011). It involves applying insecticides to kill mosquitoes resting on interior walls of houses (WHO, 2015a). In Tanzania, it has been used intermittently since the 1960s (Pringle, 1967), and is currently deployed in selected districts mostly in the northern regions where malaria burden remains very high (National Malaria Control Programme, 2014; The Presidents Malaria Initiative (PMI) VectorLink Project, 2019). Across Africa, IRS is mostly promoted by the United States (US) Presidents Malaria Initiative, and currently covers 14 countries in Africa (U.S. President's Malaria Initiative, 2019b, 2019a). According analysis by Bhatt *et al*. (2015) IRS alone contributed to 10% of averted clinical malaria cases in Africa between 2000 and 2015 (Bhatt *et al*., 2015). To counter the growing challenge of insecticide resistance (WHO, 2012), most countries have switched from using pyrethroids, and now rely mostly on organophosphates or carbamates, as well as some new insecticide classes such as neonicotinoids, which were recently introduced (WHO, 2019a). There have also been calls to introduce bed-nets with multiple active ingredients or synergists as a way to tackle resistance (Gleave *et al*., 2018; Protopopoff *et al*., 2018; Tiono *et al*., 2018).

While much of the focus is paid to finding new chemical actives and combinations, considerably less attention is paid to how malaria mosquitoes actually respond to the indoor interventions such as IRS and LLINs. This is despite the changing housing designs and structures across Africa (Tusting *et al*., 2019), and the demonstrated impact of housing on vector densities and malaria transmission (Kirby *et al*., 2013; Lindsay *et al*., 2003; Tusting *et al*., 2015, 2017). Instead indoor interventions still primarily rely on historical evidence of mosquito indoor resting habits (Smith, 1962b, 1962a), which are now due for update in light of modern transformations (Tusting *et al*., 2019). A study from early 1960s in Tanzania assessed distribution of malaria vectors on sprayable surfaces inside houses compared to household possessions usually removed during IRS (Smith, 1962b). It was observed that less than 20% of mosquitoes rested on the possessions, and that of the remaining, sprayable surfaces, the resting populations were evenly divided between substrates (Smith, 1962b). In a separate study in mud huts in northern Tanzania, 56% to 70% of all resting mosquitoes were found on the walls or hanging articles, while the remaining 30% to 40% were on the underside of the roofs (Smith, 1962a).

Other than these early studies, such investigations have become rare, yet it is likely that mosquito behaviours and survival inside houses could change with the ongoing improvements. For example, a recent study in the Gambia demonstrated that reduced mosquito survival in metal-roofed houses may lower malaria transmission (Lindsay *et al*., 2019). Elsewhere in East Africa, it was shown that despite higher temperatures inside houses with corrugated iron roofs, survival of mosquitoes resting indoors was same as in grass thatched houses (Okech *et al*., 2004).

It is, therefore, crucial to understand resting behaviours of the major malaria vectors inside houses and how much they can be affected by key indoor interventions. This way, effectiveness of techniques such as IRS can be improved, and their limitations determined. This study therefore investigated the resting behaviours of two major malaria mosquitoes (*An. funestus* and *An. arabiensis*) inside typical house types in rural south-eastern Tanzania. In this area, most malaria infections are mediated by *An. funestus*, even though *An. arabiensis* remains abundant as well (Kaindoa *et al*., 2017; Lwetoijera *et al*., 2014).

1.3. Rationale of the study

In recent years anti-malaria progress has been approaching steady rate (WHO, 2018b), partly because existing interventions, are negatively affected by multiple challenges such as insecticide resistance (Hemingway *et al*., 2016; Ranson & Lissenden, 2016), high costs and the sub-optimal coverage and usage at community and household level. This, therefore calls for alternative approach to control malaria and enhancing existing interventions to bring the fight against malaria back on track.

Indoor residual spraying has been used for many years to control malaria and was one of the key interventions during early malaria eradication in 1950s to 1960s (Kouznetsov, 1977; Nájera *et al*., 2011). It involves spraying insecticides on the walls of houses, and occasionally on roofs. Unfortunately, current procedures were developed several decades ago, when houses in Africa were still mostly thatch-roofed. Surprisingly, this practice has not changed despite improvements in housing across Africa in recent years (Tusting *et al*., 2019). Therefore, it was important to do this research to learn on indoor resting behavior of mosquito in response to current housing. This knowledge is important in the fight against malaria, as it can be used in our advantage to ensure we achieve better result in the battle against malaria mosquitoes. For example, information can be used to enhance effectiveness of house spraying as it shows where exactly we can find more mosquitoes, making it easier to introduce strategies in house spraying to achieve the great impact against malaria vectors.

1.4. Research objectives

1.4.1. Main objective

To investigate indoor resting behaviours of mosquito vectors and factors influencing their indoor resting preferences in rural south-eastern Tanzania.

1.4.2. Specific objectives

(i) To assess preferred indoor resting surfaces of major malaria vectors inside different house types in south-eastern Tanzania.

(ii) To assess associations between household factors and indoor resting preferences of mosquitoes.

1.5. Research questions

(i) What are the preferred indoor resting surfaces of major malaria vectors inside house?

(ii) What are the associations between household factors and indoor resting preferences of mosquitoes?

1.6. Significance of the study

This research created baseline knowledge on indoor resting behaviour of the major malaria vectors inside human occupied houses. Indoor residual spraying, an intervention that target resting mosquitoes involves targeting mosquitoes resting on walls however one third to two third of malaria vectors rest on surfaces that are not targeted by IRS. Thus, the knowledge generated in this study will help in planning and improving interventions targeting resting mosquitoes (especially IRS) to accelerate control and elimination efforts of residual malaria transmission.

1.7. Delineation of the study

Initially when this study was set up, collections of mosquitoes were done in the morning. This resulted to most collections of mosquitoes to be carried in the morning. It is during this time when household members participate in household chores. Thus, choice of mosquitoes to rest during morning time might have been influenced by human movement in the morning. Thus, collections in the morning might have underestimated mosquitoes resting on surfaces such as floors, furniture and utensils.

These observations of indoor resting behaviours of mosquitoes were carried out in villages that are not protected with IRS. However, evidences have shown that mosquitoes tend to change behaviour as a response to interventions. Therefore, it is important that future studies should be carried-out to assess indoor resting preference of mosquitoes in houses protected with IRS.

This study did not track movement of mosquitoes inside house rather observed where they were during time of collection. Therefore, it is acknowledged that proportion of mosquitoes resting on surfaces other than walls does not mean that these mosquitoes would never come into contact with walls or would not be killed by IRS when walls are sprayed. For this reason, additional studies may be required to examine how these resting preferences of mosquitoes inside houses impact effectiveness of IRS.

CHAPTER TWO

LITERATURE REVIEW

2.1. Vectors of malaria in Africa

In most African regions, major vectors of malaria comprise *Anopheles* mosquitoes belonging to *Anopheles funestus* group (*An. funestus* s.s.) and *Anopheles gambiae* complex (*An. gambiae* s.s., *An. arabiensis* and *An. colluzzi*) (Coetzee *et al*., 2013; Sinka *et al*., 2012). Both *An. funestus*, *An. gambiae* and *An. arabiensis* are dispersed across settings in western, eastern, southern, and central Africa (Sinka *et al*., 2012). *Anopheles arabiensis* is outspreading further to arid and semi-arid areas of Africa such as Ethiopia and northern parts of Botswana and Namibia as well as in Sahel region (Sinka *et al*., 2012). *Anopheles colluzzi* on the other hand extends from West Africa to Central Africa and some parts of east Africa, where samples of *An. colluzzi* identified in parts of Zimbabwe (Coetzee *et al*., 2013).

Anopheles gambiae have historically been most important vector among major malaria vectors, in several settings in Africa. However, its population has been reduced to undetectable levels (Bayoh *et al*., 2010; Lwetoijera *et al*., 2014). In these region *An. funestus* now presents major threats despite occurring in fewer numbers compared to *An. arabiensis.* For example, in villages of south-eastern Tanzania *An. funestus* are credited for mediating nearly nine out of 10 malaria cases in that region (Kaindoa *et al*., 2017).

2.2. Malaria control tools

Malaria control and elimination relies on tools such as insecticide treated nets, indoor residual spraying (vector control tools), effective case management and intermittent preventive treatment during pregnancy (WHO, 2018b). Insecticide treated bed-nets (ITNs) and indoor residual spraying (IRS) are regarded as important vector control strategy for malaria control and elimination, targeting indoor biting and indoor resting mosquitoes. They have demonstrated impressive impact in malaria control and elimination. According to Bhatt *et al*. (2015) among 663 million cases averted in Africa between 2000 and 2015, 68% were contributed by ITNs and 10% by IRS (Bhatt *et al*., 2015). Apart from epidemiological impact of these intervention, as mentioned earlier these tools have resulted to significant reduction of the competent vector, *An. gambiae* s.s. in several regions of Africa (Bayoh *et al*., 2010; Lwetoijera *et al*., 2014).

