UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences
School of Health Systems and Public Health

The value of house screening as an addition to long-lasting insecticidal nets in protecting against malaria in Zambia

Thesis submitted in fulfilment of the requirements for the degree

Doctor of Philosophy
(Environmental Health)

By

Kochelani Saili
(19394421)

January 2024

This thesis is presented in a manuscript format

Supervisor: Prof. Clifford M. Mutero

Co-supervisor: Prof. Christiaan de Jager

Declaration: Authorship

I declare that this thesis, which I hereby submit for the degree Doctor of Philosophy in Environmental Health at the University of Pretoria's School of Health Systems and Public Health of the Faculty of Health Sciences, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

30 January 2024

Kochelani Saili **Date**

Declaration: Publications

Journal articles published.

1. **Saili, K.,** de Jager, C.; Sangoro, O.P.; Nkya, T.E.; Masaninga, F.; Mwenya, M.; Sinyolo, A.; Hamainza, B.; Chanda, E.; Fillinger, U. and Mutero, C.M. *Anopheles rufipes* implicated in malaria transmission both indoors and outdoors alongside *Anopheles funestus* and *Anopheles arabiensis* in rural south-east Zambia. *Malaria Journal* **2023**, *22*, 95, DOI: https://doi.org/10.1186/s12936-023-04489-3

   Authors' contributions: CMM, UF, EC and FM conceived the study and wrote the main study protocol. KS, CdJ, SOP, UF and CMM designed this study. KS, MM, AS, FM, POS and BH supervised the study data collections. KS and AS performed the molecular analysis. KS performed data analysis. KS wrote the initial draft of the manuscript, which was revised by CMM, UF, FM, CdJ, POS, TEN and BH. All authors read and approved the final manuscript.

2. **Saili, K**, de Jager, C., Masaninga, F., Sangoro, O.P., Nkya, T.E., Likulunga E.L., Chirwa J., Hamainza, B., Chanda, E., Fillinger, U., and Mutero, C.M. House Screening Reduces Exposure to Indoor Host-Seeking and Biting Malaria Vectors: Evidence from Rural South-East Zambia. *Tropical Medicine and Infectious Diseases* **2024,** 9, 20. https://doi.org/10.3390/tropicalmed9010020

   Author Contributions: Conceptualization: FM, OPS, EC, UF and CMM; Data curation: KS; Formal analysis: KS and LEL; Funding acquisition: FM, EC, UF and CMM. Investigation: KS, FM, OPS, JC and BH. Methodology: KS, CdJ, SOP, LEL, JC and UF. Project administration: FM, EC and CM; Resources: CdJ, FM, EC, UF and CMM; Supervision: CdJ, OPS, UF and CMM,

Visualization: KS; Writing – original draft, KS; Writing – review & editing: KS, CdJ, FM, OPS, NTE, LEL, JC, BH, EC, UF and CMM. All authors have read and agreed to the published version of the manuscript. All the authors have read and approved the final manuscript.

3. **Saili, K.,** de Jager, C., Masaninga, F., Chisanga, B., Sinyolo, A., Chiwaula, J., Chirwa, J., Hamainza, B., Chanda, E., Bakyaita, N.N. and Mutero, C.M. Community perceptions, acceptability, and the durability of house screening interventions against exposure to malaria vectors in Nyimba district, Zambia. *BMC Public Health* ***2024,*** 24, 285. https://doi.org/10.1186/s12889-024-17750-4

Author Contributions: CMM, EC and FM conceived the study and wrote the main study protocol. KS, FM, and BC designed this study. KS, FM, BC, AS, JC, and JC collected the data. KS, BC and FM performed the analysis. KS wrote the initial draft of the manuscript, which was revised by FM, CdJ, AS, JC, JC, BH, EC, NNB and CMM. All authors read and approved the final manuscript.

DEDICATION

To my wife Natalie, my son Kochelani (Jr) and daughter Kanyanta Leilani in whose love I bask.

ACKNOWLEDGEMENTS

"If I have seen a little further, it is by standing on the shoulders of giants"- Isaac Newton, 1675.

The PhD journey is a long and lonely journey that has humbly taught me the true value of collaboration. I am truly grateful to the following people on whose shoulders I have stood.

1. My university supervisors, Prof. Clifford M. Mutero and Prof. Christiaan de Jager for their guidance and support during my doctoral training. You have been supportive from the start to the very end, and for that am grateful.

2. My *icipe* supervisors Drs Ulrike Fillinger, Peter O. Sangoro and Theresia E. Nkya. Special thanks to Dr Peter Sangoro who made several trips to Zambia to ensure progress on the WHO AFRO II project. Terry, thank you for your attention to detail and quick turnaround every time I shared a manuscript. Dr Fillinger, thank you for your ever extensive overhaul to my manuscripts; you pushed me to better the quality of my writing and that I appreciate.

3. Special gratitude to Dr Freddie Masaninga from the World Health Organisation-Zambia Country office for the invaluable technical support. You proved to be a supervisor, mentor, and friend.

4. I thank members of the National Malaria Elimination Centre (NMEC)- Dr Busiku Hamainza, Mr. Jacob Chirwa, Japhet Chiwaula, Niza Siame, and Andy Sinyolo.

5. Heartfelt thanks go to the Nyimba medical office (DMO), more specifically Dr Martin Mbewe, Mr. Mike Sinkala and Emmanuel Sikaonga.

6. The community leadership in Mkopeka and Nyimba Urban for their support of the house screening project. To all study participants for their willingness to take part in the study I say a big thank you.

7. Special thanks to the Macha Research Trust entomology team (Dr Monica M. Mburu, Limonthy Simubali and Twig Mudenda) and the entire malaria department for taking the time to teach me mosquito molecular methods and sharing their protocols with me.

8. The following were with me in the field throughout data collection and deserve special mention for their contribution. Community-based health volunteers of Mkopeka Rural health Centre: Michael Lungu, Michael Banda, Packson Tembo, Anastasia Phiri, Anastasia Jere, Nyangu Lungu, John Mwanza, Phyllis Mumba and Zelesi Mwanza. Community-based health volunteers of Nyimba Urban; Staff Lungu, Josias Lobamu, Emelia Tembo, Kennedy 'Suarez' Chipeta, Irene Chiwoko, Evenness Sakala, Timothy 'Wabwino' Nsabila and Chrispine Mwanza. Together, we formed a great team of 'soldiers against mosquitoes.' Thank you!

9. I am indebted to the Deutscher Akademischer Austauschdienst (DAAD) who supported me with a doctoral scholarship through the *icipe* African Regional Postgraduate Programme in Insect Science (ARPPIS)-DAAD scholarship programme.

10. My sincere acknowledgements go to the School of Health Systems and Public Health at the University of Pretoria for a partial doctoral bursary and all the support rendered to me during my studies. I extend my thanks to University of Pretoria Institute for Sustainable Malaria Control, more specifically Dr Taneshka Kruger and Prof. Christiaan de Jager, for the financial support to the attend the 8th Annual Southern African Malaria Conference, organised by the South African Malaria Research Council in Pretoria, South Africa and the paying for article processing costs of some published manuscripts.

11. My heartfelt thanks to i*cipe*-Capacity Building and Instituitional Development (CBID) for their support and patience throughout my studies.

12. The co-authors of my manuscripts as acknowledged in each chapter and/or acknowledgements sections. A special thanks to Dr Likulunga E. Likulunga from the University of Zambia and Dr Faith I Ebhodaghe from University of Colorado who extended their help in data analysis.

13. I could not have done this without the support of my family and friends. I thank my wife Natalie for her unwavering support. You were by my side throughout this academic journey, no matter how tough and rugged our situation was and even when I was distracted and not fully present in our marriage. My son Kochelani (Jr) and daughter Kanyanta Leilani, who give me unconditional love- you complete my world. Daddy loves you!

14. My friends Nahum Jere and Kelvin Mupeta have been of constant encouragement during this long journey.

15. My wife's family in South Africa deserve a special thank you. My trips to South Africa would not have been as pleasurable as they had been without you. My mother-in-laws Aunty Mary and Aunty Grace, thank you for being ever being so warmly welcoming, ensuring that I had home-cooked warm meals every time I was in South Africa and that I got to see Jo'burg. Shawn and Chichi (Chimfwembe), thank you guys; like parents you took me by the hand to the gates of the University of Pretoria, School of Health Systems and Public Health in Gazina, Pretoria. I am forever grateful.

16. My Dad (Kenny Saili) and Mom (Bridget Salome Chilufya-Saili) who have always encouraged me to follow my dreams and aim higher. You remain my inspiration and pillars of strength; your support and continuous belief in me are what have kept me going. Thank you!

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Abstract

Introduction

The aim of this study was to evaluate the impact of adding house screening to long-lasting insecticide-treated net (LLINs) use on malaria vector densities and malaria transmission potential in rural south-east Zambia.

Methods

The study was conducted in Nyimba district in four phases. First, baseline information on malaria vector species composition, relative abundance, sporozoite infectivity and entomological inoculation rates (EIRs) was collected. Second, the impact of combining house screening with LLINs on indoor mosquito densities and EIRs were evaluated in a randomised controlled trial. Intervention houses received LLINs plus house screening whilst the control arm households received LLINs only. Third, the durability of the window and door screens were assessed a year after screening. Fourth, community acceptability of the house screening intervention by the participants was assessed.

Results

Anopheles rufipes, *Anopheles funestus* and *Anopheles arabiensis* were the main vectors in the study area. Closing eaves and screening doors and windows reduced indoor densities by an average 65%. EIR in unscreened houses was 2.91 infectious bites/person/six months (ib/p), higher than that in screened houses (1.88 ib/p/six months). After a year, window screens were intact. However, the wire mesh on most door screens was damaged on the bottom half. Participants accepted this intervention and linked house screening to reduced malaria in their households and cited sleeping peacefully due to reduced mosquito biting.

Conclusion

House screening has the potential to reduce malaria incidence, offer prevention against diseases, and provide additional benefits against nuisance biting and must therefore be promoted as a public health intervention.

Chapter 1: Introduction

Chapter 1 presents the introduction to the thesis, providing a concise background and explaining the rationale behind the research study. It includes a clear problem statement, research aim, and objectives.

1. Introduction

Malaria is caused by a protozoan parasite belonging to the *Plasmodium* genus. The parasite is transmitted to people when they get bitten by infected female *Anopheles* mosquitoes. The species *Plasmodium falciparum* is responsible for about 99% of malaria cases reported in sub-Saharan Africa[1], while a few cases are due to *Plasmodium vivax* and *Plasmodium ovale*.[1] *Plasmodium falciparum* is also the deadliest malaria parasite globally.[1] The African region carries the heaviest burden, of malaria. In 2021, approximately 234 million cases of malaria were recorded in Africa alone, with malaria-related morbidity rates reaching an estimated high of 593,000.[1]

Since the early 2000's, indoor residual spraying (IRS) and long lasting insecticidal nets (LLINs) have formed the cornerstone of malaria vector-control globally.[2] As a result, malaria deaths reduced by over 69% between 2010 and 2018 largely attributed to the use of these vector-control methods.[3-4] Today, many malaria endemic countries, Zambia included, continue to rely on both IRS and LLINs for malaria vector-control.[5-6]

Unfortunately, the near-complete reliance on LLINs and IRS for vector-control has its limitations. Chief among these is the emergence and spread of insecticide resistance among mosquito populations.[7-8] Over the past decade Zambia has reported insecticide resistance to three classes of insecticides namely pyrethroids, carbamates and organochlorines (DDT).[7,9-12] Whilst it is not yet clear what levels of resistance triggers control failure in terms of malaria transmission[13] in some cases, sharp increases in malaria incidence rates and/or little effect on vector population densities after IRS, have been attributed to insecticide resistance.[6,12,14] A second concern, is behavioural adaptations of adult mosquito vectors. These include feeding

and resting outdoors or early evening biting, which gives malaria vectors the ability to avoid LLINs and IRS-treated walls.[15-16] Third is the high cost of implementation. Almost 70% of the malaria funding required per person at risk in Zambia is externally funded.[17] The continued increased costs of implementation may create financial support bottlenecks for a low-income country like Zambia which is highly dependent on external funding.[18-20] Forth is a lack of user compliance**.** The effectiveness of LLINs and IRS interventions is dependent on population-wide human compliance. However, sub-optimal user compliance as well as misuse of LLINs is well documented across Zambia and Africa in general.[21-24]

The four factors combined undermine the implementation and efficacy of the current chemical-based vector-control tools. This has led to increased calls for an expansion of the current malaria vector-control tools to supplement, not necessarily to replace, the traditional insecticide-based vector control interventions.[25-27] The WHO recommends the use of “supplementary interventions” defined as interventions that are applicable for specific populations, situations or settings and, as such, are not broadly applicable but more locally adaptive.[28] Included among the supplementary interventions are larviciding, topical repellents, insecticide-treated clothing and spatial or airborne repellent, space spraying and housing modification which includes house screening. The goal is for national programs to reduce chemical-use dependency by formulating strategies that are realistic, multi-faceted and environmentally friendly.[27,29]

2. Problem Statement

Malaria is endemic throughout Zambia and continues to be a major public health problem. As of 2021, approximately 29% of the children in Zambia were infected with malaria parasites.[30] To reduce the malaria burden, Zambia’s National Malaria Elimination Program (NMEP) has adopted a multi-pronged approach of combined vector-control intervention - mainly LLINs and IRS, case management and strengthening information systems for quality and timely reporting of infections.[5,31-33] However, the primary vector-control interventions credited with recent decreases in malaria, namely LLINs and IRS are faced with the previously mentioned challenges.

This situation lends support for the expansion of the current malaria vector-control toolbox to complement the traditional insecticide-based vector-control methods.[25]

House screening, which prevents mosquitoes from entering houses and hence reduces human-vector contact and malaria transmission is one such alternative.[25] However, house screening as an additional vector-control tool to LLINs remains largely unpromoted by the national malaria program. This is despite evidence showing that in rural Zambia, human-vector contact occurs primarily indoors[15] and Zambia's own past success of malaria control with house screening.[34]

Moreover, interventions of such a large undertaking should be backed by scientific evidence of its effectiveness- entomologically and/or epidemiologically in the local setting. Further, there exists knowledge gaps on community acceptance of house screening as a supplementary malaria vector-control intervention. Without community acceptance and behaviour change, even well intended and well-designed interventions will not have the desired impact or be sustainable. In Zambia, a prospective study to determine the feasibility, entomological effectiveness and community acceptability of house screening is yet to be conducted.

3. Purpose of the study

The protective efficacy of an intervention is largely a function of the behaviour of local mosquito populations.[35] Hence, basic local knowledge of the species composition of malaria vectors, insecticide resistance status, blood-feeding and resting behaviour is fundamental for the design of interventions specific to the local ecological and epidemiological situation. To fill this need, this study provided information on vector species composition, host-seeking and resting behaviour in the study area prior to intervention implementation. The study further evaluated the feasibility and any added benefits of house screening to LLINs in a high malaria transmission setting generating knowledge on the impact that house screening may have on indoor vector host-seeking, resting, and biting behavior and malaria transmission potential. Third, the study provides evidence of durability of house screening material and community acceptability of house screening as a supplementary vector-control malaria intervention. Through this study, the Zambia

NMEP will get an understanding of the effectiveness of house screening as an added vector-control intervention and its acceptability in the quest for malaria elimination.

4. Aims and Objectives

4.1 Aim

To evaluate the impact of adding house screening to long-lasting insecticide-treated net (LLINs) use on malaria vector densities and malaria transmission potential in Nyimba district, south-east Zambia.

4.2 Objectives

The objectives of this study were;

1. To determine species composition of potential malaria vectors and their relative abundance and to determine their sporozoite infectivity and entomological inoculation rates (EIRs) as measures of malaria transmission in rural southeast Zambia.

2. To examine a novel glue net trap (GNT) as a mosquito sampling method for measuring mosquito entry and exit behaviour and to determine the insecticide susceptibility of anopheline mosquitoes reared from larval collections.

3. To evaluate the feasibility and impact of house-screening on indoor vector abundance, biting behaviour and entomological inoculation rates in rural southeast Zambia.

4. To assess the durability of the house screening material a year after screening and

5. To assess community acceptability of house screening as a malaria vector-control intervention in Nyimba district.

5.Thesis Structure

This thesis is presented in seven chapters, with three chapters already published as journal articles and one chapter currently under journal peer review.

Chapter 1: **Introduction** Presents the general introduction of the study and it covers the review of literature which guided this study. This chapter also presents the study rationale, purpose of the study and the study aim and objectives.