2.3. Feeding preferences of malaria vectors and implications in malaria interventions

Vector control and elimination efforts are partly affected by preference of mosquitoes to feed indoor or outdoor. Whereby, mosquitoes that prefer obtain blood meal outside houses (exophagic) are less likely to be affected by indoor interventions. For example, *An. gambiae* s.s. and *An. funestus* s.s. represent malaria vectors with higher preference of indoor feeding (endophagic) (Pates & Curtis, 2005). Indeed, it is because of this behaviour, expansion of indoor interventions such as insecticide treated bed-nets has led to untraceable numbers of *An. gambiae* s.s. (Bayoh *et al*., 2010; Lwetoijera *et al*., 2014). However, *An. arabiensis* represents malaria vectors with higher degree of exophagic behaviour. *Anopheles arabiensis* thrives even in areas with high coverage indoor interventions, feeding on human when outdoor as well as on other animals when humans are absent or protected (Tirados *et al*., 2006). Thus, outdoors feeding mosquitoes evade fatal incidents with indoor interventions such as long-lasting insecticides treated nets and indoor residual spraying.

2.4. Resting behaviours of malaria vectors and house spraying with insecticides

Once mosquitoes obtain blood meals, they can either rest inside or outside houses for digestion of blood and development of eggs. Earlier study demonstrated that mosquitoes went indoor to obtain blood where they also rest until they are ready to lay eggs (De Meillon, 1934). House spraying utilizing this behaviour have been successful in several setting, and was the primary vector control tool during the Malaria eradication 1955-1969 (Kouznetsov, 1977; Nájera *et al*., 2011).

In control of mosquito vector using indoor interventions, it is important to understand if mosquitoes prefer to rest inside houses and to determine where exactly mosquitoes prefers to rest when they are inside human dwellings. In historical times, some studies have gone beyond indoor-outdoor resting behavior of mosquitoes and assessed where exactly malaria vectors rest inside houses. For example, in villages of Ukara Island in Lake Victoria of Tanzania, a study in huts made of mud walls and thatched roofs found that less than 40% of *An. funestus* and *An. gambiae* were resting on roofs. Percentage of mosquitoes found resting on surfaces below roof level was between 61% and 70% (Smith, 1955). In a separate study in Umbugwe, northern Tanzania, daytime collections made between 1959-1960 in mud huts with turf or thatched roofs, similar results were observed, 56% to 70% of *An. gambiae* were found resting on walls or hanging articles, while from 30% to 44% *An. gambiae* were found resting underside of roofs (Smith, 1962a). In another study from early 1960s in Tanzania assessed distribution of malaria vectors on sprayable surfaces inside houses relative to non-sprayable household possessions (Smith, 1962b). It was observed that less than 20% of mosquitoes rested on the possessions that are usually removed during IRS, and that of the remaining sprayable surfaces, resting mosquitoes were evenly divided between surfaces (Smith, 1962b).

Similarly, in earlier studies in Kenya and Uganda, resting preference of African malaria vectors was shown to be more on higher surfaces such as roof or ceiling and upper parts of walls over floor and other resting surfaces (Gibbins, 1933; Haddow, 1942). Apart from these studies in East Africa, resting behavior of mosquitoes inside houses was also investigated in a study in villages of Burkina Faso (West Africa). The purpose of this study was to assess dichlorvos using evaporators as a residual fumigant for malaria control in mud houses. In this study, it was observed that 94.6% *An. funestus* and *An. gambiae* were rested on ceiling (Mathis *et al*., 1963).

Elsewhere beyond Africa, indoor resting behavior of different species of malaria vectors have also been documented. A study by Ogata in 1990's documented preferences of *Anopheles albimanus* inside houses with thatched roofs and woody or bamboo walls. In this study mosquitoes were observed to have higher preference of resting on roofs. Whereby 53% of mosquitoes were observed resting on underside of roofs, 28.3% on walls, 13.4% on furniture, 2.1% ceiling, 2.1% on eaves, and 1.1% on exterior walls (Ogata *et al*., 1992). In east central India, resting preference of *Anopheles fluviatilis* and *Anopheles minimus* were studied. The two species had exclusive preference of resting on walls. Of all *An. fluviatilis* collected indoor 99.3% were resting on walls and 0.7% were resting on hanging objects, while all of *An. minimus* were resting on walls (Sahu *et al*., 2011). On walls, large percentage of both species were concentrated on portion of walls (3-4 ft from the floor), ranging between 45.6% and 47.3%. Below 3 ft and above 4 ft the percentage of mosquitoes were between 21.6% and 32.8% (Sahu *et al*., 2011). A more recent study in area with houses with stone/brick walls and stone slab/concrete roofs, *Anopheles stephensi* were observed to have higher preference of resting on non-sprayable household items such as cupboards, furniture, clothes, stored goods, cobwebs and floor. Percentage of mosquitoes resting on household items was between 95% and 97% while less than 5% rested on sprayable surfaces such as walls, roofs and windows (Nagpal *et al*., 2012).

It appears that resting preferences of mosquitoes inside houses varies between mosquito species and geography. Thus, it is important to assess resting preferences of mosquito species in different geography rather than relying on historical evidences. Unfortunately, other than these early studies, there have been few attempts to examine mosquito resting preferences indoors and how this may influence vector control efforts such as IRS.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study area

The study was conducted in four villages across Ulanga and Kilombero districts in south-eastern Tanzania (Fig. 1). These included, Kivukoni (-8.2021, 36.6961) and Tulizamoyo (-8.3669, 36.7336) in Ulanga district, and Sululu (-7.9973, 36.8317) and Ikwambi (-7.9833, 36.8184) in Kilombero district. The area is within a low-lying river valley extending 250 km long and up to 65 km wide, interspersed with villages and farmlands. It has two rainy seasons, short rains between November and December and long rains between March and May, while between rainy seasons spans two dry seasons. Annual rainfall and temperatures vary from 1200 mm to 1800 mm, and 16°C to 32°C, respectively (World Weather Online, 2019). Residents are mostly subsistence farmers, though some are also fishermen or owned small businesses.

During this study, typical house types in the villages were either thatch-roofed or metal-roofed (with corrugated iron sheets), and had either mud walls or brick walls, which were sometime plastered with concrete. Primary malaria vectors in this region are *An. funestus* and *An. arabiensis*, with *An. funestus* contributing more than 80% of current malaria transmission (Kaindoa *et al*., 2017). *Culex pipiens* are nuisance biters contributing 79% of all indoor biting risk (Matowo *et al*., 2019).

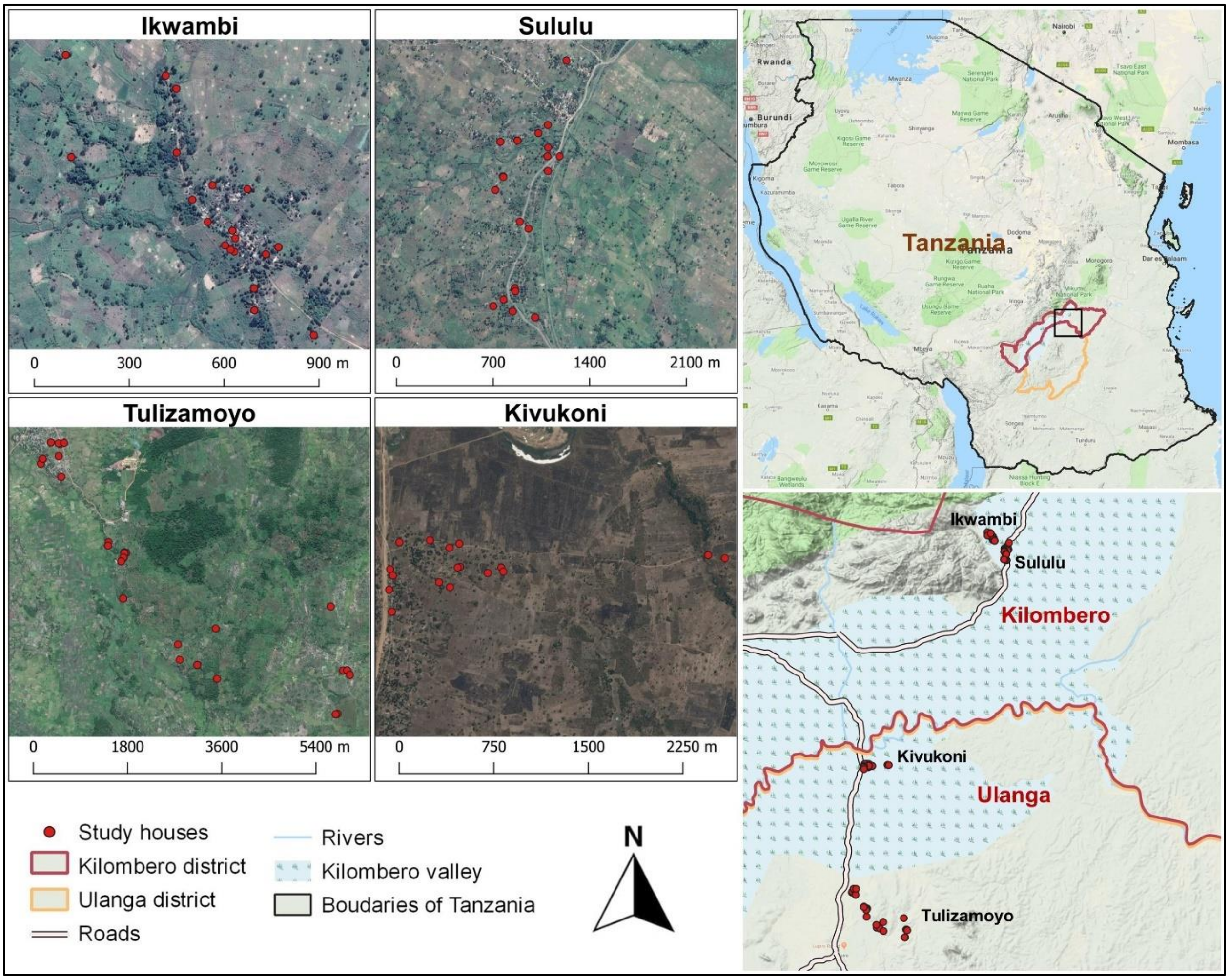

Figure 1: Map showing study villages and study households in both Kilombero and Ulanga districts, south-eastern Tanzania

3.2. Selection and characterization of study houses

Field collection of resting mosquitoes was done inside human-occupied houses, ensuring to cover the main house types. Candidate houses were selected based on construction materials for walls (mud or bricks, with or without concrete plastering) and roofs (metal or thatch). This resulted in four classes of houses (Fig. 2) commonly found in the study area, namely: (a) houses with thatched roofs and mud walls, (b) houses with thatched roofs and brick walls (none of these houses had plastered walls), (c) houses with metal roofs and un-plastered brick walls, and (d) houses with metal roofs and plastered brick walls. Ceilings were uncommon and therefore excluded in this survey. All individual houses were also geo-referenced, then characterized by other attributes, namely: (a) whether eave gaps were open or closed, (b) number of rooms in the house, (c) height of walls and (d) maximum daily temperatures (°C), recorded using Tinytag® data loggers (Gemini, UK) hung at height more than a meter from the roof, or on a dry surface away from the floor.

A sample size of 20 houses was estimated using the Cohen simulation with the pwr R package, to achieve at least 80% power with 0.5 effect size. Therefore, prior to commencement of mosquito collections, 20 houses were purposively selected in each of the four villages upon consent by household heads. These included five houses per house type.

Figure 2: Typical house types in the study villages in rural south-eastern Tanzania

3.3. Collection of mosquitoes resting on different surfaces inside the houses

Potential mosquito resting places were identified to include: (a) walls, (b) roofs (underside of the roofs) and (c) other surfaces such as floor, clothing, bed-nets and other household items. The household items were varied but generally included furniture such as beds, tables, chairs, cupboards, wood blocks, other household items such as bicycles, and utensils, wash basins, water containers, clay pots and cooking pans. The clothing included hanging garments, curtains, sacks and bags. Actual mosquito collections were done using Prokopack aspirator (Maia *et al*., 2011) by trained technicians. Collections involved hovering the aspirator systematically over the surfaces and collecting all mosquitoes. Lighting was provided using hand-held flash lights.

The sequence of collection between resting surfaces in each room was changed to minimize sampling biases. The collections were done for five days each week in each village, visiting 2-4 houses per day. Initially the collections were done between 0600 h and 12 h, from January 2019 to May 2019. Then from May to July 2019, the collections were done three times a day (in the morning (between 0700 h and 0830 h), evening (between 1800 h and 2000 h) and at night (between 2400 h and 0200 h)), to minimize variations associated with mosquitoes moving between different resting surfaces within the houses. Unlike the other collections done by trained technicians, the late evening and late-night collections were done by trained household members to avoid intrusion of their privacy.

In total, there were 277 house visits for indoor resting mosquito collections, including 76 visits to houses with thatched roofs and mud walls, 70 to houses with thatched roofs and brick walls, 70 to houses with corrugated iron roofs and un-plastered brick walls, and 61 visits to houses with corrugated iron roofs and plastered brick walls.

3.4. Morphological identification and processing of collected mosquitoes

Mosquitoes collected from each of the resting surfaces were placed in separate disposable cups and labelled appropriately. They were sorted by sex and taxa, then all *Anopheles* sorted and identified using the morphological keys (Gillies & Coetzee, 1987). Physiological status of each female *Anopheles* was determined as unfed, partly fed, fully fed, gravid or semi gravid. All records were kept by house, surface, house type and village.

3.5. Identification of sibling species of malaria vectors, blood meal analysis and detection of *Plasmodium falciparum* sporozoites in the mosquitoes

The field-collected mosquitoes were packed individually in 1.5 ml microcentrifuge tubes (BioPointe Scientific®) containing silica plugged with cotton wool. Sub-samples of *An. funestus sensu lato* (s.l.) and *An. gambiae* s.l females were further analysed for sibling species, *Plasmodium falciparum* sporozoites and blood meal sources (if the mosquitoes were blood-fed). Sibling species identification for *An. funestus* s.l and *An. gambiae* s.l was done using polymerase chain reaction

(PCR) protocols originally developed by Koekemoer and others (Koekemoer *et al*., 2002) and Scott and others (Scott *et al*., 1993) respectively. Blood meal analysis was done using enzyme-linked immunosorbent assay (ELISA) tests (Beier *et al*., 1988), and parasite infections detected by screening for the *P. falciparum* circumsporozoite proteins in salivary glands of the adult females (Wirtz *et al*., 1987). Heat-labile non-*P. falciparum* were eliminated by boiling the ELISA lysates at 100°C for 10 minutes to remove false positives (Durnez *et al*., 2011).

3.6. Determination of physiological ages of mosquitoes

Parity of mosquitoes was approximated following procedure described by Detinova (Detinova, 1962) as a proxy of physiological age of mosquitoes. A subsample of non-blood fed*, An. funestus* and *An. arabiensis*, were first immobilized in a refrigerator. Under stereo microscope abdomens of anesthetized mosquitoes were dissected to extract ovaries. Ovaries were examined under compound microscope to determine whether mosquitoes had laid eggs or not.

3.7. Data analysis

Data analysis was done using open source statistical software, R version 3.6.0 (R Core Team, 2019). Generalized linear mixed effects models (GLMM) were built using functions within the *lme4* package (Bates *et al*., 2015) to assess: (a) preferences of mosquitoes (*An. funestus*, *An. arabiensis* and *Culex*) for different resting surfaces and (b) relationships between various household risk factors and number of mosquitoes caught on different surfaces. Initially, the number of female mosquitoes of each species was modelled as a response variable against resting surfaces as a fixed factor. Since walls are typically the main target for insecticide spraying, they were used as reference against which other surfaces were compared.

To assess relationships between household risk factors and mosquitoes resting on different surfaces, the number of mosquitoes caught from each surface was modelled as function of: (a) roof type, (b) wall type, (c) whether interior walls were plastered with cement or not, (d) eave gaps, (e) number of rooms, (f) wall height and (g) daily maximum temperatures inside the houses.

In all models, households nested within villages and sampling days were used as random terms, to capture unexplained variations, and account for pseudo-replication. Poison distribution was used when fitting GLMM models, except when overdispersion was detected, in which cases, negative binomial distribution was used instead. The best fitting models were selected using Akaike Information Criterion (AIC) (Bolker, 2007), and results presented as relative rate ratios (RR) at 95% confidence intervals. In addition, the *dabestr* package for estimation statistics (Ho *et al*., 2018), was used to depict effect sizes of differences in mean numbers (at 95% confidence intervals) of mosquitoes collected on different resting surfaces relative to walls.

3.8. Ethics approval and consent to participate

Detailed explanations on aim, procedures, potential risks and benefits of the study was provided to household occupants before study commencement. Written informed consent in local language (*Swahili*) were obtained from household heads before inclusion in the surveys. Ethical approval for the study was granted by Institutional Review Board of Ifakara Health Institute (IHI/IRB/No: 007-2018) and the Medical Research Coordination Committee of the National Institute for Medical Research in Tanzania (NIMR/HQ/R.8a/Vol.IX/2895).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Results

4.1.1. Descriptive summary of mosquitoes caught in the surveys

A total of 17 870 female mosquitoes were collected, of which 31.1% (n= 5 564) were *Anopheles* mosquitoes and 68.9% (n=12 306) were culicines. Among *Anopheles* mosquitoes, 81.5% (n=4535) were *An. funestus* s.l, 17.6% (n= 977) were *An. arabiensis* and 0.9% (n=52) were other *Anopheles* species including *Anopheles custani* and *Anopheles pharoensis*. The majority of *An. funestus* (72.4%), *An. arabiensis* (87.8%) and *Culex* (58.0%) were collected in thatch-roofed houses.