Chapter 2: By means of literature review, this chapter presents evidence of the biological basis for house screening in the context of malaria vector-control and Zambia's local malaria situation. It briefly highlights the success of house screening; and discusses the gaps and opportunities that house screening offers as a supplementary vector-control tool in the Zambian context. In this chapter I argue that to promote house screening, a local shift of house construction practice may need to be implemented by individuals and families, encouraged by community leaders, enforced by local law, advocated for by the national malaria program and will need intersectoral collaboration.

Chapter 3 presents a published manuscript providing baseline information on the species composition of potential malaria vectors, their relative abundance and sporozoite infectivity and entomological inoculation rates (EIRs) as measures of malaria transmission in rural south-east Zambia. The information provided in this chapter forms the basic local knowledge of the species composition, their behavior prior to intervention implementation and addresses objective 1.

Chapter 4: Basic local knowledge of the species composition of malaria vectors, insecticide resistance status, entry and exit behavior into the house is fundamental for the design of interventions specific to the local ecological and epidemiological situation. The objectives of this study were to determine the entry and exit behaviour

of anopheline mosquitoes using a sampling tool herein referred to as the Glue Net Trap (GNT). The second objective was to determine insecticide susceptibility status of anopheline mosquitoes to commonly used insecticides Nyimba district. This Chapter thus addresses objective 2.

Chapter 5 presents published results on the entomological outcomes of the house screening intervention. The specific objective of this study was to evaluate the impact of combining house screening with LLINs on mosquito host-seeking, resting, and biting behaviour. This chapter overall thus addressed objective 3. Intervention houses received house screening plus long-lasting insecticidal nets whilst the control arm households received long-lasting insecticidal nets only. Centre for Disease Control Light traps and Pyrethrum spray collections were used to determine indoor and outdoor host-seeking and indoor resting densities respectively, in 15 sentinel houses per study arm per sampling method. Results show a significant reduction of indoor resting and host-seeking *Anopheles funestus* and *Anopheles arabiensis* in screened houses compared to unscreened houses. Estimated indoor entomological inoculation rates (EIRs) in unscreened houses was significantly higher than in screened houses. The findings of this study show that closing eaves and screening doors and windows has the potential to reduce indoor densities of malaria vectors and malaria transmission.

Chapter 6 addressed objective 4 and 5 and presents results from an assessment of the durability of the window and door screens a year after screening and acceptability of the of house screening intervention by the participants involved. This study demonstrated that in rural south-east Zambia, closing eaves and screening windows and doors was a widely accepted intervention. Participants perceived that house screening reduced human-vector contact, reduced the malaria burden and nuisance biting from other potentially disease carrying insects.

Chapter 7. This chapter presents the general discussion and conclusion of the thesis. It presents the strengths and limitations of the entire thesis followed by a

general conclusion. It concludes with recommendations for future research and/or policy formation in malaria.

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Chapter 2: Literature review

Since the early 2000s, there has been scale-up of malaria interventions namely insecticide treated nets (ITNs), indoor residual spraying (IRS) and malaria case management using artemisinin-based combination therapies (ACTs). As a result, over 2 billion malaria cases and 12 million deaths had been averted between 2000 and 2015, renewing calls for malaria elimination. The use of ITNs and IRS combined contributed about 81% to this decline. However, the rate of decline of malaria cases and malaria related deaths, has since stalled. In some cases, the gains made have been reversed. This has been attributed, in part, to the emergence and spread of insecticide-resistant mosquito populations to the available classes of insecticides, and behavioural resilience or adaptation of malaria vectors. This has led to increased calls for an expansion of the current malaria vector-control tools to supplement the traditional insecticide-based vector control interventions.

The World Health Organization recommends house screening as a supplementary malaria vector-control intervention to the core interventions of long-lasting insecticidal nets and indoor residual spraying. This is because human-vector contact primarily occurs indoors. Recent studies showed that up 80% malaria transmission took place indoors in sub-Saharan Africa. Further, open eaves, windows and doors remain an entry point for mosquitoes. The major vectors of malaria of human malaria are highly anthropophilic, endophagous and endophilic. They are also well adapted for entering houses using the gaps between walls and roofs (eaves) in traditional rural houses. Attracted to host odours emanating from humans inside houses, anopheline mosquitoes tend to fly upwards, towards the eaves and/or windows, when they meet an obstacle such as a wall. Despite this evidence, house screening remains largely unpromoted by the Zambia national malaria program. This hesitance to promote house screening, may be due to a lack of evidence on the impact of the intervention on local malaria transmission and prevalence, paucity of data on community acceptance, cost-effectiveness, and the mode of implementation.

This chapter focuses on understanding the relevance of three strategies which are key for successful implementation of house screening intervention, namely:

community acceptance, intersectoral collaboration and engaging community leaders. These three strategies function as the pillars of any successful integrated vector management (IVM) initiative in the broader context. We also recognize that the gap on the cost-effectiveness of house screening as an intervention must be bridged for meaningful promotion of the intervention. Whilst these may be perceived as gaps, they also create the opportunities that house screening specifically offers as a supplementary vector-control tool in the Zambian context.

Keywords: House screening, malaria, vector-control, Zambia, *Anopheles,* eaves

House screening as a strategy for malaria vector-control in Zambia: Gaps and opportunities

Introduction

Since the early 2000's, indoor residual spraying (IRS) and long lasting insecticidal nets (LLINs) have formed the cornerstone of malaria vector-control globally.[1] As a result, malaria deaths reduced by over 69% between 2010 and 2018 largely attributed to the use of these vector-control methods.[2-3] Today, many malaria endemic countries, continue to rely on both IRS and LLINs for malaria vector-control.[4-5] However, the emergence and spread of insecticide-resistant mosquito populations to the available classes of insecticides[6-12] has led to increased calls for an expansion of the current malaria vector-control tools to supplement the traditional insecticide-based vector control interventions.[13-15]

The World Health Organization (WHO) recommends the use of "supplementary interventions" which are defined as interventions that are locally adaptive and applicable only for specific populations and situations.[16] The goal is for national programs to reduce chemical-use dependency by formulating strategies that are realistic, multi-faceted and environmentally friendly.[15,17] Included among the supplementary interventions are housing modifications and within it, house screening. House screening is defined as the covering of potential entry points (ceilings, eaves, doors, windows gable ends) with either PVC-coated fibreglass or metal mesh, or with alternative materials found around the home including old mosquito netting.[18-19]

In the past two decades, various scholars in Africa and beyond have demonstrated the link between house screening and reduced vector densities and malaria infection rates. Two recent Cochrane systematic reviews[19-20] summarize these findings, and it is not the intention of the authors to repeat those findings. Important among the findings of those reviews is that house screening reduces malaria parasite prevalence by more than 30% (RR 0.68, 95% CI 0.57 to 0.82).[19] Houses screening also reduces indoor mosquito density by about 40% (rate ratio 0.63, 95% CI 0.30 to 1.30) based on four randomized controlled trials across sub-Saharan Africa.[19]

In spite of this documented evidence, house screening remains largely unpromoted in the Zambia National Malaria Elimination program (NMEP).[21] In The Gambia, health promotion by the national malaria control programme has resulted in more houses being built with closed eaves houses and screened windows than in the past.[22] The hesitance by Zambia's NMEP to promote house screening, may be due to lack of local evidence of impact- entomologically and epidemiologically, and the paucity of data on community acceptance, cost-effectiveness and the mode of implementation.[21-22] A PUBMED check using the search terms "House screen" OR "house screening" OR "eaves" OR "house modification" AND "Zambia" (conducted on October 10, 2023) reveals only six publications; three of which were falling outside the scope of this topic and hence ineligible for any review. This revelation shows that in Zambia, a prospective trial on the effects of house-screening on vector densities and malaria parasite prevalence is yet to be evaluated. It also reveals a dearth of information on house screening that is specific to Zambia.

In this chapter, three strategies with potential to promote successful implementation of house screening intervention were reviewed to ascertain their relevance in the Zambian context. The three strategies, form the pillars of any successful integrated vector management (IVM) in the broader context. Whilst these may be perceived as gaps, they also create the opportunities that house screening specifically offers as a supplementary tool for malaria vector control in Zambia.

House Screening- Why the Need?

The success of an intervention is dependent on the sound understanding of the bionomics of the main vectors in a given loclity.[23] In Zambia, the primary malaria vectors are *Anopheles funestus, Anopheles gambiae s.s* and in some cases, *Anopheles arabiensis.*[5,24-25] These mosquito species are efficient malaria vectors partly because they are highly anthropophilic (prefer to bite humans), endophagic (feed indoors) and endophilic (rest indoors).[26-27] Most blood meals obtained by these mosquitoes are from human hosts.[28-30] Thus, the inside of the house remains a high-risk space for the transmission of malaria.[1] Huho *et al*.[31] showed that close to 80%

malaria transmission took place indoors in sub-Saharan Africa.[31] In Zambia, Seyoum *et al.*, provide evidence that most malaria transmission in a rural setting with *An. funestus* as a primary vector largely occurred indoors.[32] This holds true for Tanzania; a recent review showing that up to 99% human-vector contact occurs inside the house after 22:00hrs mediated by *An. funestus* and *An. arabiensis*.[33] A recent systematic review and meta-analysis estimated the percentage of mosquito bites taken when people are indoors in sub-Saharan Africa to vary between ~40 to 100% with a median of 87.5%.[34]

Second, open eaves, windows and doors remain an entry point for mosquitoes. The aforementioned major vector mosquito species of human malaria are well adapted for entering houses using the gaps between walls and roofs (eaves) in traditional rural houses.[22,35-37] Attracted to host odours emanating from humans, anopheline mosquitoes tend to fly upwards when they meet an obstacle such as a wall.[38] They thus, use open eaves to enter the house and locate their human hosts. They may also utilize open windows to access the indoor space and blood hosts.[22,39] Hence, closing eaves and using screens as physical barriers on windows and doors to make the house refractory to mosquito entrance, holds a promise in malaria and other vector-borne disease control.[40-42]

House Screening- Gaps and Opportunities

Community acceptability- an important first step. Acceptability refers to how well an intervention will be received by the target population and the extent to which the new intervention will meet the needs of this population.[43] Without community acceptance and behavioural change, even well intended and well-designed interventions will not have the desired impact or be sustainable. Studies in the Gambia, Ethiopia and Tanzania have taken this approach and explored the factors that affect acceptability of house screening such as lighting, ventilation, effect on disease burden (malaria), aesthetics, security, and durability.[44-47] National malaria programs wishing to implement house screening must follow this process. As shown by Kayendeke *et al.* [46] and Jones *et al.*[48] researchers and policy makers should go beyond the physical features of house screening. They should explore and

interrogate the religious and cultural context of what would make house screening acceptable or not.[49] With culture, no one size fits all. Thus, well designed qualitative and/or quantitative and ethnographic studies specific to the area are required.[49] A recent report from southeast Tanzania underscores this need.[50] Despite building improved housing with house screening and raised floors for the residents of Mtwara, few house owners occupied them and instead only made sporadic use of them. Qualitative research conducted later revealed that rumours circulated about the study team being involved with the 'Freemasons', a supposedly secret society of 'evil' men. The study team further discovered that decisions about occupying the improved homes were not taken by the householders alone and, sometimes, disagreements about the 'Freemasons' stories led to family breakdown.[50] The lesson here, is as Mshamu *et al.,* [50] conclude; *"These experiences highlight the critical need for pilot studies to understand the expectations, fears and hopes of participants in specific contexts. Community engagement must also be adapted to the local context".* It is also worth noting that community acceptance and perceived effectiveness of vector-control tools have been rarely conducted in Zambia.[51]

Intersectoral collaboration. House screening offers the malaria space the rare opportunity for intersectoral collaboration and transdisciplinary research.[23,52] In the spirit of integrated vector management, house screening offers an opportunity for collaboration of the health sector and various public and private agencies and communities.[23,52-53] In the Zambian context, the following ministries (which may change names occasionally), can be brought on board for support.

Ministry of local government. This ministry is responsible for making district level by-laws and approving local design construction. With the right engagement, the local councils can make it a policy or a law to ensure all newly constructed houses have closed eaves and screened windows and doors. Indeed, the government could enforce house screening through appropriate legislation. Laws and regulations played a critical part in eliminating malaria in China.[54] As argued by Ogbonna,[55] monitoring adherence to this law would not be difficult because 'a house cannot run'; it's only a matter of visual observations. This has been achieved in the past in

Zambia. Houses in the copper mining towns of Mufulira, Luanshya, Chingola and Kitwe, were mostly constructed with external doors that were screened and all windows fitted with wire mesh.[1,56] Of course, that may be more difficult to achieve in the rural areas. That is where involvement of the *Ministry of chiefs and cultural affairs* may be key. Key collaboration and advocacy through this ministry may enable traditional leadership to play a cardinal role in this fight against malaria.

Engaging community leaders There is no denying that community leaders, namely chiefs and headmen and the spiritual leaders (clerics) have a key role to play in the community acceptance of an intervention. They are gatekeepers of communities and act as a point of contact between the government, non-governmental institutions (NGOs) or research institutions implementing a project.[57] By virtue of their position, traditional leaders can wield influence across different age groups and political divides and can be the 'make or break' of a community accepting a health intervention. They have the power to generate momentum and support from different stakeholders, including ministers of state, elected councillors, headmen and the media.[58] An example is seen from community-led total sanitation (CLTS). Zulu *et al.,*[59] reports of how Chief Macha of Choma district in the Southern province, Zambia, used his status to advocate for improved sanitation with a multitude of stakeholders, including government ministers, elected councillors and fellow chiefs. As a result, Choma district, and his chiefdom specifically, was among the first to be declared open defecation free.[59] Community leaders must be engaged and informed in a formal and culturally sensitive manner to garner their support. Mutual respect and trust must be built with key messages and frequently asked questions on house screening prepared and added on for a holistic approach to the community leaders. Where possible, community leaders must be invited to trainings and workshops.[58-59]

Increased Urbanisation. Another factor worth considering for the future of house screening and the road to malaria elimination is the increased rate of urbanisation in SSA.[52] A recent review shows that housing structure in Africa has changed rapidly with many grass-thatched roofed houses being replaced by iron-sheet roofs with cement blocks and burnt bricks.[60] Houses with metallic roofs and concrete or burnt brick walls make screening of houses more feasible and less costly.[61-62] Houses in

many parts of rural Africa are generally built with gaps between the roofs and the walls mainly because grass thatched roofs which protrude beyond or below the wall make it nearly impossible to close these gaps (eaves).[41,63] The increase in iron-sheet roofed housing increases the very feasibility of house screening having a ripple effect in the reduction of malaria transmission.[61-62] It must be recognized that increased urbanisation on its own may not be good news for malaria control. Urbanization has been associated with increased breeding sites due to increased artificial breeding sites such as discarded tyres, plastic bottles and plastic caps, irrigating wells and shallow wells.[64] For this reason, an integrated approach is encouraged, implementing house screening through the multi-sectoral strategies mentioned earlier rather than a silo.[52]

Further opportunities. The WHO recognises house screening as an intervention with the potential for public health.[16] However, specific evidence-based recommendations and cost-effectiveness are yet to be made concrete.[18] Another consideration for the future of house screening is the cost effectiveness and who pays for it.[65] As of 2019, the WHO had commissioned a systematic review of housing and vector-borne diseases to fill this gap.[16,18] A recent report by Chisanga *et al.*[62] may start to address this gap. Further, questions arise on the effect that house-screening has on outdoor vector density and behaviour, effects on residual transmission and overall contribution in mitigating insecticide resistance.[21] Filling this gap will require long term, retrospective, and well-designed studies.

Conclusion

It must be acknowledged that there will be no '*silver bullet'* to malaria elimination, and we do not propose house screening to be such. However, Zambia, as the rest of Africa, through scale up and sustained distribution of and use of LLINS and IRS, has made steady gains in the fight against malaria.[4] These gains must be accelerated and sustained. This may require a shift from the over-dependence on chemical-based vector-control interventions to a more holistic and multi-sectorial approach.[23,52] We agree with the WHO Director, Dr Tedros A. Ghebreyesus who said, after the realization that global malaria reduction rates were just starting to plateau in 2018: *"If we continue with the business as usual approach- employing the*

same level of resources and the same interventions- we will face near-certain increases in malaria cases".[66] House screening has the potential to reduce the entry of mosquitoes into the house thereby reducing human-vector contact and reducing malaria infection rates. This intervention has in the past demonstrated its effectiveness against indoor biting vectors and formed an integral component of malaria control programs.[1] House screening thus, remains a vector-control tool worth exploiting.[21-22] This is especially true for highly malaria endemic countries like Zambia where primary vectors continue to spread malaria primarily indoors. However, the successful integration of house screening in malaria control programs will require multiple stakeholder engagement. A local shift of house construction practice will have to be implemented by individuals and families, encouraged by community leaders, enforced by local law, and advocated for by the national malaria program.