4.1.2. Resting preferences of mosquitoes inside the houses

There was an uneven distribution of mosquitoes between the four house types and between the different resting surfaces (Tables 1 & 2; Fig. 3). Only 26.1% of *An. funestus,* 18.2% of *An. arabiensis* and 27.9% of *Culex* mosquitoes rested on walls. Proportions resting on the undersides of the roofs included 32.9% of *An. funestus,* 42% of *An. arabiensis* and 33.6% of *Culex* mosquitoes. Surprisingly, as many as 41% *An. funestus*, 40% of *An. arabiensis* and 39% of *Culex* mosquitoes rested on surfaces other than either the walls or roofs, i.e. surfaces that are not typically sprayed during IRS. The actual distribution of the two malaria vector species and the *Culex* mosquitoes also depended on house construction materials. Nearly 80% of *An. funestus* and *An. arabiensis* were collected in grass-thatched houses and the remainder in the metal-roofed houses. However, once inside the houses, proportions resting under the roof surfaces was generally lower in metal-roofed houses (*An. funestus*, 16.0% - 20.0%; *An. arabiensis*, 7.6% - 30.0%) than in grass-thatched houses (*An. funestus*, 32.5% - 55.2%; *An. arabiensis*, 43.1-49.8%). The proportions of mosquitoes resting on surfaces not typically sprayed were approximately one third in grass-thatched houses, and between one half and two third in metal-roofed houses. Full details including distribution of *Culex* mosquitoes are shown in Table 1.

Table 1: Numbers and percentages of mosquitoes of different species collected from different surfaces inside houses of different types

Species	**Resting surfaces inside houses**	**Thatched roofs and mud walls**	**Thatched roofs and brick walls**	**Metal roofs and un-plastered brick walls**	**Metal roofs and plastered brick walls**	**Totals**
		n (%)	n (%)	n (%)	n (%)	N (%)
Anopheles funestus	Walls	168 (17.9)	573 (24.5)	385 (37.1)	59 (27.4)	1185 (26.1)
	Roofs	519 (55.2)	762 (32.5)	166 (16.0)	43 (20.0)	1490 (32.9)
	Other surfaces	253 (26.9)	1008 (43.0)	486 (46.9)	113 (52.6)	1860 (41.0)
	Total	**940**	**2343**	**1037**	**215**	**4535**
Anopheles arabiensis	Walls	111 (21.0)	42 (12.7)	21 (26.6)	4 (10.0)	178 (18.2)
	Roofs	227 (43.1)	165 (49.8)	6 (7.6)	12 (30.0)	410 (42.0)
	Other surfaces	189 (35.9)	124 (37.5)	52 (65.8)	24 (60.0)	389 (39.8)
	Total	**527**	**331**	**79**	**40**	**977**
Culex mosquitoes	Walls	1089 (25.2)	700 (25.4)	683 (32.2)	929 (31.1)	3401 (27.9)
	Roofs	1926 (44.6)	1352 (49.0)	389 (18.3)	431 (14.4)	4098 (33.6)
	Other surfaces	1300 (30.1)	707 (25.6)	1051 (49.5)	1630 (54.5)	4688 (38.5)
	Total	**4315**	**2759**	**2123**	**2990**	**12 187**

Table 2 shows the extent to which mosquitoes preferred roofs and other internal house surfaces, compared to walls. Generally, the proportion of mosquitoes resting on non-sprayed surfaces (other surfaces) was always higher than proportions resting on walls regardless of house type. However, proportions resting on roofs was higher than on walls for grass-thatched houses, but lower for metal-roofed houses (Table 2).

When the data was examined for different house types, it became clear that wall surfaces, at best had only one third of mosquitoes resting. Depending on house construction materials, proportions of mosquitoes resting on roofs and other surfaces was often higher than on walls, except in metal-roofed houses, where walls tended to harbour more mosquitoes (Fig. 5 & 6). Data for *Culex* mosquitoes is shown in Tables 1 & 2, and Fig. 4 and 7. When the other surfaces were examined in detail, it was observed that significant proportions of mosquitoes on these surfaces were resting on bed-nets, floors, and on furniture, but also on hanging clothes. Full details are provided in Table 3.

Table 2: Summary statistics for comparison of the number of mosquitoes of different species collected from walls, roofs and other surfaces inside the different house types

House type	Resting surfaces	***Anopheles funestus***		***Anopheles arabiensis***		***Culex* mosquitoes**	
		RR (95% CI)	**p value**	**RR (95% CI)**	**p value**	**RR (95% CI)**	**p value**
Thatched roofs and mud walls	Walls	1.00		1.00		1.00	
	Roofs	2.72 (2.11-3.50)	0.001	1.93 (1.33-2.79)	0.001	1.77 (1.50-2.08)	0.001
	Other surfaces	1.49 (1.14-1.94)	0.003	1.73 (1.20-2.51)	0.004	1.31 (1.11-1.54)	0.001
Thatched roofs and brick walls	Walls	1.00		1.00		1.00	
	Roofs	1.17 (0.94-1.45)	0.170	2.27 (2.27-2.28)	0.001	1.72 (1.37-2.16)	0.001
	Other surfaces	1.63 (1.31-2.02)	0.001	1.97 (1.96-1.97)	0.001	1.16 (0.92-1.47)	0.210
Metal roofs and un-plastered brick walls	Walls	1.00		1.00		1.00	
	Roofs	0.42 (0.32-0.57)	0.001	0.29 (0.12-0.71)	0.007	0.51 (0.37-0.69)	0.001
	Other surfaces	1.25 (0.96-1.63)	0.100	1.60 (0.93-2.78)	0.090	1.50 (1.15-1.97)	0.003
Metal roofs and plastered brick walls	Walls	1.00		-	-	1.00	
	Roofs	0.73 (0.50-1.07)	0.110	-	-	0.64 (0.48-0.83)	0.001
	Other surfaces	1.92 (1.41-2.59)	0.001	-	-	1.98 (1.56-2.52)	0.001

Very few *An. arabiensis* were caught in houses with metal roofs and plastered brick walls, it was not possible to fit GLMM model to *An. arabiensis* data

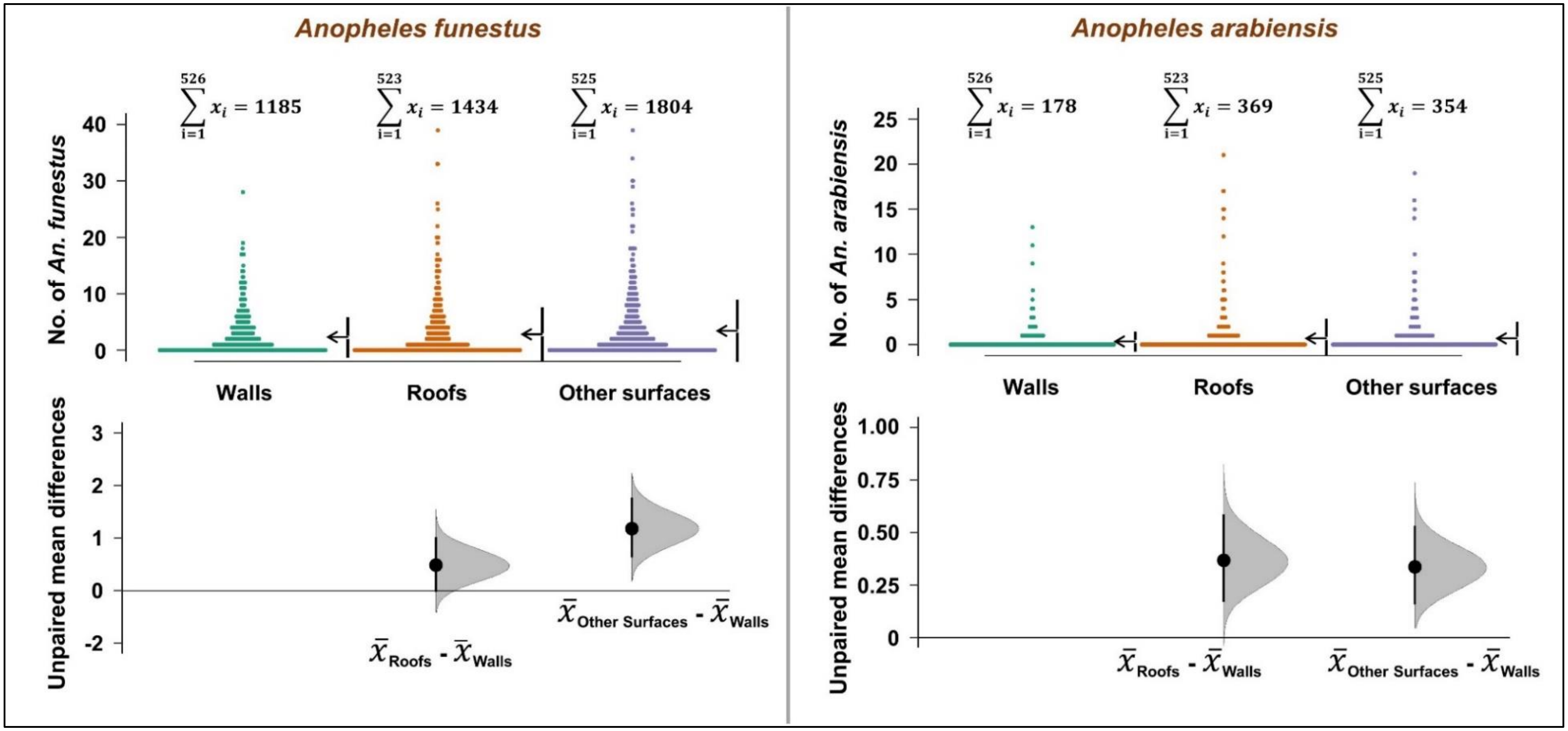

Figure 3: Overall nightly densities of malaria vectors *Anopheles funestus* and *Anopheles arabiensis*, from different resting surfaces inside the houses