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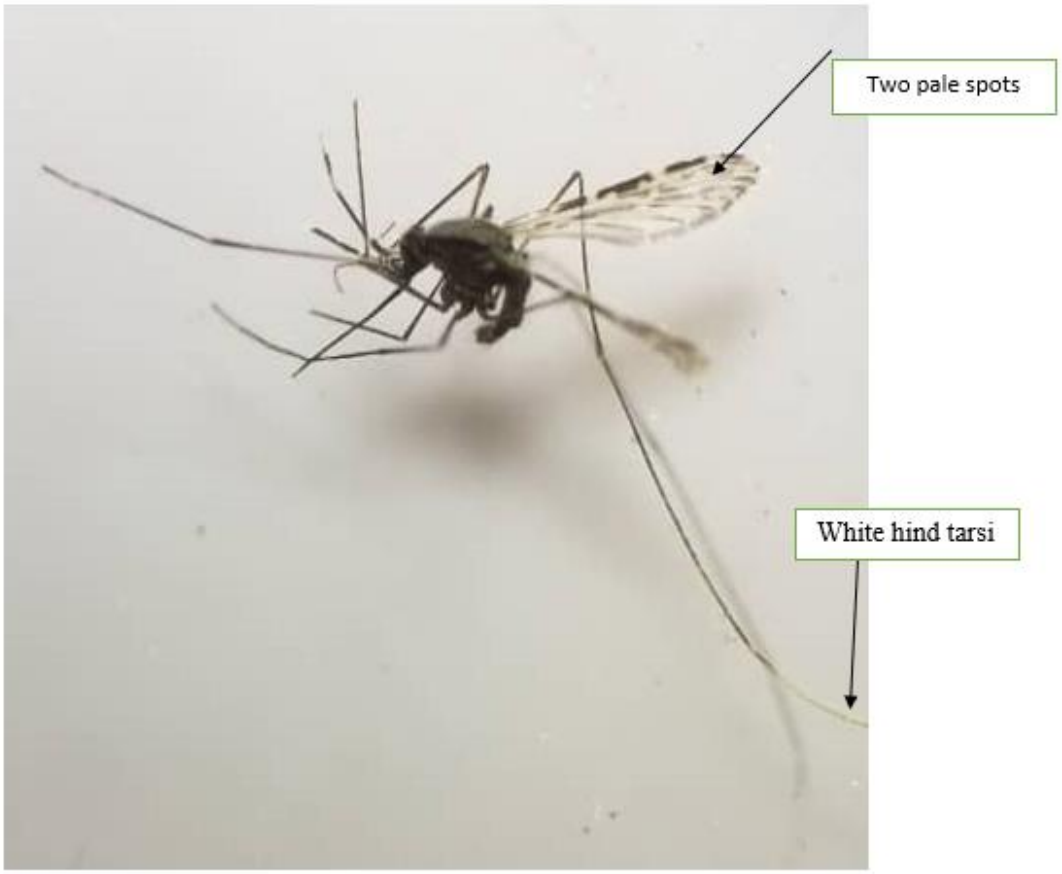
Chapter 3: *Anopheles rufipes* implicated in malaria transmission both indoors and outdoors alongside *Anopheles funestus* and *Anopheles arabiensis* in rural south-east Zambia

The protective efficacy of a mosquito control intervention is largely a function of the behaviour of local mosquito populations. Hence, knowledge of the species composition of malaria vectors, their insecticide resistance status, blood-feeding and resting behaviour is fundamental for the design of interventions specific to the local ecological and epidemiological situation. This chapter presents baseline information prior to the implementation of the house screening intervention, on the species composition of potential malaria vectors, their relative abundance and sporozoite infectivity and entomological inoculation rates (EIRs) as measures of malaria transmission in rural south-east Zambia. It thus addresses the first objective highlighted in this thesis.

In recent years, Nyimba district in Eastern province Zambia has benefitted from increased vector-control interventions, primarily indoor residual spraying, and long-lasting insecticidal nets. Malaria cases with the wider population, however, persist with a reported incidence rate of 467 cases per 1000 persons per year as of 2018 for the entire district. The current interventions are primarily intra-domiciliary and target mosquito species that prefer to feed and rest indoors.

In this study, *Anopheles funestus* was identified as the main driver of both indoor and outdoor malaria transmission in Nyimba district. *Anopheles funestus* is a long-lived species, highly anthropophilic with strong endophagic and endophilic behaviour. In the absence of insecticide resistance and/or improved formulations of current insecticides, this species may be controlled by long-lasting insecticidal nets and indoor residual spraying. However, previous studies have reported insecticide resistance in *An. funestus* to pyrethroids and carbamates. Thus, house screening as a supplementary vector-control intervention remains a viable option. The findings of this study also note that *Anopheles rufipes,* long considered a secondary vector due to its largely zoophilic, exophilic and exophagic tendencies, is gaining prominence in malaria transmission.

The findings of this study have been published in the *Malaria Journal* under the title: "*Anopheles rufipes* implicated in malaria transmission both indoors and outdoors alongside *Anopheles funestus* and *Anopheles arabiensis* in rural south-east Zambia" and was presented at the Public Health Association of South Africa (PHASA) Conference in February 2021 (oral presentation, virtual) and the 1st National symposium for the Entomological Society of Zambia, held at the University of Zambia, School of Veterinary Sciences in December 2022 (in-person oral presentation).



Graphical abstract: *Anopheles rufipes* showing; (1) the characteristic two pale spots on R1 vein in the median (second) dark area. The preapical or third dark area has no pale interruption. (2) White hind tarsi are another key characteristic of this species.

Saili et al. Malaria Journal (2023) 22:95
https://doi.org/10.1186/s12936-023-04489-3

Malaria Journal

RESEARCH Open Access

Anopheles rufipes implicated in malaria transmission both indoors and outdoors alongside *Anopheles funestus* and *Anopheles arabiensis* in rural south-east Zambia

Kochelani Saili[1,2*], Christiaan de Jager[2], Onyango P. Sangoro[1], Theresia E. Nkya[1,3], Freddie Masaninga[4], Mwansa Mwenya[5], Andy Sinyolo[5], Busiku Hamainza[5], Emmanuel Chanda[6], Ulrike Fillinger[1] and Clifford M. Mutero[1,2]

Abstract

Background The primary malaria vector-control interventions, indoor residual spraying and long-lasting insecticidal nets, are effective against indoor biting and resting mosquito species. Consequently, outdoor biting and resting malaria vectors might elude the primary interventions and sustain malaria transmission. Varied vector biting and resting behaviour calls for robust entomological surveillance. This study investigated the bionomics of malaria vectors in rural south-east Zambia, focusing on species composition, their resting and host-seeking behaviour and sporozoite infection rates.

Methods The study was conducted in Nyimba District, Zambia. Randomly selected households served as sentinel houses for monthly collection of mosquitoes indoors using CDC-light traps (CDC-LTs) and pyrethrum spray catches (PSC), and outdoors using only CDC-LTs for 12 months. Mosquitoes were identified using morphological taxonomic keys. Specimens belonging to the *Anopheles gambiae* complex and *Anopheles funestus* group were further identified using molecular techniques. *Plasmodium falciparum* sporozoite infection was determined using sandwich enzyme-linked immunosorbent assays.

Results From 304 indoor and 257 outdoor light trap-nights and 420 resting collection, 1409 female *Anopheles* species mosquitoes were collected and identified morphologically; *An. funestus* (n = 613; 43.5%), *An. gambiae* sensu lato (*s.l.*)(n = 293; 20.8%), *Anopheles pretoriensis* (n = 282; 20.0%), *Anopheles maculipalpis* (n = 130; 9.2%), *Anopheles rufipes* (n = 55; 3.9%), *Anopheles coustani s.l.* (n = 33; 2.3%), and *Anopheles squamosus* (n = 3, 0.2%). *Anopheles funestus* sensu stricto (*s.s.*) (n = 144; 91.1%) and *Anopheles arabiensis* (n = 77; 77.0%) were the dominant species within the *An. funestus* group and *An. gambiae* complex, respectively. Overall, outdoor CDC-LTs captured more *Anopheles* mosquitoes (mean = 2.25, 95% CI 1.22–3,28) than indoor CDC-LTs (mean = 2.13, 95% CI 1.54–2.73). Fewer resting mosquitoes were collected with PSC (mean = 0.44, 95% CI 0.24–0.63). Sporozoite infectivity rates for *An. funestus*, *An. arabiensis* and *An. rufipes* were 2.5%, 0.57% and 9.1%, respectively. Indoor entomological inoculation rates (EIRs) for *An. funestus s.s*, *An. arabiensis* and *An. rufipes* were estimated at 4.44, 1.15 and 1.20 infectious bites/person/year respectively. Outdoor EIRs for *An. funestus s.s.* and *An. rufipes* at 7.19 and 4.31 infectious bites/person/year, respectively.

*Correspondence:
Kochelani Saili
ksaili@icipe.org
Full list of author information is available at the end of the article

Conclusion The findings of this study suggest that *An. rufipes* may play an important role in malaria transmission alongside *An. funestus s.s.* and *An. arabiensis* in the study location.

Keywords *Anopheles rufipes*, *Anopheles funestus*, *Anopheles arabiensis*, Vector-control, Entomological inoculation rate, Zambia

Background

Malaria is endemic throughout Zambia, where it continues to be a major public health concern. In 2018, Zambia reported a national average malaria parasite prevalence of 9.1% in children under the age of five years [1, 2]. While this signifies progress compared to previous years (2010: 16.0%, 2012:14.9% and 2015:19.4% [1, 3]), this progress is not uniform across the country. In the southern regions, i.e., Lusaka and Southern provinces, malaria incidences have steadily decreased to less than 1% [1]. However, the disease remains intractable in the northern and eastern regions where parasite prevalence can be as high as 30% in children under the age of five years [1]. This is despite high coverages of primary vector-control interventions, namely indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) [4–8]. The 2018 nationwide malaria indicator survey indicated that in the southern regions, more than 83% of households had at least one LLIN or had received IRS the previous year. Coverages were higher in the northern and eastern regions; approximately 94% of households had at least one LLINs or had received IRS [1, 2].

The high malaria prevalence has been attributed, in part, to the development of insecticide resistance to commonly used insecticides for malaria vector control [4, 8–10]. Resistance to carbamates, pyrethroids and the organochlorine DDT has been reported in multiple sites in Zambia in the primary malaria vectors *Anopheles funestus* and *Anopheles gambiae* sensu stricto (*s.s.*) [9, 11–14]. Insecticide resistance undermines the continued efficacy offered by both LLINs and IRS by reducing mosquito susceptibility to the insecticides used in the two vector-control methods [15]. Further, behavioural resistance, such as outdoor vector biting and resting behaviour to avoid contact with insecticides, such as the increased exophagy observed in *An. funestus* [16, 17], poses a threat to malaria control and elimination efforts. And whilst increased vector-control interventions have led to a population decline of the primary vectors *An. funestus* and *An. arabiensis* [18, 19], this suppression has sometimes led to a proportionally increased role in malaria transmission by secondary vectors, such as *Anopheles squamosus* and *Anopheles coustani s.l.* [20–23]. In the Southern and Northern provinces of Zambia, *An. coustani s.l.* and *An. squamosus* exhibited anthropophilic tendencies with a high human blood index [23, 24] and were found harbouring malaria parasites [21, 25]. In the Eastern province, Lobo et al. [22], found a larger than expected number of sporozoite infected *An. coustani s.l.* mosquitoes. As many of the secondary vectors are exophilic and exophagic [26], they may have minimal contact with insecticides sprayed on the inside walls of houses or impregnated in LLINs. Subsequently, *An. coustani* s.*l.*, *An. squamosus* or other secondary vectors may evade current vector-control interventions and thus sustain residual malaria transmission after the main endophilic and endophagic vectors have been reduced by IRS and/or LLINs [26, 27].

In recent years, Nyimba district in Eastern province Zambia has benefitted from increased vector-control interventions, primarily IRS and LLINs [13, 28, 29]. The current interventions are primarily intra-domicilliary and target mosquito species that prefer to feed and rest indoors. Thus, malaria vectors which feed, and rest outdoors may elude vector control interventions and be responsible for residual malaria transmission. This phenomenon, therefore, calls for entomological surveillance of all mosquito populations to understand which species might be responsible for transmission and whether, based on their behaviour, they will be sufficiently targeted by current interventions [30]. This study aimed to contribute to the understanding of the species composition of potential malaria vectors and their relative abundance and to determine their sporozoite infectivity and entomological inoculation rates (EIRs) as measures of malaria transmission in rural south-east Zambia and whether they will respond to current interventions.

Methods

Study area

This study was conducted in Nyimba district, located in south-eastern Zambia (Fig. 1) between January-May 2019 and July 2019 to January 2020. Nyimba is predominantly a rural area with an estimated population of 108,637 persons [6]. Geographically, Nyimba district is divided into two parts; the eastern part of the district lies on a plateau whilst the western is in the Luangwa River valley. It shares an international boundary with Mozambique [31]. Nyimba district experiences three distinct seasons. Warm and wet from December to April; cool and dry winter from May to August and, hot and dry

from September to November. Malaria transmission is perennial with a reported incidence rate of 467 cases per 1000 persons per year as of 2018 for the entire district [District Health Information System [DHIS]). Malaria transmission peaks after the rainy season between March and May [1].

Two neighbouring health facility catchment areas were selected for this study: Mkopeka and Nyimba Urban (Fig. 1). In 2018 Mkopeka and Nyimba Urban had malaria incidence rates of 414 and 161 cases per 1000 persons/year respectively (Nyimba District Medical Office [DMO]). The houses in the study area were largely of two types: traditional mud or fire brick walls and grass thatched roof and mud or fire brick walls with metallic roofs.

IRS is the frontline vector-control intervention with annual spraying done since 2009 [28]. Starting 2014, IRS had been conducted using blanket application of the organophosphate, pirimiphos-methyl (PM) between the years 2013 and 2018 [13, 28, 32]. In this district LLIN distributions were only done in 2014 and 2018 [33]. However, starting 2019, continuous distribution of LLINs through antenatal care (ANC) clinics and school-based distribution continued as per national guidelines. During the study period, no IRS was conducted in the study area.

Adult mosquito collection

Longitudinal mosquito surveys were conducted between January-May 2019 and July 2019 to January 2020. No collections were made in June 2019 due to logistical challenges. Households in Mkopeka and Nyimba Urban were enumerated, mapped and each household individually assigned a unique identification number. From the household list generated, 60 houses were randomly selected to serve as sentinel houses for entomological surveillance. Twenty-five served as sentinel houses for Centre for Disease Control and prevention light traps (CDC-LTs; Model 512, John W Hock, Florida, USA); 10 were in Nyimba Urban and 15 in Mkopeka. Another 35 houses were used for pyrethrum spray catches (PSC); 15 in Nyimba Urban and 20 in Mkopeka [13, 34]. The houses were spread across 20 villages. Each village had a minimum of two sentinel houses, 50 m apart, with one house

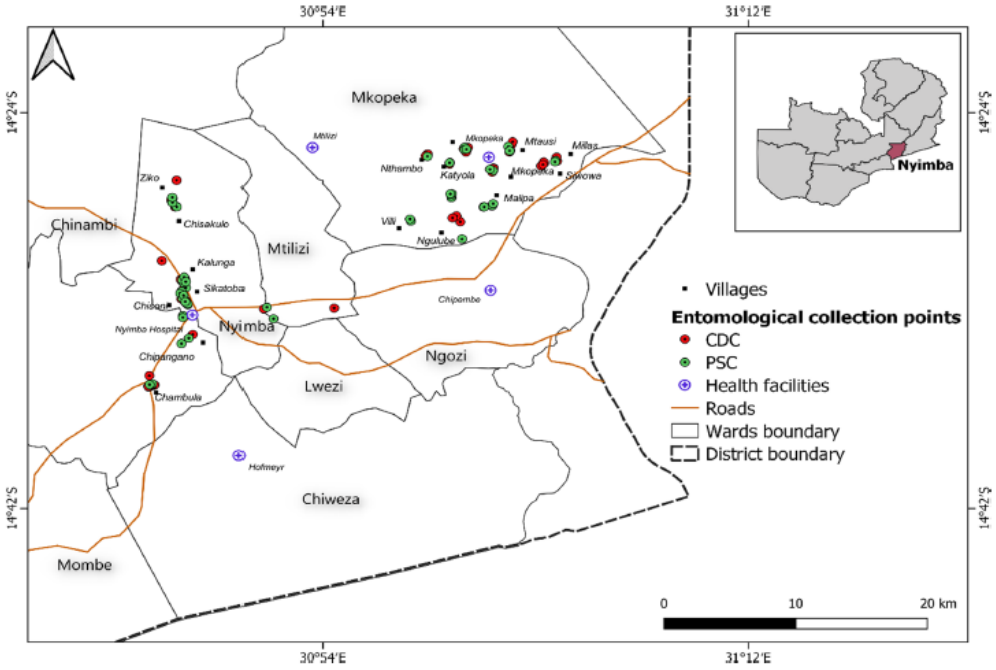


Fig. 1 Map of Nyimba district showing the location of households that were used for entomological collection. Insert: Map of Zambia showing the location of Nyimba district

serving for CDC-LT collections and another serving for PSC collections. At least 15 villages had three houses with two for PSC collections.