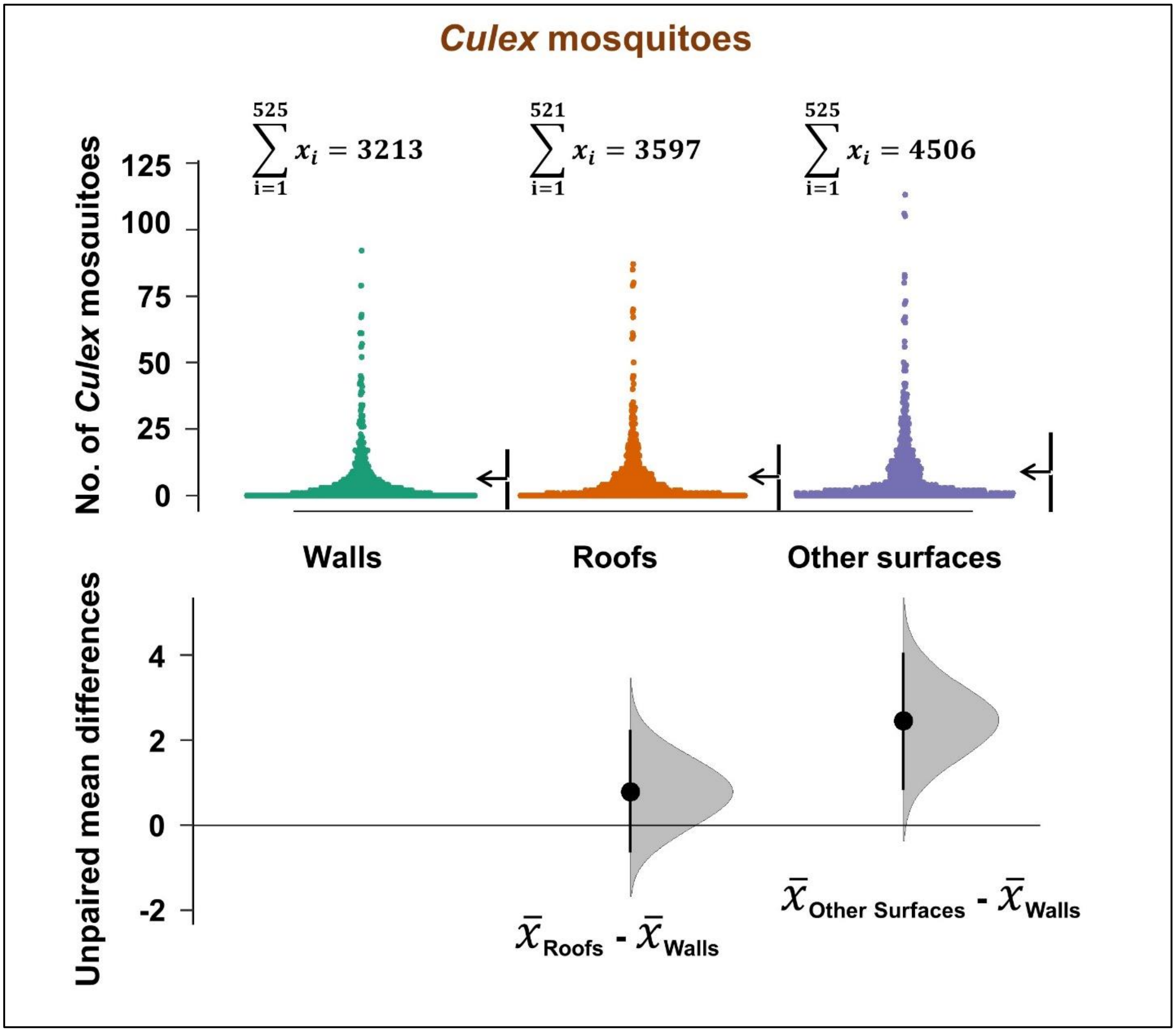

Figure 4: Overall densities of *Culex* mosquitoes, from different resting surfaces in houses

Table 3: Numbers and percentages of mosquitoes of different species collected from surfaces typically not targeted by IRS inside different houses types

Species	**Resting surfaces inside houses**	**Thatched roofs and mud walls**	**Thatched roofs and brick walls**	**Metal roofs and un-plastered brick walls**	**Metal roofs and plastered brick walls**	**Totals**
		n (%)	n (%)	n (%)	n (%)	N (%)
Anopheles funestus	Floor	129 (12.8)	29 (25.7)	125 (25.7)	48 (19.0)	331 (17.8)
	Furniture	186 (18.5)	18 (15.9)	80 (16.5)	87 (34.4)	371 (19.9)
	Bed-nets	587 (58.2)	25 (22.1)	79 (16.3)	59 (23.3)	750 (40.3)
	Clothes	74 (7.3)	31 (27.4)	134 (27.6)	32 (12.6)	271 (14.6)
	Utensils	32 (3.2)	10 (8.8)	68 (14.0)	27 (10.7)	137 (7.4)
	Total	**1008**	**113**	**486**	**253**	**1860**
Anopheles arabiensis	Floor	25 (20.2)	8 (33.3)	16 (30.8)	36 (19.0)	85 (21.9)
	Furniture	18 (14.5)	7 (29.2)	6 (11.5)	54 (28.6)	85 (21.9)
	Bed-nets	63 (50.8)	1 (4.2)	15 (28.8)	24 (12.7)	103 (26.5)
	Clothes	9 (7.3)	3 (12.5)	9 (17.3)	41 (21.7)	62 (15.9)
	Utensils	9 (7.3)	5 (20.8)	6 (11.5)	34 (18.0)	54 (13.9)
	Total	**124**	**24**	**52**	**189**	**389**
Culex mosquitoes	Floor	209 (29.6)	458 (28.1)	275 (26.2)	261 (20.1)	1203 (25.7)
	Furniture	189 (26.7)	470 (28.8)	191 (18.2)	461 (35.5)	1311 (28.0)
	Bed-nets	100 (14.1)	123 (7.5)	236 (22.5)	100 (7.7)	559 (11.9)
	Clothes	125 (17.7)	368 (22.6)	175 (16.7)	236 (18.2)	904 (19.3)
	Utensils	84 (11.9)	211 (12.9)	174 (16.6)	242 (18.6)	711 (15.2)
	Total	**707**	**1630**	**1051**	**1300**	**4688**

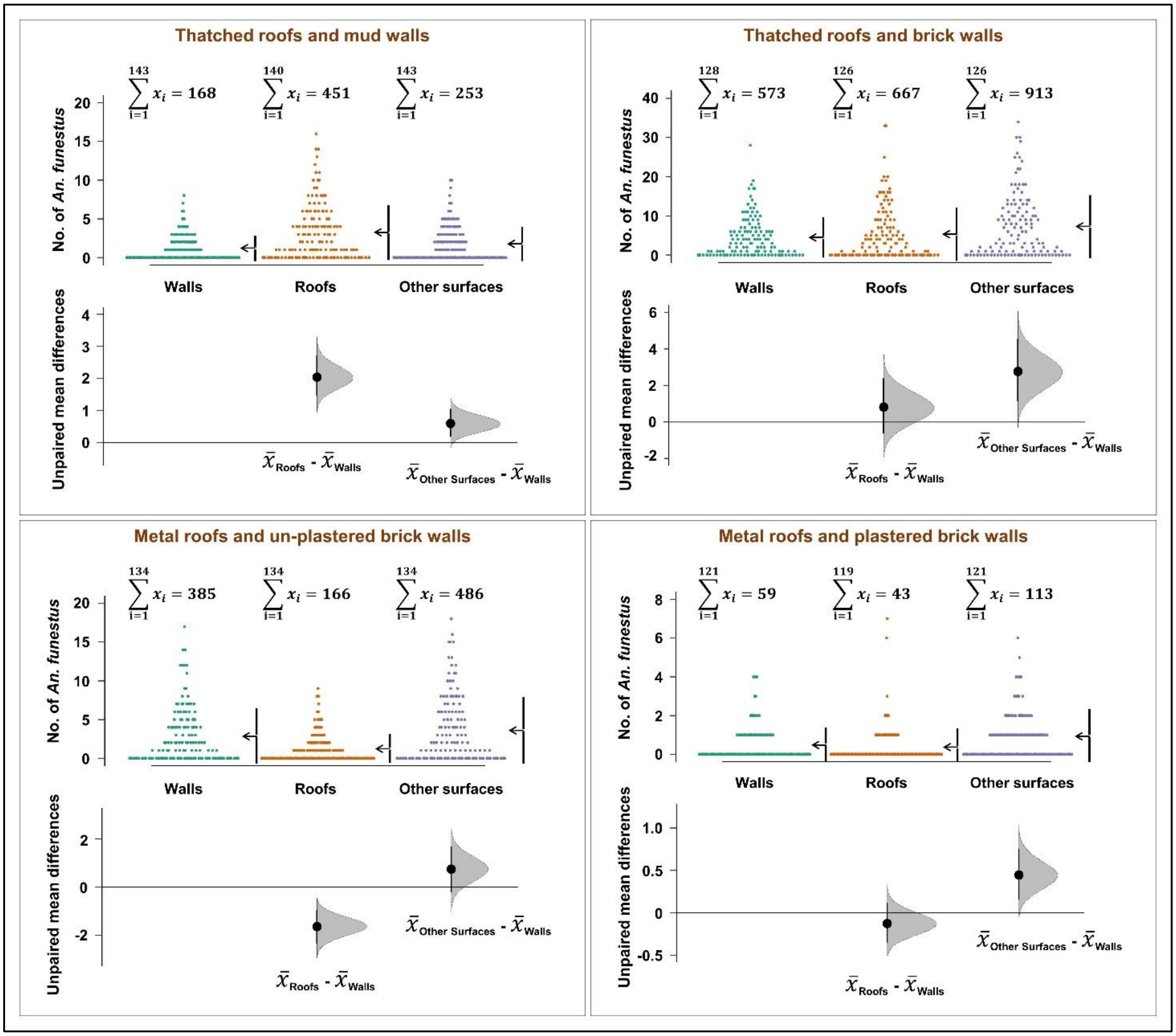

Figure 5: Comparison of *Anopheles funestus* densities on different resting surfaces in different house types

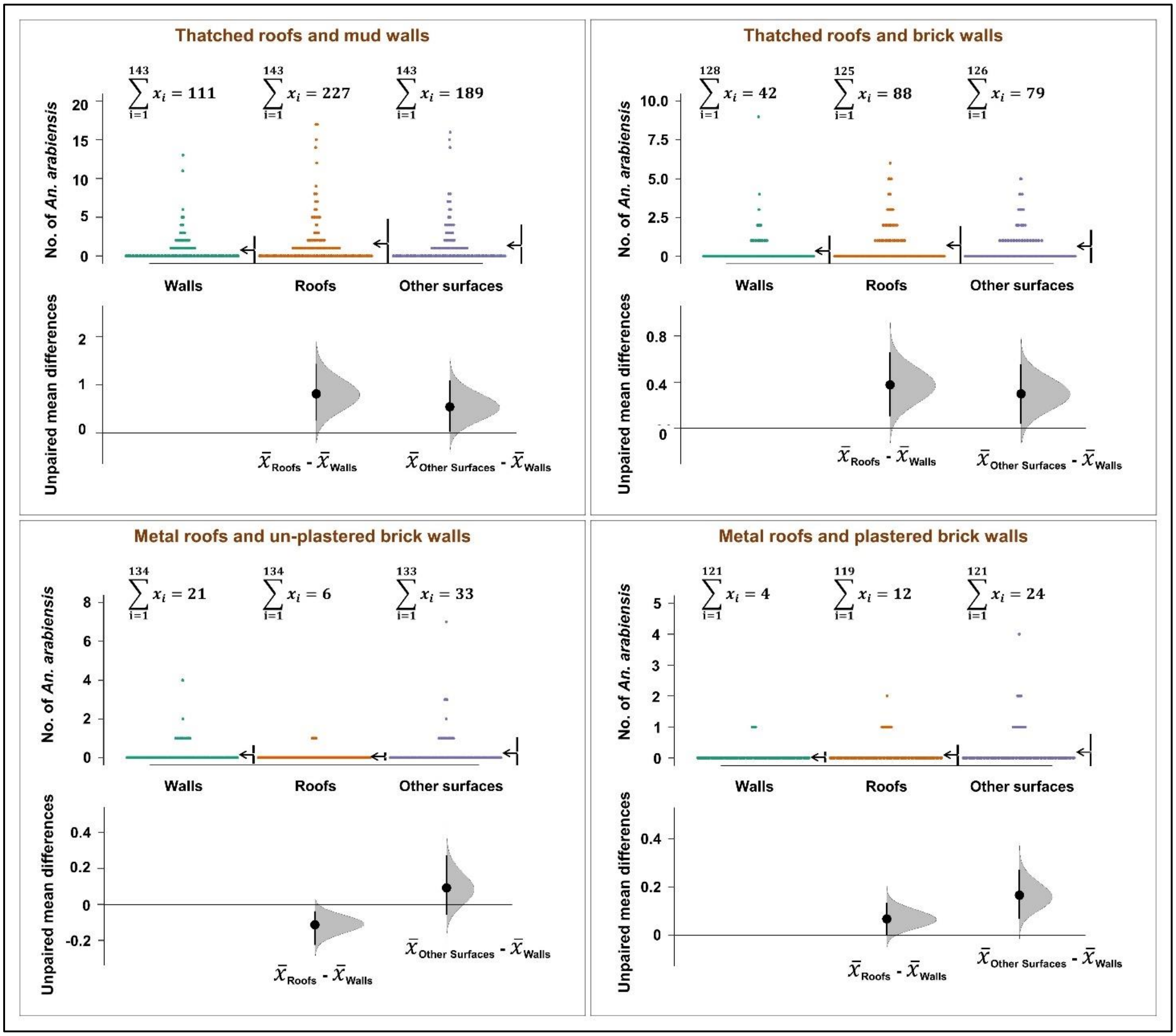

Figure 6: Comparison of *Anopheles arabiensis* densities on different resting surfaces in different house types

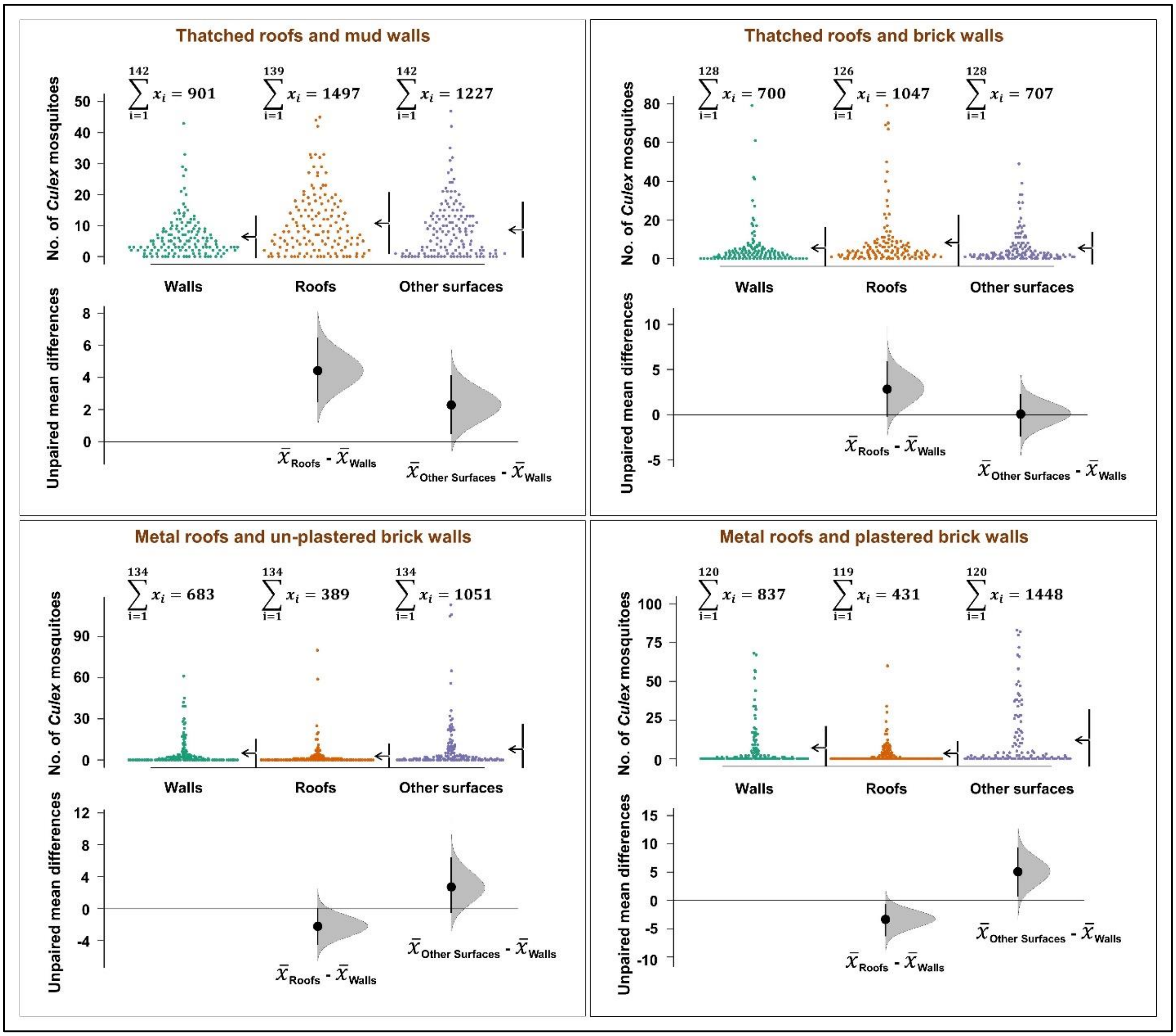

Figure 7: Comparison of densities of *Culex* mosquitoes, from different resting surfaces in different house types

4.1.3. Effects of household variables on preferences of mosquitoes for different resting surfaces inside houses.

Associations between household risk factors and proportions of mosquitoes in different resting surfaces are summarized in Table 4. Generally, compared to metal-roofed houses, grass-thatched houses had more mosquitoes of all taxa, and on all surfaces. In most cases, the number of mosquitoes in grass-thatched houses was more than double that in metal-roofed houses. Compared to brick walled houses, the mud-walled houses had less mosquitoes of all taxa, on any surface assessed. These differences varied but were significantly four times less for *An. funestus* (p=0.01) (Table 4). Leaving walls un-plastered was also associated with greater *Anopheles* density on the walls, significantly more so with *An. funestus*. This effect was less evident when considering mosquitoes collected from roofs or other surfaces. Similarly, leaving the eave spaces open was associated with higher vector densities on the walls and other surfaces, but not on roofs. Finally, there were more mosquitoes on walls below one metre. Full details of this analysis are provided in Table 4.