Mosquito collections were undertaken both indoors and outdoors using CDC-LTs. On each night of collection, two CDC-LTs were deployed per household; one inside and another outside. For indoor collections, the CDC-LT was set up between 18:00 and 06:00 h by hanging the trap, with its entrance 1.5 m above the floor and about 1.5 m away from the feet of a person sleeping under a treated mosquito net [35]. For outdoor collections, the CDC-LT was hung 5–10 m from where the family would usually sit to eat and/or spend evenings before going to bed. This distance allows for the effective range for CDC-LT whilst preventing inhabitants from acting as unprotected bait [36]. The trap was switched on at 18:00 h and switched off at 06:00 h. Both indoor and outdoor CDC-LTs, collections were made in five nights to complete the 25 houses. For each house, collections were made once per month.

Indoor mosquito resting densities were estimated monthly using pyrethrum spray collections (PSC; Mortein Energy ball®, Reckitt Benckiser) [40]. During each collection, the number of people who slept in the house the previous night and bed net use were made were recorded. PSC collections were made monthly in each of the sentinel houses. Five houses per day were sprayed, requiring 7 days to complete.

Morphological identification of mosquitoes

All collected mosquitoes were morphologically identified [37] and the physiological status of each female was noted as either unfed, fed or gravid. All morphologically identified *Anopheles* mosquitoes were then individually placed in clearly labelled 1.5 ml microcentrifuge tubes containing silica gel desiccant (Fisher Scientific) and cotton wool and stored for molecular analysis. All culicine mosquitoes were counted and discarded.

DNA extraction and PCR amplification for species identification

DNA was extracted using a modified salt extraction method [38]. Members of the *An. funestus* group (n = 236; 38.5%) and *An. gambiae* complex (n = 110; 37.5%) were further identified to sibling species level by polymerase chain reaction (PCR) [39–41]. Specimens that did not amplify on either the Gambiae-PCR or Funestus-PCR were confirmed using the internal transcribed spacer-2 ribosomal-DNA polymerase chain reaction i.e., ITS2 PCR. The ITS2 PCR technique targets the ITS2 region of nuclear ribosomal deoxyribonucleic acid (rDNA) to produce amplicons of varying band sizes depending on the mosquito species [21, 40, 44, 45]. In each month of collection, a subset of between 25–60% of the total collected female mosquitoes per species separated by collection method was targeted for species identification by PCR. In months where less than 10 mosquitoes were collected, all were subjected to species identification through PCR.

Blood meal analysis

Blood meal analysis was performed on blood-fed *An. funestus* (n = 81), *An. gambiae* s.l. (n = 33) and *An. rufipes* (n = 7). PCR analysis was used to detect and identify host blood from 121 mosquito abdomens from which DNA was extracted using the multiplex PCR assay [38] which targeted the cytochrome b region of the hosts mitochondrial DNA [38].

Detection of *Plasmodium falciparum* infection in mosquitoes

A random subsample, by sampling method and month of collection of female *An. funestus* (n = 360/613; 58.7%), *An. gambiae s.l.* (n = 174/293; 59.4%), *An. pretoriensis* (n = 72/282; 25.5%), *An. rufipes* (n = 42/55; 76.3%), *An. coustani s.l.* (n = 18/33; 54.5%) and *An. squamosus* (n = 3/3; 100%) mosquitoes were tested for *P. falciparum* circumsporozoite proteins (CSPs) using sandwich enzyme-linked immunosorbent assays (ELISA) [46]. To avoid false CSP positives common in zoophilic species the ELISA lysates were heated [47]. Sporozoite infectivity was determined separately for mosquitoes caught indoors and outdoors.

Statistical analyses

All data were entered and stored into an Excel spreadsheet (Microsoft Office 2018) and exported to open-source statistical software R version 3.51 [48] for analysis. Descriptive statistics namely mean catches per trap per night and proportions of mosquitoes caught per sampling method per catchment area were used to summarize the data. Species-specific mean catches were calculated by dividing the total number of mosquitoes caught by the number of trap-nights. The human blood index (HBI), sporozoite infectivity rate (SIR) and entomological inoculation rate (EIR) were calculated as a measure of malaria transmission intensity using the following formulae.

Human blood index (HBI)

The human blood index (HBI) was calculated as the proportion of mosquitoes fed on human blood meals out of the total mosquitoes that successfully amplified for blood meals [49].

$$\text{Human Blood Index} = \frac{\text{Number of mosquitoes with human blood}}{\text{Total number of mosquitoes amplified for blood meal}}$$

Mixed (human + domestic animal) blood meals were added to the number of human blood meals when calculating the HBI.

Sporozoite infectivity rate (SIR)

Sporozoite infectivity rate (SIR) is defined as the proportion of *Anopheles* mosquitoes with sporozoites in their salivary glands to the total number of mosquitoes examined for sporozoites [50]. Sporozoite infectivity was determined separately for each species. This was determined using the following formula:

$$\text{Sporozoite infectivity rate} = \frac{(\text{Number of mosquitoes with sporozoites})}{(\text{Number of mosquitoes examined})}$$

Sporozoite infectivity rates were determined separately for indoor (PSC and CDC-LTs) and outdoor (CDC-LTs only) collection methods and were species-specific. The Pearson's Chi-square tests were used to evaluate the difference in proportions and infectivity rates at an $\alpha = 0.05$ level of significance.

Entomological inoculation rate (EIR)

Entomological inoculation rate (EIR) is defined as the number of infectious bites per person per unit time, usually expressed per year or month [51]. Species-specific EIR was calculated based on the mean number of female *Anopheles* mosquitoes caught per trap/night, without adjusting for room occupancy [10, 50]. Annual EIR was calculated separately for indoors and outdoors using the formula:

$$EIR = SIR \times \frac{\#\ of\ mosquitoes\ collected\ by\ CDC - LT}{\#of\ CDC - LT\ trap\ nights} \times 365\ days$$

For PSC collections, EIRs was calculated using the formula described in [52].

EIR = Human Biting Rate (HBR) x SIR × 365 days where SIR as defined above and the human biting rate as shown below.

$$HBR = HBI \times \frac{Number\ of\ blood - fed\ mosquitoes}{Number\ of\ occupants\ on\ night\ of\ collection}$$

Results

Species composition of *Anopheles* mosquitoes

The sampling design of this study resulted in an overall 304 indoor and 257 outdoor CDC light trap-night collections. Less frequent outdoor CDC-LTs collections were due to the rainy season when heavy rains would interfere with trapping. A total of 420 resting collections were done using the pyrethrum spray catch (PSC) method. The average number of human occupants during PSC collections was three.

A total of 1409 female *Anopheles* mosquitoes were collectively sampled in 977 collections. Overall, seven species were identified morphologically. The *An. funestus* group (n = 613; 43.5%) represented the predominant malaria vectors in the study area followed by *An. gambiae s.l.* (n = 293; 20.8%). Other species were *Anopheles pretoriensis* (n = 282; 20.0%), *Anopheles maculipalpis* (n = 130; 9.2%), *An. rufipes* (n = 55; 3.9%), *An. coustani s.l.* (n = 33; 2.3%), and *An. squamosus* (n = 3, 0.2%). Table 1 summarizes the species composition and mean collections per sampling method per night. Only eight male *Anopheles* mosquitoes were collected: *An. gambiae* s.l. (n = 3) and *An. pretoriensis* (n = 5). At the same time 2052 female culicine mosquitoes were collected.

Polymerase chain reaction was performed on a random subsample of 236 (38.5%) of all collected female *An. funestus* mosquitoes. Of these, 158 specimens successfully amplified. A total of 74 specimens did not amplify and four gave non-specific amplification on the ITS2-PCR (n = 2, 700 base pairs and n = 2, 900 bp). Overall, collections from both sites revealed the predominant species found was *An. funestus* sensu stricto (*s.s.*) (n = 144/158; 91.1%); PSC (n = 61/61), indoor CDC-LT (n = 36/36) and outdoor CDC-LT (n = 47/61). There was a significantly higher occurrence of *An. funestus s.s.* in indoor versus outdoor traps ($\chi^2 = 7.73$, $df = 1$, $P = 0.03$). Other species identified within the *An. funestus* group were *Anopheles leesoni* (n = 8; 5.1%), *Anopheles parensis* (n = 4; 2.5%) and *Anopheles vaneedeni* (n = 2; 1.2%). *Anopheles leesoni, An. parensis* and *An. vaneedeni* amplified from specimens caught only outdoors. Figure 2 shows the different proportions of species within the *An. funestus* group per sampling method per site.

Polymerase chain reaction (PCR) was performed on a random subsample of 110 (37.5%) female *An. gambiae s.l.* mosquitoes. Of these 100 successfully amplified. Eight did not amplify and two gave non-specific amplifications on the ITS2-PCR (n = 2, 280 bp) upon further analyses.

Within the *An. gambiae* complex, the predominant species was *An. arabiensis* (n = 77; 77.0%); PSC (n = 15/15), indoor CDC-LT (n = 48/58) and outdoor CDC-LT (n = 14/27). *Anopheles gambiae s.s.* (n = 20; 20.0%) and *Anopheles quadriannulatus* (n = 3; 3.0%) were the two other species within this complex in the study area. No *An. gambiae s.s.* were found in PSC with few occurring in indoor (n = 9/61) and outdoor (n = 11/27) CDC-LT collections. Likewise, no *An. quadriannulatus* were collected using PSC with few collected in indoor (n = 1/61) and outdoor (n = 2/27) CDC-LT collections. Figure 3 shows species composition and proportions

Table 1 *Anopheles* species composition and mean collections per sampling method in the study area

Species	Overall	CDC LT-IN		CDC LT-OUT		PSC	
	N	n	Mean (95% CI)	n	Mean (95% CI)	n	Mean (95% CI)
An. funestus group	613	331	1.09 (0.92–1.25)	140	0.55 (0.46- 0.65)	142	0.34 (0.19–0.42)
An. gambiae s.l	293	167	0.55 (0.38–0.71)	107	0.42 (0.35–0.49)	19	0.04 (0.02–0.06)
An. pretoriensis	282	82	0.27 (0.15–0.39)	183	0.71 (0.46–0.97)	17	0.04 (0.01–0.07)
An. maculipalpis	130	53	0.17 (0.06–0.29)	74	0.29 (0.22–0.36)	3	0.01 (0–0.01)
An. rufipes	55	6	0.03 (0.02–0.04)	47	0.18 (0.14–0.22)	2	0.004
An. coustani	33	8	0.03 (0–0.05)	25	0.10 (0.08–0.11)	0	0
An. squamosus	3	1	0	2	0.01 (0–0.02)	0	0

CDC Centers for Disease Control and Prevention, *LT* Light Trap, *PSC* Pythrerum Spray Catches, *IN* Indoor *OUT* Outdoor

within *An. gambiae s.l.* per collection method and separated by study site.

Indoor and outdoor host-seeking and resting collections

Similar numbers of host-seeking *Anopheles* mosquitoes were trapped with light traps outdoors (mean = 2.25, 95% CI 1.22–3.28) and indoors (mean = 2.13, 95% 1.54–2.73) per trap. Fewer mosquitoes were collected per PSC trap night (mean = 0.44, 95% CI 0.24–0.63).

At the species level, more host-seeking mosquitoes of the *An. funestus* group were trapped using indoor CDC-LTs (95% CI 0.92–1.25) per night per house than outdoors (mean 0.55; 95% CI 0.46–0.65) (Table1). Indoor resting densities of *An. funestus* group were slightly lower with a mean of 0.31 (95% CI 0.19–0.42) per house. Only 23.2% of all collected female *An. funestus* mosquitoes (n = 142/613) were caught resting indoors with most of these blood-fed (n = 123/142, 87.6%).

The mean number of *An. gambiae s.l.* mosquitoes trapped with indoor CDC-LTs (mean = 0.55, 95% CI 0.38–0.71) per night per house was slightly higher than collected outdoors (mean = 0.42, 95% CI 0.35–0.49) (Table 1). Only 6.5% of all collected female *An. gambiae*

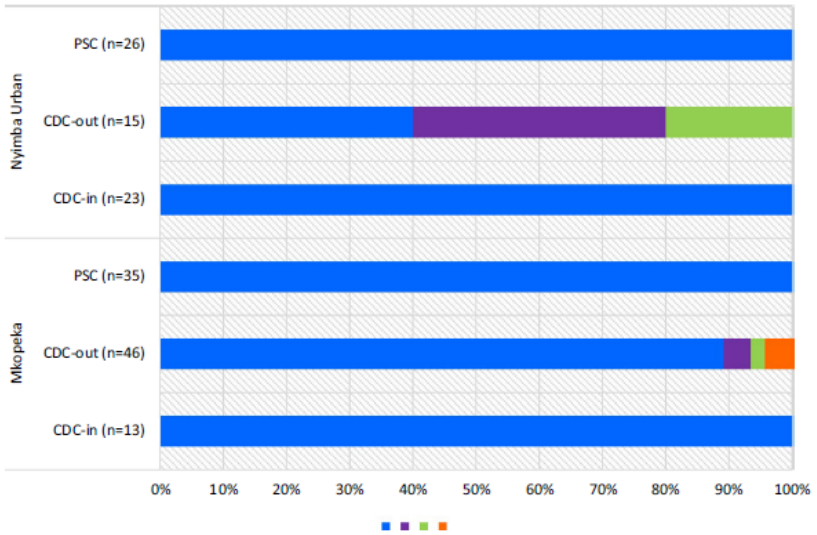


Fig. 2 Proportions of species within the *Anopheles funestus* group in the two study areas. The numbers in parentheses indicate the total number of specimens that successfully amplified per collection method per study site

s.l. mosquitoes (n = 19/293) were caught resting indoors with most of these being blood-fed (n = 16/19, 84.2%).

The 503 other anopheline specimens, included the species *An. pretoriensis, An. maculipalpis, An. rufipes, An. coustani s.l.* and *An. squamosus*. Most of these were caught outdoors (n = 318/503, 63.2%) rather than indoors (n = 150/503, 29.8%). Taken together, a larger proportion of these specimens were outdoor host-seeking ($\chi^2 = 21.1$, df = 4, $P < 0.01$). Few of the other anopheline specimens were caught resting indoors (n = 22/503, 4.4%) with zero blood-fed.

Blood meal sources

Of the 121 blood-fed mosquitoes analysed, only 18 (14.9%) amplified successfully. Of these, 13 blood meals were from humans and three had mixed human-goat blood meal host (Table 2). The overall human blood index from resting collections and CDC-LT collections both indoors and outdoors was found to be 0.89. Due to the small sample size of mosquitoes that amplified on the blood meal analysis, these results are interpreted with caution.

Sporozoite infectivity and entomological inoculation rates

A total of 360 (58.7%) female specimens of the *An. funestus* group were tested for the presence of *P. falciparum* circumsporozoite protein (*Pf* CSP). Of these, nine mosquitoes tested positive for sporozoites giving an overall sporozoite infectivity rate of 2.5%. The nine sporozoite infected mosquitoes came from samples collected in February 2019 (n = 3), March 2019 (n = 2), July 2019 (n = 1) and January 2020 (n = 3). All sporozoite infected mosquitoes were *An. funestus s.s.* Other species within the *An. funestus* group, namely *An. leesoni, An. parensis* and *An. vaneendeni* tested negative for *P. falciparum* sporozoites.

A total of 174 (59.4%) female *An. gambiae s.l.* mosquitoes were tested for the presence of the *Pf* CSP. One tested positive giving an overall sporozoite infectivity rate of 0.57%. The sporozoite infected mosquito was *An. arabiensis* trapped in March 2019. The other members within the *An. gambiae* complex namely, *An. gambiae s.s.* and *An. quadriannulatus* tested negative for *P. falciparum* sporozoites.

Other anopheline mosquitoes, namely *An. pretoriensis* (n = 70/282; 24.8.0%), *An. coustani s.l.* (n = 17/33; 51.5%), *An. rufipes* (n = 33/55; 94%) and *An. squamosus* (n = 3/3; 100%) were analysed for *Pf*-CSP. Three *An. rufipes* specimens tested positive for sporozoites, giving an overall sporozoite infectivity rate of 9.1% for *An. rufipes* (Table 3). The three sporozoite infected *An. rufipes* were trapped indoors using CDC-LTs in February 2019 (n = 1) and outdoors using CDC-LTs in March and February 2019 (n = 2) in the Mkopeka study sites. The morphological identification of the *An. rufipes* mosquitoes was confirmed using the ITS2-PCR, resulting in an amplification of 500 bp. In all the above, heating the ELISA lysate did not change the *Pf*-CSP positive result.