Table 4: Relationship between of household risk factors and indoor temperatures on mosquito resting preference on different surfaces

Variable	Categories	*Anopheles funestus*		*Anopheles arabiensis*		*Culex* mosquitoes	
		RR (95% CI)	p value	RR (95% CI)	p value	RR (95% CI)	p value
Number of mosquitoes caught resting on walls							
Roof type	Iron sheets	1.00		1.00		1.00	
	Grass thatch	2.20 (0.87-5.56)	0.090	1.93 (0.46-8.10)	0.400	1.04 (0.43-2.46)	0.940
Wall type	Brick	1.00		1.00		1.00	
	Mud	0.17 (0.07-0.41)	0.001	0.33 (0.09-1.24)	0.100	0.97 (0.42-2.26)	0.950
Interior walls	Plastered	1.00		1.00		1.00	
	Un-plastered	3.66 (1.34-10.02)	0.010	1.65 (0.22-12.25)	0.620	0.96 (0.38-2.48)	0.940
Eave space	Closed	1.00		1.00		1.00	
	Open	0.38 (0.13-1.13)	0.080	1.68 (0.23-12.26)	0.610	1.04 (0.40-2.74)	0.940
Increasing No. rooms		1.51 (1.08-2.11)	0.020	2.48 (1.38-4.47)	0.002	1.16 (0.86-1.58)	0.330
Increasing wall height		0.38 (0.13-1.11)	0.080	0.12 (0.02-0.79)	0.030	1.05 (0.42-2.65)	0.910
Increasing max. temp.		0.97 (0.93-1.02)	0.220	1.16 (1.07-1.25)	0.001	1.02 (0.97-1.07)	0.410
Number of mosquitoes caught resting on the underside of roofs							
Roof types	Iron sheet	1.00		1.00		1.00	
	Grass thatch	6.07 (1.78-20.70)	0.004	92.16 (9.90-857.94)	0.001	3.96 (1.51-10.36)	0.005
Wall type	Brick	1.00		1.00		1.00	
	Mud	0.27 (0.09-0.77)	0.010	0.39 (0.09-1.68)	0.210	0.80 (0.32-2.01)	0.630
Interior walls	Plastered	1.00		1.00		1.00	
	Un-plastered	1.55 (0.44-5.46)	0.500	0.29 (0.02-4.74)	0.390	0.74 (0.26-2.12)	0.570
Eave space	Closed	1.00		1.00		1.00	
	Open	0.25 (0.06-0.98)	0.046	0.91 (0.06-14.53)	0.910	0.53 (0.18-1.54)	0.240
Increasing No. rooms		1.48 (0.97-2.26)	0.070	2.49 (1.25-4.96)	0.010	1.44 (1.03-2.00)	0.030
Increasing wall height		0.25 (0.06-0.95)	0.040	0.55 (0.06-4.71)	0.570	0.87 (0.31-2.41)	0.790
Increasing max. temp.		1.02 (0.97-1.07)	0.410	1.13 (1.06-1.20)	0.001	0.98 (0.93-1.02)	0.300
Number of mosquitoes caught resting on other surfaces inside the houses							
Roof types	Iron sheet	1.00		1.00		1.00	
	Grass thatch	2.12 (0.85-5.31)	0.110	3.75 (0.88-16.03)	0.070	1.66 (0.68-4.02)	0.260
Wall type	Brick	1.00		1.00		1.00	
	Mud	0.22 (0.09-0.55)	0.001	0.59 (0.13-2.78)	0.510	0.92 (0.38-2.21)	0.840
Interior walls	Plastered	1.00		1.00		1.00	
	Un-plastered	0.92 (0.33-2.54)	0.870	2.77 (0.44-17.51)	0.280	0.81 (0.30-2.13)	0.660
Eave space	Closed	1.00		1.00		1.00	
	Open	2.91 (1.00-8.46)	0.049	1.95 (0.28-13.71)	0.500	1.23 (0.46-3.33)	0.680
Increasing No. rooms		1.16 (0.84-1.60)	0.370	1.61 (0.90-2.88)	0.110	1.25 (0.91-1.71)	0.160
Increasing wall height		0.84 (0.29-2.41)	0.750	2.68 (0.46-15.65)	0.270	3.18 (1.21-8.33)	0.020
Increasing max. temp.		0.93 (0.90-0.97)	0.001	1.12 (1.06-1.19)	0.001	1.03 (0.98-1.08)	0.280

4.1.4. *Anopheles* sibling species and *Plasmodium* infections

A subsample of 191 *An. gambiae* s.l and 623 *An. funestus* s.l were assayed for identification of sibling species, and presence of infectious stages of *P. falciparum*, i.e. sporozoites in the salivary glands. In the *An. gambiae* s.l samples, there was an overall PCR amplification of 93.2% (n = 178), of which 100% were *An. arabiensis*, and none had sporozoite infections. For *An. funestus* s.l, PCR amplification was 89.1% (n = 555), of which 93.1% were *An. funestus sensu stricto* (s.s.) (n = 517), and 6.8% were *Anopheles rivulorum* (n = 38). None of the *An. rivolurum*, nor the un-amplified samples had sporozoites infections, but four of the *An. funestus* s.s. mosquitoes were sporozoites positive (0.8%).

4.1.5. Mosquito blood meal sources and parity statuses

Based on the blood-meal ELISA assays done on 45 blood-fed *An. arabiensis*, more than half had human blood (55.56%; n = 25). The rest had blood from cattle (20%; n = 9), dogs (15.6%; n = 7), chickens (2.2%; n = 1) as well as mixed blood from dogs and cattle (4.4%; n = 2) and from humans and dogs (2.2%; n = 1). For *An. funestus* s.s., 224 blood-fed females were tested, the majority of which had obtained blood from humans (90.6%; n = 203). The rest of the *An. funestus* had blood from chicken (2.2%; n = 5), cattle (1.8%; n = 4), dog (0.9%; n = 2), mixtures of human and cattle blood (2.7%; n = 6) or human and chicken blood (1.8%; n = 4). Lastly, for *An. rivulorum*, only seven samples were tested, six of which had human blood in their guts (85.7%), the other having fed on cattle (14.3%).

Of 67 *An. arabiensis* dissected, 53.7% (n=36) were parous and 46.3% (n=31) were nulliparous. While of 160 *An. funestus* dissected, only 36.9% (n=59) were parous and the rest were nulliparous.

4.2. Discussion

This research investigated the resting behaviours of malaria mosquitoes inside typical house types in rural south-eastern Tanzanian villages where *An. arabiensis* and *An. funestus* are the main vectors, the latter contributing more than 80% of all cases (Kaindoa *et al*., 2017). The main finding was that consistently less than one third of mosquitoes that enter houses typically rest on walls, which are the main target for IRS campaigns. In fact, significant proportions regularly rest on surfaces other than walls or roofs (which are also sometime sprayed). These other surfaces include household items such as furniture, utensils, clothing and also on floors, places that are rarely sprayed. As historically observed (Smith, 1962b, 1962a), this current study determined that malaria vectors do not rests only on walls, where they can be targeted with IRS. Instead, all surfaces inside houses are potential resting site for mosquitoes. Majority of *An. funestus* and *An. arabiensis* rest on surfaces other than walls, such as on the underside of roofs, bed-nets, floors, furniture, utensils and clothes. However, variations were observed between vector species and house designs.