The species-specific estimated indoor and outdoor annual EIR based on CDC-LT catches for *An. arabiensis, An. funestus s.s.* and *An. rufipes* mosquitoes is shown in Table 3. Indoor EIRs for *An. funestus* s.s, and *An. arabiensis* were estimated at 4.44 and 1.15 infectious bites per

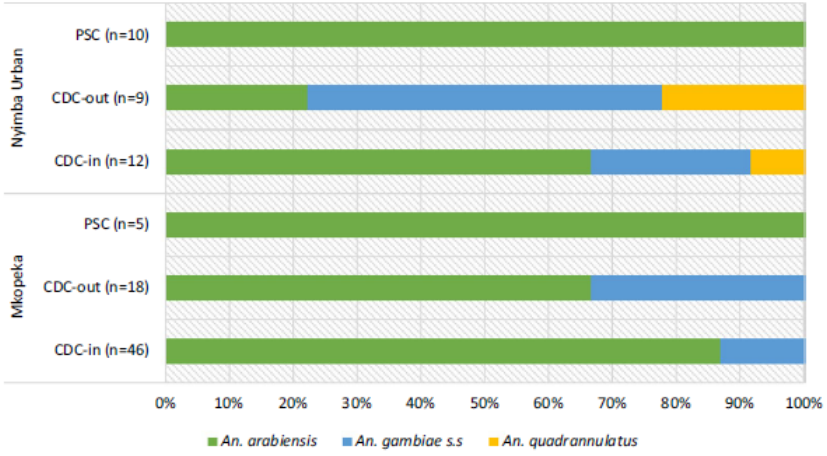


Fig. 3 Proportions of species within the *Anopheles gambiae* complex in the two study areas. The numbers in parentheses indicate the total number of successfully amplified specimens per collection method per study site

Table 2 Blood meal sources of *Anopheles* mosquitoes per sampling method

Method	*Anopheles* species	# analysed	Human	Mixed: human/goat	Dog	Unamplified	Human blood index
PSC	*An. funestus*	40	3	1	0	36	1.00
	An. gambiae	14	2	0	0	12	1.00
CDC LT indoors	*An. funestus*	20	3	1	0	16	1.00
	An. gambiae	10	2	0	0	8	1.00
CDC LT outdoors	*An. funestus*	21	3	1	0	17	1.00
	An. gambiae	9	0	0	0	9	0.00
	An. rufipes	7	0	0	2	5	0.00
Total		121	13	3	2	103	0.89

person per year (ib/p/y), respectively. Indoor EIR for *An. rufipes* in the study area was estimated at 1.20 ib/p/y. Outdoor EIR for *An. funestus s.s* and *An. rufipes* were estimated at 7.19 and 4.31 ib/p/y, respectively (Table 3). Only *An. funestus* specimens, collected with PSC, tested positive for sporozoites. Indoor EIRs for *An. funestus s.s,* collected with PSC, was estimated at 1.19 ib/p/y. However, these results are interpreted with caution due to the extremely low number of blood meals that were amplified in the blood meal analysis.

Discussion

Anopheles funestus group made up the majority of anopheline mosquitoes collected in this study. Species identification by PCR further revealed that this group was predominantly made up of *An. funestus s.s.* (henceforth simply referred to as *An. funestus*). This confirms previous reports that describe *An. funestus* as the main driver of malaria transmission in the study area [22, 28, 53]. *Anopheles funestus* is historically highly anthropophilic with strong endophagic and endophilic behaviour [54, 55]. Thus, in the absence of insecticide resistance and/or improved formulations of current insecticides, this species may be controlled by LLINs and IRS [55]. This is supported by the fact that the indoor EIR by *An. funestus* reported in this study (4.4 ib/p/y) was 16 times lower than previously reported in the same location. An EIR of 70.1 ib/p/y was observed between the years 2011–2013 [53]. This decreased EIR may highlight suppression of sporozoite infectivity following increased vector-control interventions, namely LLINs and IRS with pirimiphos-methyl (IRS-PM). These observations are consistent with previous studies conducted in other parts of Zambia which demonstrated the impact of increased IRS-PM and population-wide coverage of LLINs in reducing sporozoite infection rates of *An. funestus* [11, 19]. Similar findings have been reported in neighbouring Mozambique [56], north-western Tanzania [57] and western Kenya [58]. However, that malaria transmission persists, albeit at low levels, shows that these core interventions cannot be deployed solely.

The persistence of malaria has been associated with behavioral changes observed in anopheline mosquitoes. Findings of this study indicate that *An. funestus* may also be transmitting malaria outdoors. In this study, *An. funestus* outdoor EIR, estimated at 7.19 ib/p/y was higher than EIR indoor. The higher outdoor EIR in *An. funestus* may highlight suppression of the highly endophagic species, thereby increasing the proportions of outdoor host seeking mosquitoes [16, 17]. This behavioural modification may be as result of the increased use of LLINs or IRS in the study area [16, 59]. The outdoor malaria

Table 3 Annual EIR estimation based on CDC-LT and PSC catches for *An. arabiensis*, *An. funestus s.s* and *An. rufipes* mosquitoes

Method	Species	# assayed	Sporozoite positive	Proportion of mosquitoes infected (SIR)	EIR (ib/p/yr)
CDC-LT Indoors	*An. funestus* group	179	2	0.01	4.44
	An. gambiae s.l	91	1	0.01	1.15
	An. rufipes	6	1	0.17	1.20
CDC-LT Outdoors	*An. funestus* group	83	3	0.04	7.19
	An. gambiae s.l	83	0	0.00	0.0
	An. rufipes	27	2	0.07	4.31
PSC	*An. funestus* group	98	4	0.05	1.19

transmission described in this study has implications for malaria control and eradication in Zambia and in sub-Saharan Africa. A recent study shows that a 10% increase in outdoor biting would result in 58.2% increase in malaria cases per year on the African continent, assuming a "perfect scenario" of 100% LLINs coverage and zero insecticide resistance [60]. Outdoor biting vectors, thus pose a significant threat to elimination efforts by sustaining malaria transmission. Subsequently, indoor-vector control interventions such as LLINs and IRS alone may not be enough to eliminate malaria [61, 62].

Secondary vectors may also play a role in continued malaria transmission. In this study sporozoite infected specimens of *An. rufipes* were found. Similar findings of *An. rufipes* harbouring sporozoites have been reported in southern Zambia [25], Kenya [63], Cameroon [64–66], Burkina Faso [67] and Nigeria [68]. This study thus incriminates *An. rufipes* as a potential malaria vector in rural south-east Zambia [69] with estimated EIRs of 1.20 and 4.31 ib/p/y indoors and outdoors, respectively. The estimated EIR for *An. rufipes* was higher than that of *An. arabiensis,* indicating the need for further studies to investigate the role of secondary malaria vectors in maintaining malaria transmission [26, 70]. Sporozoite infected *An rufipes* mosquitoes were collected during the peak malaria season in Zambia, between February and April [1, 70] when vectors were most abundant. That this species is largely zoophilic and exophagic [25] makes it a threat to achieving malaria elimination as it may evade indoor-centric vector-control interventions [26].

Anopheles gambiae s.l., which was primarily *An. arabiensis,* confirming previous results [71], was found with lower sporozoite infectivity when compared to *An. rufipes*. Thus, in Nyimba district, *An. arabiensis* may be considered a vector of secondary importance when compared to *An. funestus* and *An. rufipes*. This study also confirms previous observations that in cases where *An. arabiensis* and *An. funestus* occur in sympatry, the latter appears to be the more competent malaria vector [55, 72, 73]. Nonetheless, that *An. arabiensis* was found in both indoor and outdoor traps suggest that it can forage both indoors and outdoors thereby making it less amenable to the traditional indoor-based vector-control interventions [19, 74].

The mosquito community in this study included diverse species. Within the *An. funestus* group, were found *An. leesoni, An. parensis* and *An. vaneedeni*- largely zoophilic species [27] all of which tested negative for malaria parasites. Similarly, other members of the *An. gambiae* complex, namely, *An. quadriannulatus* and *An. gambiae s.s.* also tested negative for malaria parasites. However, Lobo et al. [22] found sporozoite infected *An. quadriannulatus, An. pretoriensis* and *An. coustani* from the same study locations. Thus, in this region of Zambia, the vector population plasticity, species diversity and co-occurrence of both primary and secondary vectors with different behaviours, may sustain malaria transmission and calls for more integrated vector-control approaches. Future research should determine the bionomics, morphology, and breeding habitats of potential secondary vectors for a comprehensive understanding of their roles in malaria transmission [21–27]. Additionally, the period (less than a year) and geographical scope of sampling was not extensive and may explain some of the low vector densities observed in this study. More sampling sites are required to establish malaria transmission by *An. rufipes* and other potential secondary vectors. A further limitation of this study was the lack of amplification of some specimens for PCR species identification. This may be attributed to specimen degradation or morphological misidentification, attributed to damaged mosquito specimens. This is common with CDC-LT collections [22]. This calls for improvement in and coupling of morphological identifications with molecular methods of identification. Furthermore, molecular identification was not performed beyond the ITS2 PCR. A two-step procedure for species identification was carried out; first morphological identifications based on morphological keys [37] similar to methods used by Tabue et al.[64] and Awono-Ambene et al. [65]. Second, confirmation of the identification using the ITS2 PCR to ensure that the specimens identified as *An. rufipes* were indeed such. Additional molecular identifications- perhaps by ITS2 gene sequencing to adequately incriminate and identify vectors of malaria [22, 27] should be included in future research.

Findings of this study are limited by several factors. An extremely small number of samples amplified for the blood-meal analyses. Several re-runs were made without success. This might be due to storage conditions. Possibly, DNA of the blood meal host may have been degraded since specimens were stored for several months on silica gel before molecular analysis. Further, mosquitoes may have had incomplete blood meals or the blood meal may have been digested resulting in degradation of host DNA [75]. The successful identification of blood meal hosts by PCR depends on the quality and quantity of the host´s DNA contained in the abdomen of mosquitoes [75]. Yet another possiblity is that mosquitoes fed on hosts other than those included in the primer set e.g., avian-specific primers. Further investigations in blood meal studies in Zambia to document the range of blood meal hosts of malaria vectors are strongly recommended.

Conclusion

This study confirms earlier reports that *An. rufipes* might be involved in malaria transmission in rural south-east Zambia. Whilst for long, the species has been considered of secondary importance in Zambia due to its largely zoophilic, exophilic and exophagic tendencies, recent successes in vector control require a new evaluation of the remaining vectors. Based on these findings, increased routine entomological surveillance and *Plasmodium* sporozoite infectivity screening for all potential malaria vectors is recommended. Additionally, vector-control interventions should be diversified to include outdoor interventions for improved control and efforts towards malaria elimination.

Abbreviations

LLINs	Long-lasting insecticidal nets
IRS	Indoor residual spraying
IRS-PM	Indoor residual spraying with pirimiphos-methyl
DMO	District medical office
CDC LTs	Centres for disease control and prevention light traps
PSC	Pyrethrum spray catches
PCR	Polymerase chain reaction
CSP	Circumsporozoite protein
ELISA	Enzyme-linked immunosorbent assays
SIR	Sporozoite infectivity rate
EIR	Entomological inoculation rates

Acknowledgements

The authors are grateful to the communities in Nyimba Urban and Mkopeka in whose households collections were made. We thank the Ministry of Health through the National Malaria Elimination Centre (NMEC), Nyimba District Medical Office and traditional leadership in Nyimba Urban and Mkopeka for their co-operation and support. We thank Limonthy Simubali and Twig Mudenda from Macha Research Trust for their help on the molecular analysis.We thank Emily Kimathi for her help with the map and Dr. Cheryl Tosh for editing.

Author contributions

CMM, UF, EC and FM conceived the study and wrote the main study protocol. KS, CdJ, POS, UF and CMM designed this study. KS, MM, AS, FM, POS and BH supervised the study data collections. KS and AS performed the molecular analysis. KS performed data analysis. KS wrote the initial draft of the manuscript, which was revised by CMM, UF, FM, CdJ, POS, TEN and BH. All authors read and approved the final manuscript.

Funding

The authors gratefully acknowledge the financial support for this research by the following organizations and agencies: Global Environmental Fund (GEF) through United Nations Environmental Programme (UNEP) and the WHO-Africa Regional Office (WHO-AFRO) (Grant number: 4668); the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Australian Centre for International Agricultural Research (ACIAR); the Federal Democratic Republic of Ethiopia; and the Government of the Republic of Kenya. The views expressed herein do not necessarily reflect the official opinion of the donors. Kochelani Saili was supported by a German Academic Exchange Service (DAAD) In-Region Postgraduate Scholarship and a University of Pretoria doctoral bursary.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol and informed consent forms were reviewed and approved by the ERES Converges IRB Zambia (Reference: 2018-Oct-007 and 2020-Jul-018), the National Research Health Authority (Ref: NHRA00002/23/04/2021 and Health Researcher Registration #: NHRAR-R-119/27/05/2022) and the research ethics committee of the University of Pretoria (Ref: 242/2020). Written permission to undertake the study was obtained from the Ministry of Health through the National Malaria Elimination Centre (NMEC) and Nyimba District Medical office. Local and traditional leadership were also informed about the purposes of the study. Participation in the study was voluntary, and informed consent was obtained from household heads and every participant above the age of 18 years. Verbal consent was obtained from household heads before routine mosquito collections.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]International Centre of Insect Physiology and Ecology (Icipe), P.O. Box 30772-00100, Nairobi, Kenya. [2]University of Pretoria Institute for Sustainable Malaria Control, School of Health Systems and Public Health, University of Pretoria, Pretoria, South Africa. [3]Mbeya College of Health and Allied Sciences, University of Dar es Salaam, Mbeya, Tanzania. [4]World Health Organization, Lusaka, Zambia. [5]National Malaria Elimination Centre, Lusaka, Zambia. [6]World Health Organization, Regional Office for Africa, Brazzaville, Congo.

Received: 2 August 2022 Accepted: 12 February 2023
Published online: 16 March 2023

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Publisher's Note

Chapter 4: Evaluation of Glue Net trap and Window Entry and Exit Traps for monitoring the entry and exit behaviour of *Anopheles* mosquitoes in rural southeastern Zambia

Determining the house entry and house exit behavior of malaria vectors is fundamental for the design and implementation of vector-control interventions. Anopheline mosquitoes can also be collected through larval collections which prove the presence or absence of a species, determines the preferred breeding sites of each vector species, and assess the effectiveness of the vector-control program. Further, mosquitoes collected as larvae can be reared into adults and used for insecticide susceptibility testing. This is crucial for the design of effective vector-control interventions, specifically, selection of appropriate vector-control interventions, informing resistance prevention strategies or prompting changes in vector-control strategies when resistance emerges.

In the previous chapter, baseline information on the species composition of potential malaria vectors, their relative abundance and sporozoite infectivity and entomological inoculation rates (EIRs) were presented. Chapter 4 builds on that information. This study determined (1) the entry and exit behaviour of anopheline mosquitoes using a sampling tool herein referred to as the Glue Net Trap (GNT) and (2) the insecticide susceptibility status of anopheline mosquitoes to commonly used insecticides in Nyimba district. Overall, this chapter addresses the second specific objective of this thesis.

The ability to trap mosquitoes using the GNT was first tested using a two-chamber system, separated by the GNT. The effectiveness of the GNT was then assessed and compared to window exit or entry traps during the wet and dry seasons. Three villages within Mkopeka and Nyimba Urban catchment areas were randomly selected. In each selected village, two sets of three houses were randomly selected in which a Latin square rotation sequence was followed. Immature mosquito sampling was conducted in breeding sites using dippers. Bioassays were conducted on F1 adult mosquitoes that emerged from immature collections. Three insecticide-

impregnated papers were used: organochloride (DDT) and two pyrethroids (deltamethrin and permethrin).

Cage experiments revealed an average trap rate of 88.9% (95% CI 88.8-90.0%). Most mosquitoes caught on the GNT did not have legs, wings, or maxillary palps upon removal from the glue net trap. In 27 trap-nights in the wet season and 18 trap-nights in the dry season, no mosquitoes were trapped in window traps and GNTs. Larval collections were dominated by *Anopheles pretoriensis* (n=392; 89.7%). Bioassays revealed 100% mortality rates of *Anopheles pretoriensis* to DDT, deltamethrin, and permethrin. This study shows that both GNTs and window traps may not be effective sampling tools for studying the entry and exit behaviour of anthropophilic mosquitoes in rural Zambia. Findings of this study also showed that during the study period, *Anopheles pretoriensis* remained susceptible to pyrethroids and DDT. This chapter will be prepared for submission to a target journal.

Keywords

Glue Net trap, Zambia, Window exit traps, *Anopheles pretoriensis*, insecticide resistance.

Evaluation of Glue Net trap and Window Entry and Exit Traps for monitoring the entry and exit behaviour of *Anopheles* mosquitoes in rural southeastern Zambia.

Kochelani Saili[1,2*], Christiaan de Jager[2], Mwansa Mwenya[3] Freddie Masaninga[4], and Clifford M. Mutero[1,2]

[1]International Centre of Insect Physiology and Ecology *(icipe),* P.O. Box 30772-00100, Nairobi, Kenya

[2] University of Pretoria Institute for Sustainable Malaria Control, School of Health Systems & Public Health, University of Pretoria, Pretoria, South Africa

[3] National Malaria Elimination Centre, Zambia

[4] World Health Organization, Lusaka, Zambia

Corresponding author: ksaili@icipe.org

Introduction

Entomological monitoring and surveillance of adult and immature vector populations remains an integral component of malaria control and elimination programs. It is important in determining the behaviour, distribution, abundance, and insecticide resistance status of malaria vectors.[1] Entomological monitoring is also important in establishing if there is a spatial and temporal overlap between humans and mosquitoes and that mosquitoes are taking their blood meals from humans.[2-3]

To monitor the entry and exit behaviour of anopheline mosquitoes into houses occupied by humans, Window Entry or Exit Traps (henceforth simply referred to as window traps or WTs) have been used.[3-5] When used as entry traps, these catch mosquitoes entering houses. When used as exit traps, they catch female mosquitoes leaving houses at sunrise for oviposition or outdoor resting e.g., in *Anopheles arabiensis.*[3-4] Window traps hence provide vital information on the physiological state of the species under investigation, before or after interaction with human or animal hosts hence providing insight into malaria transmission dynamics.[3-5]

Window Traps however, have limitations. They can be cumbersome to transport and install and may inconvenience the householders.[6] Collecting trapped mosquitoes from a WT using mouth aspirators is highly dependent on the skill of a technician and is also labour intensive.[4,7] Further compounding the problem is that, even in a single study village, homes and animal shelters may have entry and exit points in different sizes, shapes, and numbers.[4-5] This makes it difficult to be consistent in the use of the trap and/or find a suitable space that can be used as an entrance or escape route for endophilic mosquitoes.[5,7-8] Another limiting factor of WTs is that they can only be used as either “exit traps” or “entry traps” but never as entry and exit traps simultaneously over the same window.[8] Some studies have shown that reversing an “exit trap” to become an “entry trap” or vice versa, yields lower catches than anticipated.[8] What is desirable then, is a tool that simultaneously and unbiasedly measures the species-specific entry and exit behaviour.[8]

This paper describes the use of a relatively simple sampling tool called the Glue Net Trap (GNT) as a mosquito entry and exiting behaviour sampling tool. First described

by Müller *et al.*[8] in Mali and more recently by Liao[9] and Yalla *et al.,*[10] in Kenya, this trap is economical, easy to transport and assemble. The trap can be constructed from locally purchased plastic garden greenhouse fencing material with 0.2 cm wide spaces, separated by 0.8 cm square holes. The frames are then constructed by overlapping two layers of thin plywood fixed with nails, screws and/or wood glue with the netting sandwiched between the two wooden frames. The net is then painted or sprayed with insect glue to capture mosquitoes trying to enter or leave the house.

The holes on the netting material are large enough for a mosquito to walk through. As such, the net without glue does not act as a control or sampling tool against mosquitoes as would a mosquito-impenetrable wire or plastic mesh. Video studies of mosquito behaviour on mosquito nets with various hole sizes show that if the holes are close or smaller than 0.8 mm by 0.8 mm in size, the mosquitoes would land on the netting and then walk or squeeze through.[11] The GNT exploits this behaviour. With the addition of sticky glue, the fence material acts as a mosquito sampling tool to measure the entry and exit behaviour of mosquitoes inside human dwellings.

Another method of entomological monitoring and surveillance involves larval collections i.e., collection of immature mosquitoes. In malaria endemic areas, larval collections prove presence or absence of a species, determine the preferred breeding sites of each vector species, and assess the effectiveness of the vector-control program.[2-3] Mosquitoes collected as larvae can also be reared into adults used for insecticide susceptibility testing, which is the preferred method for insecticide resistance testing.[12]

This study formed part of a baseline study carried out in Nyimba district, Zambia, with the following objectives: (1) To evaluate the use of GNT as a sampling tool to monitor the entry and exit behaviour of endophilic and endophagic mosquitoes in comparison to traditional entry and exit traps placed on windows. The hypothesis was that the two trapping methods applied in the study would successfully collect sufficient numbers of mosquitoes entering or exiting a house occupied by humans. (2) To profile the insecticide resistance or susceptibility status of malaria vectors in the Nyimba district, southeast Zambia.

Methods

Cage Experiments

Before deployment into the field, the ability to trap mosquitoes using the GNT was tested in cage experiments in January and February 2020. The cage experiments were also useful to determine the "damage state' i.e., how damaged the mosquitoes caught using this method would (or not) be as they were removed from the GNT.

To achieve this objective, a two-chamber system (Figure 1a and b) was constructed using 6-inch radius metal rods and mosquito netting. Two chambers were used. The design was similar to that described by Muller *et al.*[8]

Figure 1a: A two-chamber system to establish the proof of concept that the GNT would trap mosquitoes.

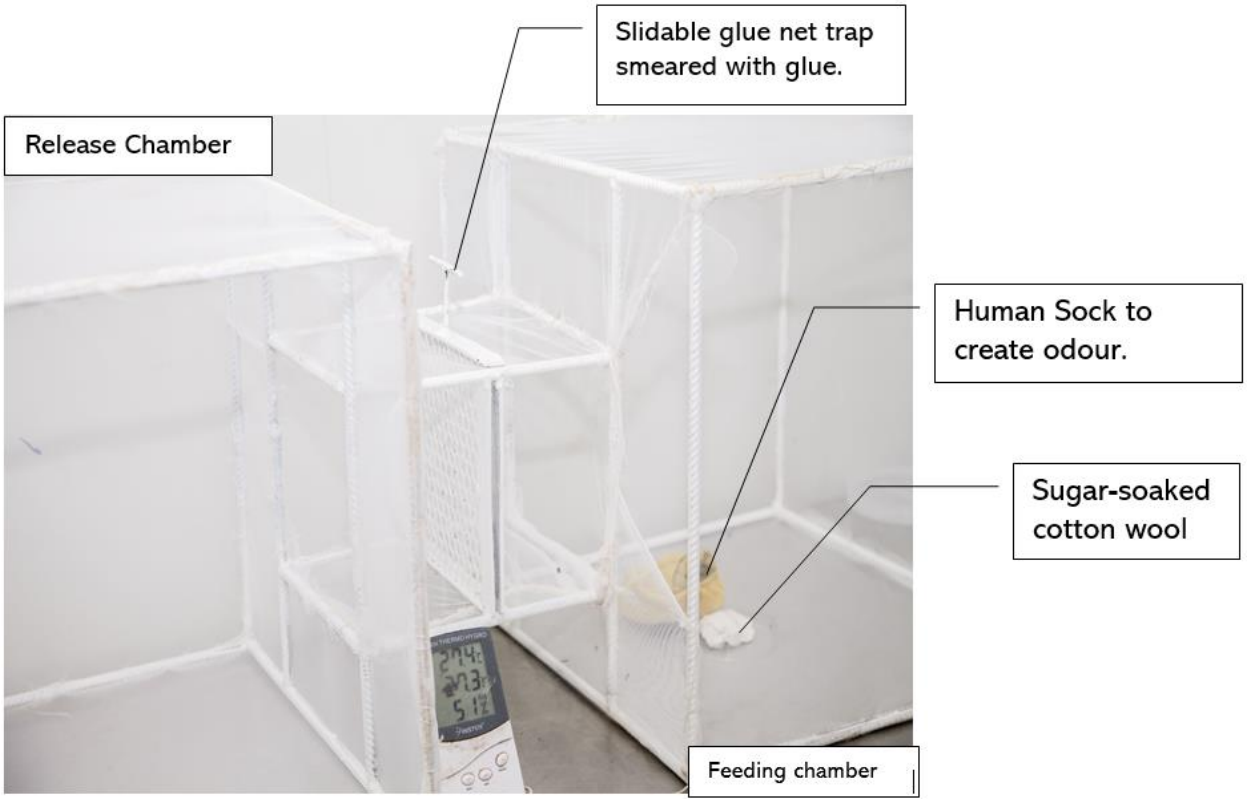

Figure 1b: A close of the two-chamber system to establish the proof of concept that the GNT would trap mosquitoes.

The cages comprised two chambers, the release chamber, and the feeding chamber. These were replicas of each other, connected by a square passage made of the same material and 220 mm in length, width, and height. In the middle of this connection (at about 110mm) from either side was a slidable piece of the garden fence used as a replica of the GNT (Figure 1b). The slidable screen was slightly smaller in length and width (140mm by 18mm) due to improvised grooves meant for easy sliding in and out. This piece of fence acted as a 'barrier' between mosquitoes trying to move between either chamber. During the trap experiments, the fence was smeared on the release chamber side with glue to trap mosquitoes trying to cross to the feeding chamber. The glue used was Tanglefoot® Glue, (Tanglefoot Company, Grand Rapids, MI, USA). During control experiments, no glue was smeared on the garden fence. However, the feeding chamber still contained the human sock and sugar solution.

Cage experiments were conducted using insectary-reared female *An. gambiae* Kisumu strain (available at the Zambia National Malaria Elimination insectary in Lusaka) using similar methods as described by Muller *et al.*[13] Between 25-30 female anopheline mosquitoes, 3-5 days old and fed only on sugar solution were placed in the "Release chamber". The release chamber contained only distilled water. The mosquitoes were released at 17:00 on each experimental day and allowed to acclimatise for an hour. At 18:00, a nylon or cotton sock worn in the previous 24 hours to create human-based odour[14] and sugar-soaked cotton wool solution (placed on a petri dish) were placed in the feeding chamber and left overnight in the dark. Trap experiments were replicated ten times, whilst control experiments were replicated five times.

In the morning, at 06:00 hrs, the number of mosquitoes caught on the sticky trap were counted and carefully removed. To determine "damage state" i.e., how damaged the individual mosquito was after removal, the following was noted on each mosquito; presence and number of wings, number of legs, presence of at least one hind leg, and the presence or absence of the palps. All four parts of the mosquito are key parts in the morphological identification of anopheline mosquitoes.[15]

Field Experiments

Field experiments were conducted in April and May 2020 (wet season) and August and September 2020 (dry season) in Nyimba district, located in the south-eastern part of Zambia (Figure 2). It is predominantly a rural area with an estimated population of more than 108 637 persons. Malaria transmission is perennial, mediated largely by *An. funestus* as a primary vector.[16] As in many parts of Zambia, malaria transmission peaks after the rainy season between March and May when water bodies become more stable allowing a proliferation of anopheline mosquito species.[17]

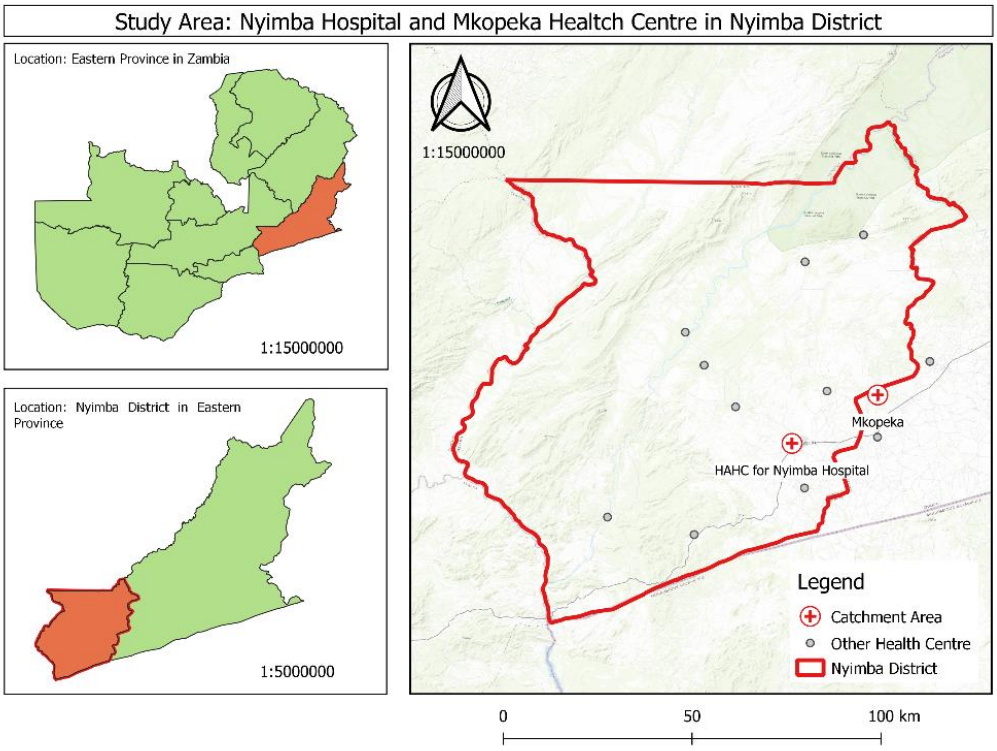

Figure 2: Map of Nyimba district, south-eastern Zambia showing the location of Mkopeka and Nyimba Urban catchment areas.

Two health zones or catchment areas within Nyimba were selected for this study; Mkopeka and Nyimba Urban (officially called Hospital Affiliated Centre for Nyimba or HAC for Nyimba but commonly referred to as Nyimba Urban). The two study areas have been described in greater detail elsewhere.[16]

For this study, three housing types were identified, namely traditional (grass thatched roof with mud walls), semi-modern (iron roof sheet and mud walls) and modern (iron roof with brick walls). The GNTs were constructed as described by Muller *et al.* and mounted on the windows and/or eaves of houses of the semi-modern houses and modern houses. It was not feasible to place the GNTs on grass thatched houses due to their having too many openings.[18] Experimental houses with many large openings had the larger spaces covered with dark linen.[8]

In the Mali experiments by Muller *et al.,* field experiments were conducted for a total period of six days.[8] The most mosquitoes were caught during the first three days. The fourth to sixth experimental days yielded negligible to no mosquitoes.[8] Based on that study, the GNT in this study was used only for three nights on a single house. A

GNT with the side facing the inside the house marked “EXIT” and the side facing outside marked “ENTRY”, was hung outside the possible entry points to trap any mosquitoes attempting to enter or exit the house during the night (Figure 3). The GNT were hung using a nail and where needed, strings. Each morning, the GNT was removed and trapped mosquitoes removed, counted, and stored. Mosquitoes trapped on the side marked “EXIT” would have been attempting to exit the house whilst those trapped on the side marked “ENTRY”, were caught while attempting to enter the houses. Each home had at least one person sleeping inside the house under an LLIN.

Figure 3a: Placing the GNT over a ventilation space in a room used for sleeping.

Figure 3b: A version of the GNT placed over a ventilation space.

Window Traps were designed, mounted and used as described by Govella *et al.*[4] but without the plywood (Figure 4). Spaces were covered using the flaps from the window traps. Like the GNT, WTs were set concurrently as entry traps or exit traps for three nights. Each home had at least one person sleeping inside the house under an LLIN.

Figure 4: A house showing window traps placed over the windows.

The evaluation of the GNT was a 3 × 3 Latin square design, performed over the malaria peak transmission season when malaria vectors are most abundant in April and May 2020 (wet season). In each catchment area, three villages were randomly selected for this experiment. In each selected village, two sets of three houses were randomly selected to form two distinct groups identified as an experimental block, each with a self-contained trio of numbered (1, 2 and 3) houses in which a Latin square rotation sequence was followed. Three treatments, namely GNT, Window Exit traps and Window Entry Traps were applied in periods of three experimental days. In the first treatment, houses identified as “House 1” had an GNT stuck on at least one of their windows or ventilation spaces. The second treatment had Window Exit Trap placed on all houses identified as “House 2” whilst houses identified as “House 3” had the third treatment, Window Entry Traps. The treatments were rotated on experimental day 4 and replicated for a further three days with another rotation occurring on experimental day 7, with a replication of another three days i.e., day 7-9. This was to ensure that all three experimental blocks receive the three treatments in the 12-day experimental period. This is illustrated below in Table 1.