Indoor residual spraying and long-lasting insecticide-treated nets, despite having been tremendously impactful (Bhatt *et al*., 2015), are now perceived as inadequate for the goal of malaria elimination (Rabinovich *et al*., 2017; The malERA Consultative Group on Vector Control, 2011; The malERA Refresh Consultative Panel on Tools for Malaria Elimination, 2017), partly due to the rise of insecticide resistance (Hemingway *et al*., 2016; Ranson & Lissenden, 2016) and changes in mosquito biting behaviours (Finda *et al*., 2019; Monroe *et al*., 2019; Sherrard-Smith *et al*., 2019). These challenges may result from, and can be compounded by extensive and improper implementation of the insecticide based strategies (Killeen & Chitnis, 2014; Protopopoff *et al*., 2008; Stump *et al*., 2004). For example, incomplete coverage of all mosquito resting surfaces with IRS inside houses could lead to lower coverage of indoor surfaces with insecticides, sub-optimal dosing of the mosquitoes and hence reduced communal impact of the interventions. Therefore, to attain malaria elimination targets, current interventions need improvements to maximize effectiveness. This requires extensive understanding of mosquito behaviours inside houses, and how these mosquitoes would respond to indoor interventions, notably IRS and ITNs.

The composition of indoor resting mosquitoes observed in this study was of fairly different physiological ages and few infectious *Anopheles*. Also, bloodmeal sources suggests that even outdoor biting mosquitoes rested indoor. This study therefore provides evidence that expanding target surfaces inside houses when spraying insecticides would increase impact of IRS on mosquito populations. Where this is not possible, a behaviour change communication program can be implemented to sensitize and educate people on dangers of mosquitoes resting indoors on surfaces such as hanged clothes. IRS campaigns usually involve removal of household items before spraying is conducted (WHO, 2015a). However, once these items are returned to the houses, they form important resting surfaces free of insecticides. Since the study involved multiple collections at different times of day and night, the observed resting patterns are likely the natural patterns. It is however unclear whether there are any frequent movements of mosquitoes between resting surfaces, and how such movements may influence overall impact of IRS.

More importantly, these findings highlight specific gaps and limitations of IRS, and the need for more comprehensive interventions such as house improvement. As an example, house screening would not be affected by mosquito resting behaviours but would instead reduce overall densities in the homes. Another way would be to expand, as much as possible, the IRS targeted surfaces to include undersides of roofs and other sprayable surfaces (such as underneath beds, tables and other furniture) to have increased impact on the mosquitoes. Thirdly, coupling IRS with strategies to minimize mosquito resting on non-sprayable surfaces might also enhance impact. Such strategies may include, but are not limited to proper storage of household items, e.g. by placing these items inside enclosures such as cupboards. This could reduce potential surfaces for mosquito to rest, which may maximize mosquito contacts with treated surfaces. Without considering surfaces other than walls, our current efforts, targeting mosquito vectors with IRS might limit the impact of IRS on elimination and control outcomes. However, one would argue that proportion of mosquitoes resting on surfaces other than walls does not mean that these mosquitoes would never come into contact with walls or wouldn't be killed by IRS.

Indoor residual spraying remains one of the mainstays of malaria control in Africa, and is widely popular despite high costs. It is currently promoted in Africa mostly through the US Presidents Malaria Initiative (U.S. President's Malaria Initiative, 2019b) and national programs often alongside LLINs, but was historically the most dominant tool in Africa and elsewhere starting from the Global Malaria Eradication period (Kouznetsov, 1977; Nájera *et al*., 2011). It has indeed been associated with major reductions in malaria cases in the southern Africa region in past decades (Mabaso *et al*., 2004; Sharp *et al*., 2007), and remains an important component of their malaria control arsenal. The spraying procedures are generally standardized to achieve scale and reduce costs (WHO, 2015a), and generally target walls and ceilings occasionally where these exist. As a result, the spraying operations may not adequately capture the full-spectrum of resting spaces used by malaria vectors or others.

The findings of this current study are in line with previous studies on resting preference of *Anopheles* mosquitoes inside houses (Smith, 1962a). However, this study extended the mosquito collections to cover more potential sites inside human inhabited dwellings, and also examined differences between different house types. It also described relationships between house designs and microclimate, with resting preferences of the *An. funestus*, *An. arabiensis*, and *Culex* mosquitoes. For example, grass thatched roofs were associated with higher proportions of *An. funestus* on roofs. When houses had open eaves, proportion of *An. funestus* increased on other surfaces, but increase in indoor maximum temperature was associated with decrease in proportion of *An. funestus* on other surfaces.

Insecticide resistance has led to a change of insecticides used in IRS to non-pyrethroid insecticides such as pirimiphos-methyl and neonicotinoids (Hemingway *et al*., 2016; WHO, 2018b). However, recently a countrywide survey in Tanzania detected resistance against pirimiphos-methyl in several sites within the country (Kisinza *et al*., 2017). It was observed that out 20 sites, three sites had *An. gambiae* s.l population resistant to pirimiphos-methyl and elevated levels of glutathione S-transferases, nonspecific esterases, acetyl- cholinesterase and mixed function oxidases enzymes (Kisinza *et al*., 2017). Suspected cause of this resistance was common use of insecticides classes in agriculture as that in malaria vector control (Kisinza *et al*., 2017). Results of this study indicates that current IRS practices clearly miss several surfaces where mosquitoes rest, a situation, which could exacerbate the challenge of insecticide resistance and further compromise IRS. As mentioned earlier in this paper, understanding the resting behaviours of malaria vectors is crucial, if at all IRS is going to be widely used in malaria endemic countries including Tanzania. The gaps identified in this study can be compounded by insecticide resistance, and therefore need urgent attention to ensured effectiveness.

Though mostly successful, this study also had a few limitations. First, most collections of mosquitoes were done in the morning, when people were active participating in household chores. This might have influenced the choice of mosquitoes on resting surfaces during the day. Collections during the day might also have underestimated mosquitoes resting on surfaces such as floors and utensils. Second, the type and number of possessions inside houses are related to house types, since both are linked to wealthy/income. Mud houses are unlikely to have bigger furniture and rarely items inside these houses are properly arranged. It is likely that resting patterns of mosquitoes between individual house type was influenced by type and number of surfaces inside houses. Thus, influencing observed differences in resting preference among house types involved. Unfortunately, this phenomenon was not assessed in this study. Third, this study was conducted in villages which are not protected with IRS. However, mosquitoes have been shown in multiple studies to change their behaviours with interventions. Therefore, it is important that future studies should be carried-out to assess indoor resting preference of mosquitoes in houses protected with IRS.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

This study has demonstrated that while IRS typically uses contact insecticides against adult mosquitoes on walls, and occasionally roofs and ceilings, significant proportions of malaria vectors rest on other surfaces not usually sprayed during IRS campaigns. The study also demonstrated that the spraying gaps are influenced by house designs. For example, in grass-thatched houses, up to one third of mosquitoes consistently rest on surfaces other than walls or roofs, are therefore not effectively controlled by contact insecticides. These gaps can reach two-thirds of mosquitoes in metal-roofed houses.

Given that roofs are often ignored during IRS, it is likely that there are even wider gaps in IRS operations. Similarly, any attempts by IRS implementers to reduce the surface area sprayed (e.g. to reduce costs and increase coverage), should be analyzed based on local evidence of mosquito-resting preferences. Expanding IRS targeted surfaces inside houses can also be impactful. However, given the costs of IRS and logistical challenges associated with spraying non-standard surfaces, this approach in resource limited settings may not sustainable. It remains unclear how the observed mosquito habits could impact overall effectiveness of IRS.

Most importantly therefore, there is need to incorporate locally-obtained data on mosquito resting behaviours to maximize potential of IRS. Besides, other interventions such as improved housing should be prioritized to more comprehensively tackle indoor-biting and indoor-resting mosquitoes.

5.2. Recommendations

Based on the observation made in this study that, only less than one-third of mosquito were found rest on walls and significant proportions of vectors rest on surfaces not usually sprayed. In houses with thatch roofs mosquitoes resting on surfaces that are not sprayed can exceed one-third and in houses with metal roofs can be more than two-third. Also, with observation that house structure significantly influences indoor mosquito density and resting preferences, recommendations from this study include the following:

(i) Indoor residual spraying should rely on house designs for targeting IRS activities. House design significantly influence indoor mosquito density and resting preferences. Therefore, IRS activities should rely on house design and focus its activities in houses that tend to have higher densities of mosquitoes, for example e.g. by focusing on thatch-roofed houses and houses without plastered walls.

(ii) For houses with thatched roofs, target surfaces for spraying residual insecticide should be expanded to include roofs, especially in occasions when roofs are not considered for spraying. In houses with thatch roofs, roofs were observed to harbor large proportion of mosquitoes. Therefore, by targeting this surface majority of mosquitoes in these houses can be impacted with IRS, thereby increasing the impact of IRS.

(iii) Implement behaviour change communication programs to raise awareness of indoor mosquito resting places. These programs can be implemented to sensitize and educate people on dangers of mosquitoes resting indoors on surfaces such as hanged clothes.

(iv) Coupling IRS with other strategies to minimize mosquito resting on non-sprayable surfaces. As an example, by minimizing the number of resting surfaces that are not targeted by indoor residual spraying inside houses. This could reduce potential surfaces for mosquito to rest, which may maximize mosquito contacts with treated surfaces.

(v) Consider other options that target all mosquitoes. For example, option such as:

a) House improvement will reduce overall mosquito densities inside houses.

b) Insecticide-treated eave ribbons which target mosquitoes before they enter houses, thus increasing impact.

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