Table 1: A 3 × 3 Latin square design and rotational design for the three mosquito sampling methods for one round cycle of mosquito collection in each study site in Nyimba district.

Experimental nights	**“House 1”**	**“House 2”**	**“House 3”**
Nights 1, 2, 3	GNT	WT-Exit	WT-Entry
Nights 4, 5, 6	WT-Exit	WT-Entry	GNT
Nights 7, 8, 9	WT-Entry	GNT	WT-Exit

GNT: Glue Net trap, WT: Window Trap

All adult mosquitoes caught in the above-mentioned methods were singly stored in Eppendorf tubes containing paper above the silica. These were morphologically identified using the morphological keys provided by Coetzee.[19] Damage state of all collected mosquitoes was noted as earlier described.

Mosquito larval sampling and rearing

Parallel to adult mosquito collections, immature mosquito sampling was conducted in breeding sites during the wet season (April and May 2020). Water bodies within 500m of the villages that were used for the longitudinal entomological surveillance[16] were sampled. Some village boundaries overlapped and as such, so did the water bodies.

Anopheline mosquito larvae and pupae were collected using dippers (Figure 5) as per World Health organisation recommendation.[20] In small habitats where dippers were not effective, larvae were collected individually using plastic pipettes and/or disposable plastic cups. At each site, collections were made for two days between 09:00h-12:00hr over a stretch of 100m in length.

Figure 5: Anopheline larval collections being conducted on Mtilizi River using dippers (Photo: Authors KS and MM)

For each site, we recorded; the Global positioning system (GPS) coordinates using a handheld device, type of breeding site (e.g., stream, pond, puddle, or hoof print), villages surrounding the breeding sites and/or distance to the closest village and the presence/absence of vegetation. For all sampled sites, visible presence of surface films and presence of algae was noted, and the surface area covered was estimated as a percentage.

All collected larvae and pupae were transported to the National Malaria Elimination Centre (NMEC) insectaries. Rearing of mosquito larvae and pupae to adults was conducted under standard insectary conditions with a temperature range of 26±2°C and 70-80% relative humidity. All larvae were fed on ground fish food. Morphological species identification of all anopheline mosquitoes collected at larval or pupa stage was conducted only at adult stage i.e. once they emerged.[15]

Insecticide resistance testing

Bioassays were conducted using WHO tube kits to assess susceptibility or resistance of the F1 adult mosquitoes that were raised from immature collections in the study site. To test for insecticide resistance (IR), emerged male mosquitoes of species were removed using a mouth aspirator and placed in separate paper cups covered by fine netting material and cotton wool. These were later discarded. Only females were used for IR testing.

We adapted our procedure from the WHO manual for testing for insecticide resistance.[12] Two insecticide-impregnated papers were used: organochlorine (4% DDT) and a pyrethroids (0.75% permethrin), obtained from a WHO-collaborating centre in Malaysia through WHO-Zambia office. The two insecticides were prioritized based on the long history of use of pyrethroids in Eastern province Zambia through IRS and LLINs.[7,21] Further, at the time of data collection, the Zambia National Malaria Elimination Program (NMEP) was considering reintroducing the use of DDT for IRS operations in some parts of Zambia (NMEC, pers. communication). This was as a measure of preventing the development of insecticide resistance to the organophosphate pirimiphos-methyl (Actellic™) which had been used since 2013.[21]

As of 2020, no insecticide resistance had been confirmed against organophosphates in malaria vectors in Zambia.

As per WHO-recommendation on insecticide resistance profiling, 3-5 days old female mosquitoes in batches of between 20-25 mosquitoes and in four to five replicates were used. In addition, two batches of 20-25 adult mosquitoes were exposed to untreated test papers which served as negative controls.[12]

Data analysis

The number of specimens caught in the sticky nets in the cage experiments were expressed as a percentage of the total number released. The mean trap rates were calculated as the total number of mosquitoes trapped divided over the period of experimentation. Despite the intention to use Analysis of Variance (ANOVA) for the field experiments, data analysis was not feasible due to extremely low catches as is evident in the results below.

For Insecticide resistance testing, the data were analysed using Microsoft Excel® (Microsoft Corporation, Redmond, WA) software. The prevalence of insecticide resistance in anopheline mosquitoes was defined as per the standard WHO protocol by calculating mortality rate percentage 24 hours post-exposure.

Results

Cage experiments

On the ten experimental days, cage experiments revealed an average trap rate of 88.9% (95% CI 88.8-90.0%) (Table 2). These results showed that the glue on the screen worked well. Additional file 1 is an image showing mosquitoes stuck on the GNT. No mosquitoes were trapped on the control screen. However, mosquito specimens from the GNT were always damaged, even when concerted efforts were applied to reduce the amount glue that was being applied on the Glue net trap. Of the 263 mosquitoes caught on the GNT all (100%) did not have legs, which got stuck on the glue trap. Similarly, 76.8% (n=202) of the mosquitoes did not have either wing while 62.7% (n=165) were without maxillary palps upon removal from the glue net.

Further, it was difficult to individually store mosquitoes due to the residual glue remaining on the carcasses.

Table 2: Trap rates on Glue Net Trap cage experiments.

Treatment	**Age of mosquitoes (days)**	**# of mosquitoes in release Chamber**	**# of mosquitoes trapped on screen after experiment ended**	**# of mosquitoes in release chamber after experiment**	% catch
GNT	3	25	21	3	84.0%
GNT	5	32	28	4	87.5%
GNT	5	29	26	3	89.7%
GNT	5	30	26	4	86.7%
GNT	5	30	30	0	100.0%
GNT	5	30	30	0	100.0%
GNT	5	30	20	10	66.7%
GNT	3	30	28	2	93.3%
GNT	3	30	25	5	83.3%
GNT	3	30	29	1	96.7%
Control (no glue)	3	30	0	15	0.0%
Control (no glue)	3	30	0	20	0.0%
Control (no glue)	3	30	0	16	0.0%
Control (no glue)	5	30	0	2	0.0%
Control (no glue)	5	30	0	2	0.0%

Field experiments

Window traps

Field experiments with WTs did not yield results. Despite a combined collection effort of 27 trap-nights in April, May, and June (wet season) and 18 trap nights in Sept and October 2020 (dry season), the window exit/entry traps did not trap any mosquitoes. Despite our best efforts, at times switching houses and restarting the experiment, and changing the type of GNT, not a single mosquito was caught with the window entry and exit traps.

Glue Net traps

From all collections' efforts, only five mosquitoes were caught on the GNT. Morphological identifications however, proved a challenge. Like the cage experiments, vital body parts like wings, legs and maxillary palps were missing from the mosquito specimens. As such, it was not possible to entirely tell which species these were. The GNT, however, did catch other non-targeted organisms, such as butterflies and moths (Lepidoptera) mayflies (Ephemeroptera), true bugs (Hemiptera) and the common house fly (Diptera).

Larval collections

Mosquito larvae was collected at two main water bodies in Mkopeka catchment area only. All water bodies surveyed from Nyimba Urban did not have any anopheline larvae. Larval collections were made at Mtilizi river and its tributary the Mukombwe stream which were in proximity. Please see additional file 2 a for a summary of the habitat characteristics of the sampled sites.

Throughout the study period, both in terms of distribution and abundance, the anopheline population from the combined larval collections from Mtilizi river and the Mukombwe stream was dominated by the potential secondary vector *Anopheles pretoriensis* (n=392; 89.7%). Other species were *Anopheles rufipes* (n=42; 9.6%), and *Anopheles coustani* (n=3; <1%). Also collected was A*nopheles gambiae* s.l. (n=8; 1.8%). Males of the species were also collected but not necessarily counted as they are not important for IR testing.[12] There was no difference in species composition between male and female anopheline mosquitoes.

Insecticide susceptibility tests

Insecticide resistance (IR) tests were performed only on *An. pretoriensis* as only this species had the sufficient numbers for insecticide resistance testing according to WHO recommendation.[12] Results are shown in figure 6.

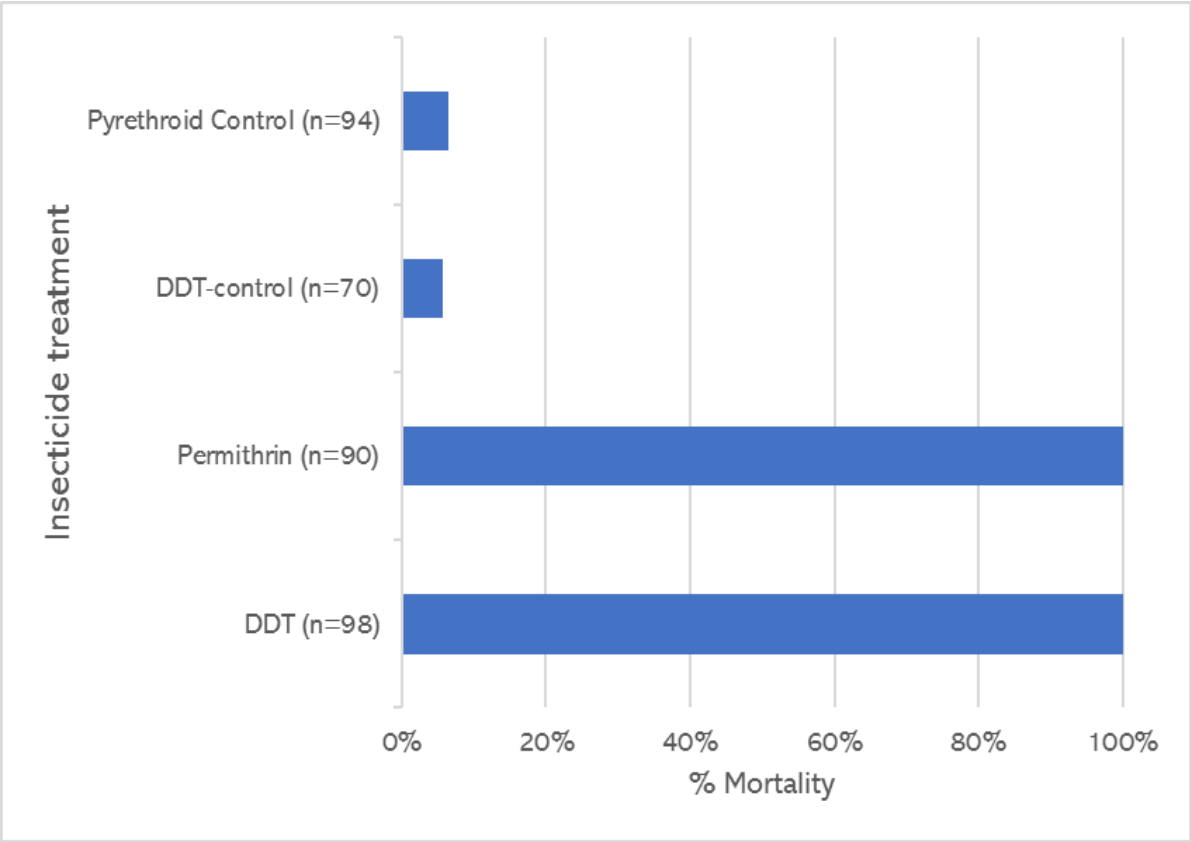

Figure 6: Percentage mortality of *An. pretoriensis* 24-hours post-exposure to two different insecticide classes. Numbers in parenthesis show the total number of mosquitoes tested.

Based on the WHO criteria of 2016[12], the 100% mortality rates of *An. pretoriensis* confirmed that this species remains susceptible to insecticides DDT (organochloride) and permethrin (pyrethroid). Mortality rates in controls was low (>10%) confirming that mortality in treatment tubes was induced using insecticide impregnated paper.

Discussion

Results of this study provide evidence that during this study (1) GNT and WET may not be effective mosquito sampling tools in Zambia's rural settings and (2) *An. pretoriensis* remains susceptible to organochlorines and pyrethroids.

The GNT may not be an effective mosquito sampling tool in Zambia's rural areas. Negligible number of mosquitoes were caught using this method in field surveys on rural houses in rural southeastern Zambia. The results are similar to those of Liao[9] in Kenya who caught no mosquitoes using a modified version of the GNT. The weak performance of the GNT may be attributed to the following reasons. First, use of the GNT requires placing the trap over a window or ventilation space. To increase chances of the mosquito actually using the window, other exit points such as eaves and ventilation spaces may need to be closed with preferably dark cloth.[8] In this study, some household owners removed the cloth due to reduced ventilation and light, thereby making both window traps and the glue net trap less effective to collect mosquitoes. Second, mosquitoes removed from the GNT, both during the cage experiments and field collections, were in damaged state. With vital parts like wings, legs maxillary palps missing, this made morphological identifications impossible.[15] The findings of this study differ from those of Muller *et al.,*[8] who make no mention of damaged specimens. Yalla *et al.*[10] also used GNT in a semi-field study settings for release-capture experiment using a known species, *An. gambiae* Kisumu strain. As such damaged specimens may not have been of importance in that study. Third, it may be possible that that anthropophilic mosquitoes such as *An. funestus* and *An. gambiae* may utilise the eaves more than windows to enter human dwellings.[22-23] A fourth reason is that GNT could not be used on traditional grass thatched houses due to the many large spaces attributed to the architecture of the building. Entomological monitoring needs to representative of all forms of housing structures found in a study area.[4] This ensures equity in research and that all at risk housing structures are represented.

No mosquitoes were collected by window entry or, when reversed, exit traps. Our findings are similar to those of Mpofu[24] in Botswana, Sikaala *et al.*[7] in eastern Zambia and Govella *et al.*[4] in Tanzania, who caught negligible absolute numbers of

mosquitoes using this sampling method. Similar to the observations of Sikaala *et al.*[7] and Govella *et al.,*[4] houses in the two study areas had highly variable entry and exit points. Thus, fitting WT on homes proved difficult and laborious.[7-8] It is also possible that mosquitoes initially trapped may have escaped during the removal using a mouth aspirator.[4] Based on the findings of this study, WT are not recommended as entomological sampling tools for rural Zambia. To improve collections using this method, we recommend lining the WT internally with sticky surface.[25] Further, based on a parallel longitudinal study conducted in the study area we recommend that Centre for Disease Light Traps (CDC-LTs) and knock-down spray collections as sampling tools for endophagic and endophilic mosquitoes.[16]

This paper reports largely discouraging results from this evaluation, where an initial hypothesis was not verified. Indeed, only an estimated 19% researchers publish negative data.[26] To the best of our knowledge, this is the first time that the glue net trap has been evaluated as a mosquito sampling tool on houses. Thus, reporting negative results may help improve the methodology of similar evaluations.[27] Based on these reported findings, Zambian entomological researchers may avoid evaluations with WTs and GNT altogether, thereby saving money, time, and resources.[4,27-28]

Anopheles pretoriensis, a potential secondary vector,[29] dominated mosquitoes from larval collections. Similar findings of secondary vectors dominating over primary vectors in larval collections have been recorded elsewhere in Zambia[30] and Kenya.[31] This may be indicative of larval habitats preferred by *An. pretoriensis* and other secondary vectors but not of the primary vectors *An. funestus s.s* and *An. gambiae s.l*. *Anopheles gambiae* prefer temporary sunlit pools whilst *An. funestus* prefer large permanent or semi-permanent body of fresh water, usually with emergent vegetation such as swamps and lake edges.[32] The absence of such larval habitats in the study area provides a plausible explanation of the absence of the primary malaria vectors in this study. Knowledge of the presence or absence of larval habitats with specific species may be useful for targeted larviciding.[30]

Findings from this study show that populations of *An. pretoriensis* are susceptible to DDT and pyrethroids. These findings are similar to those described in Kenya where the secondary vectors *Anopheles pharoensis* and *An. coustani* remained susceptible to commonly used insecticides.[31] This may be expected as the majority secondary vectors are exophilic and exophagic hence have minimal contact with insecticides used on nets and/or sprayed on walls during IRS.[2,31,33] However, secondary vectors are not immune to the selective pressure caused by exposure to insecticides used in agriculture.[34] Insecticides used in malaria vector control and agriculture share targets sites and modes of action.[34-35] A case in point is seen in *Anopheles rufipes* in Cameroon. Although this species is largely zoophagic and exophagic, Awono-Ambene *et al.*[36] found different populations of this species resistant to the pyrethroid deltamethrin. This was attributed in part to the high use of pesticides for agricultural purposes in the study area.[36] Whilst the present findings reveal no resistance to DDT and the pyrethroid permethrin, this study advocates for an all-inclusive entomological and insecticide resistance monitoring. Most national malaria programs are biased towards the major or primary vectors.[2] However, if elimination of malaria is to be achieved, all-encompassing knowledge on vector populations, diversity, biology, insecticide susceptibility and genetic structure may be needed to implement vector control measures in areas of both high and low malaria transmission.[2,16,30]

There are some limitations to this study. Insecticide resistance tests were limited to a secondary vector, namely *An. pretoriensis.* In the absence of primary vectors from larval collections, the ideal would-be collection of resting blood-fed mosquitoes inside people's homes through mechanical or mouth aspirations for future propagation.[37] However, due to the Covid-19 pandemic at the time of data collection, safety precautions including social and physical distancing,[38] limited our entrance into people's homes for this exercise. Larval collections were thus a safe alternative.

Conclusion

Findings of this study show that the GNT and WETs may not be effective sampling tools to study the house entry and exit behaviour of malaria vector mosquitoes in rural Zambia. Whilst the GNT is inexpensive to assemble, transport and easy to fix on the windows or ventilation spaces of rural houses, many rural houses have other

large openings. This makes the GNT and the WTs inefficient. It is also more likely that mosquitoes caught on the GNT will be damaged and difficult to store and/or morphologically identify. Findings of this study also show that *An. pretoriensis* remains susceptible to pyrethroids and the organochloride, DDT. We recommend that national malaria programmes extend insecticide resistance monitoring to secondary vectors especially when these are found in more abundance than the primary vectors.

Ethical considerations

The study protocol and informed consent forms were reviewed and approved by the ERES Converges IRB Zambia (Reference: 2018-Oct-007 and 2020-Jul-018), the National Research Health Authority (Ref: NHRA00002/23/04/2021) and the research ethics committee of the University of Pretoria (Ref: 242/2020). Written permission to undertake the study was obtained from the Ministry of Health through the National Malaria Elimination Centre (NMEC) and Nyimba District Medical office. Local and traditional leadership were also informed about the purposes of the study. Participation in the study was voluntary, and informed consent was obtained from household heads and every participant above the age of 18 years.

Data availability

Saili, Kochelani et al. (2023). Percentage mortality of Anopheles pretoriensis 24 hours post-exposure to two different insecticide classes. figshare. Figure. ***https://doi.org/10.6084/m9.figshare.24457603.v1***

Saili, Kochelani et al. (2023). Additional file 1 Anopheles mosquitoes stuck on Glue Net Trap.jpg. figshare. Figure. *https://doi.org/10.6084/m9.figshare.24457318.v2*

Saili, Kochelani et al. (2023). Habitat characteristics of two mosquito breeding sites in Mkopeka, Nyimba district, Zambia. figshare. Dataset. *https://doi.org/10.6084/m9.figshare.24457522.v1*

Competing interests

The authors declare no competing interests.

Grant information

The authors gratefully acknowledge the financial support for this research by the following organizations and agencies: the Global Environmental Fund (GEF) through United Nations Environmental Programme (UNEP) and the WHO-Africa Regional Office (WHO-AFRO) (Grant number: 4668); the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Australian Centre for International Agricultural Research (ACIAR); the Norwegian Agency for Development Cooperation (Norad); the Federal Democratic Republic of Ethiopia; and the Government of the Republic of Kenya. The views expressed herein do not necessarily reflect the official opinion of the donors. Kochelani Saili was supported by a German Academic Exchange Service (DAAD) In-Region Postgraduate Scholarship and a partial University of Pretoria doctoral bursary. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

The authors are grateful to the communities in Nyimba Urban and Mkopeka in whose households collections were made. We thank the Ministry of Health through the National Malaria Elimination Centre (NMEC), Nyimba District Medical Office and traditional leadership in Nyimba Urban and Mkopeka for their co-operation and support. We thank Willard Mutoka Jr for his help with the map. We thank Dr Ulrike Fillinger for sharing the original idea to evaluate the Glue Net trap for monitoring entry and exit of mosquitoes on houses.

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Additional file 1: *Anopheles gambiae* (Kisumu strain) stuck on the glue net trap during cage experiments.

Additional file 2: Habitat characteristics of mosquito breeding in Mkopeka, Nyimba district.

Location	**Mtilizi River**	**Mukombwe stream**
GPS coordinates	-14° 30'19.5"S, 30° 57' 38.9"E	-14°28'17.4"S 30°59'50.2"E
Village/s surrounding the sites	Masanchi	Vizimumba and Kapotwe
Distance to nearest village	<1km	~500m
Habitat type	Both large and small ponds of water after drying up of river	Small stream
Relative depth	Deep stagnant ponds (~30cm) to very shallow (~5cm)	Shallow running waters (<10cm)
PH	7.8	8
Presence of vegetation	No	No
Surface films (approximate % covered)	Yes (30)	Yes (30)
Presence of algae (approximate % covered)	Yes (50)	Yes (75)

Chapter 5: House Screening Reduces Exposure to Indoor Host-seeking and Biting Malaria Vectors: Evidence from Rural South-East Zambia

The study phase presented in this chapter was conducted after the baseline information, which was presented in chapters 3 and 4, was collected. Chapter 5 thus addresses objective 3 highlighted at the beginning of this thesis, specifically, to evaluate the impact of combining house screens with long-lasting insecticidal nets (LLINs) on mosquito host-seeking, resting, and biting behavior.

Before implementing the house screening intervention, all households in the two neighbouring health facility catchment areas, Nyimba Urban and Mkopeka, were mapped, and household lists were generated. From this list, 800 households were randomly selected, and each provided with at least one LLIN per two persons. Half of the households were then assigned to the treatment arm (n=400, LLINs and house screening) and the other half to the control arm (n=400, LLINs only). Centre for Disease Control light traps (CDC-LTs) and pyrethrum spray collections (PSC) were used to assess the densities of indoor/outdoor host-seeking and indoor resting of malaria vectors in 15 sentinel houses per intervention group per sampling method. Species-specific biting behavior and host-searching times were determined using paired indoor and outdoor human landing catches (HLCs) in the three villages in Mkopeka catchment area. HLC collections took place during two periods: the wet season and the dry season. All collected *Anopheles* mosquitoes were morphologically identified using dichotomous keys. Sporozoite infectivity was determined for *Anopheles* mosquitoes using sandwich enzyme-linked immunosorbent assays. The protective efficacy of house screening was estimated using entomological inoculation rates (EIRs).

There were 68% fewer indoor host-seeking *Anopheles funestus* (RR = 0.32, 95% CI 0.20–0.51, $p < 0.05$) and 63% fewer *An. arabiensis* (RR = 0.37, 95% CI 0.22–0.61, $p < 0.05$) in screened houses than in unscreened houses. There was a significantly higher indoor biting rate for unscreened houses (6.75 bites/person/h [b/p/h]) than for screened houses (0 b/p/h) ($\chi^2 = 6.67$, df = 1, $p < 0.05$). The estimated indoor EIR in unscreened houses was 2.91 infectious bites/person/six months, higher than that in

screened houses (1.88 infectious bites/person/six months). Closing eaves and screening doors and windows has the potential to reduce indoor densities of malaria vectors and malaria transmission.

The findings of this study were published in *MDPI Tropical Medicine and Infectious Diseases*. The title of the manuscript is *"House screening reduces exposure to indoor host-seeking and biting malaria vectors: Evidence from rural south-east Zambia"*. The findings of this study were also presented at the 1st National symposium for the Entomological Society of Zambia, held at the University of Zambia, School of Veterinary Sciences in December 2022 (in-person oral presentation) and the 8th Annual Southern African Malaria Conference, organised by the South African Malaria Research Council in Pretoria, South Africa (in-person oral presentation) in August 2023.

Tropical Medicine and Infectious Disease

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Article

House Screening Reduces Exposure to Indoor Host-Seeking and Biting Malaria Vectors: Evidence from Rural South-East Zambia

Kochelani Saili [1,2,*], Christiaan de Jager [2], Freddie Masaninga [3], Onyango P. Sangoro [1], Theresia E. Nkya [1,4], Likulunga Emmanuel Likulunga [5], Jacob Chirwa [6], Busiku Hamainza [6], Emmanuel Chanda [7], Ulrike Fillinger [1] and Clifford Maina Mutero [1,2]

[1] International Centre of Insect Physiology and Ecology (icipe), Nairobi P.O. Box 30772-00100, Kenya; psangoro@icipe.org (O.P.S.); theresia.nkya@gmail.com (T.E.N.); ufillinger@gmail.com (U.F.); cmutero@icipe.org (C.M.M.)
[2] University of Pretoria Institute for Sustainable Malaria Control, School of Health Systems & Public Health, University of Pretoria, Pretoria 0028, South Africa; tiaan.dejager@up.ac.za
[3] Country Office, World Health Organization, P.O. Box 32346, Lusaka 10101, Zambia; masaningaf@who.int
[4] Mbeya College of Health and Allied Sciences, University of Dar es Salaam, Mbeya 35063, Tanzania
[5] Department of Biological Sciences, University of Zambia, Great East Road Campus, P.O. Box 32379, Lusaka 10101, Zambia; likulungae@yahoo.com
[6] National Malaria Elimination Centre, P.O. Box 32509, Lusaka 10101, Zambia; chirwa.jack@gmail.com (J.C.); bossbusk@gmail.com (B.H.)
[7] WHO Regional Office for Africa, Cite du Djoue, Brazzaville P.O. Box 06, Congo
* Correspondence: ksaili@icipe.org

Abstract: This study evaluated the impact of combining house screens with long-lasting insecticidal nets (LLINs) on mosquito host-seeking, resting, and biting behavior. Intervention houses received house screens and LLINs, while control houses received only LLINs. Centre for Disease Control light traps, pyrethrum spray collections and human landing catches were used to assess the densities of indoor and outdoor host-seeking, indoor resting, and biting behavior of malaria vectors in 15 sentinel houses per study arm per sampling method. The protective efficacy of screens and LLINs was estimated through entomological inoculation rates (EIRs). There were 68% fewer indoor host-seeking *Anopheles funestus* (RR = 0.32, 95% CI 0.20–0.51, $p < 0.05$) and 63% fewer *An. arabiensis* (RR = 0.37, 95% CI 0.22–0.61, $p < 0.05$) in screened houses than unscreened houses. There was a significantly higher indoor biting rate for unscreened houses (6.75 bites/person/h [b/p/h]) than for screened houses (0 b/p/h) ($\chi^2 = 6.67$, df = 1, $p < 0.05$). The estimated indoor EIR in unscreened houses was 2.91 infectious bites/person/six months, higher than that in screened houses (1.88 infectious bites/person/six months). Closing eaves and screening doors and windows has the potential to reduce indoor densities of malaria vectors and malaria transmission.

Keywords: *Anopheles* mosquitoes; eaves; entomological inoculation rate; sporozoite infectivity rate

Citation: Saili, K.; de Jager, C.; Masaninga, F.; Sangoro, O.P.; Nkya, T.E.; Likulunga, L.E.; Chirwa, J.; Hamainza, B.; Chanda, E.; Fillinger, U.; et al. House Screening Reduces Exposure to Indoor Host-Seeking and Biting Malaria Vectors: Evidence from Rural South-East Zambia. *Trop. Med. Infect. Dis.* **2024**, *9*, 20. https://doi.org/10.3390/tropicalmed9010020

Academic Editor: Arthur Sovi

Received: 22 November 2023
Revised: 31 December 2023
Accepted: 9 January 2024
Published: 15 January 2024

1. Introduction

Malaria is endemic throughout Zambia. In 2021, Zambia's malaria burden was estimated at 7,050,968 cases, with an incidence rate of 340 cases per thousand per year [1]. The prevalence in children under the age of five years, based on malaria rapid diagnostic tests (RDTs), was found to be 29%, much higher than that recorded in 2018 (9%) [2]. While this increase may reflect the impact of COVID-19 on malaria service delivery [3], it may also indicate a need for additional vector-control methods other than long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) [4,5].

The principal vector mosquito species of human malaria, *An. funestus*, *An. gambiae s.s.*, and in some cases, *An. arabiensis*, have a strong preference for feeding on people and resting inside houses [6]. These species are well adapted for entering traditional rural houses using the gaps between walls and roofs (eaves) and may also use open windows and doors

to access indoor spaces and blood hosts [7,8]. These behavioral characteristics increase human-vector contact, making these mosquito species efficient malaria vectors [6,9]. More than 80% of human exposure to malaria vectors in sub-Saharan Africa (SSA) is estimated to occur indoors [10]. In southeastern Zambia and Tanzania, approximately 78% of all malaria transmission is estimated to occur indoors [11,12]. Thus, modifying houses to reduce mosquito entry can potentially reduce malaria transmission and provide additional health benefits [5,13]. Such modifications or house improvements include closing eave gaps and screening windows and doors [5,7].

House screening using non-insecticide-treated screens (wire mesh or mosquito netting) as physical barriers on windows and eaves have shown significant protection against malaria [7,14–17], dengue [18,19], and lymphatic filariasis [20,21]. Despite well-established benefits, house screening has not been encouraged on a large scale by national malaria programs and remains neglected by public health policy. Generating evidence showing the benefits of house screening on vector densities, host-seeking, and biting behavior in specific local settings, particularly under program implementation settings, is thus important. This study evaluated the additive impact of combining house screens with LLINs on mosquito densities and host-seeking and resting behavior. We further evaluated the impact of combining house screens with LLINs on sporozoite infectivity and entomological inoculation rates (EIRs) as a proxy measure of malaria transmission.

2. Materials and Methods

2.1. Study Area

This study was conducted in Nyimba district, located in the Eastern province of Zambia (4°21′0″ S; 30°35′0″ E) (Figure 1). Two neighboring health facility catchment areas were selected for this study: Mkopeka and Nyimba Urban. The study area has been described in detail as part of an entomological baseline study [22] and elsewhere [23,24].

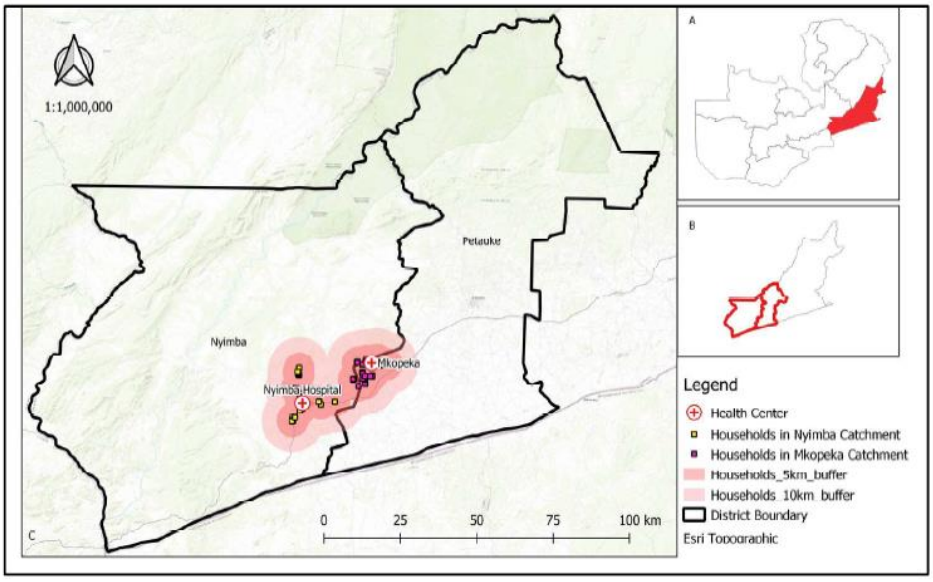

Figure 1. Study area in Nyimba District, Zambia. Insert: (**A**). Map of Zambia showing the location of Nyimba district. (**B**). Location of Nyimba in Eastern province. (**C**). Location of households that were used for entomological collections.

2.2. Study Design

This study was a cluster randomized controlled trial using a generalized randomized block design, with the village as the block. This study was part of a larger community-based house screening trial, and the protocol has been reported previously [24]. A total of 89 villages were included in the main study [23].

2.3. Community Sensitization and Consent to Participate

Community sensitization meetings with community leaders were held before screens were installed in houses. The chiefs and village headmen were informed about the purposes of the study. Before the installation of screens, voluntary informed verbal consent was obtained.

2.4. Study Households, Enumeration, and Participants

Before sampling and implementing interventions, all households in the two neighboring health facility catchment areas, Nyimba Urban and Mkopeka, were mapped, and household lists were generated. Nyimba Urban is the peri-urban region of the district and is relatively close to the central district administration offices, while Mkopeka is a largely rural region. From the list of households, 800 eligible households were randomly selected. The following inclusion criteria were used: (i) at least two children with ages ranging between 6 months and 13 years; (ii) the house should be semi-modern, defined as a house with a roof made of corrugated iron sheets and with walls that were either mud or fire-burnt bricks (Figure 2); and (iii) houses should not have already had screens.

Figure 2. A semi-modern house with firebrick walls and a metal roof showing screened windows and ventilation spaces.

The 800 households were then randomly assigned to the treatment arm (400 houses to receive screens) or the control arm (400 houses), stratified according to region. From March to April 2019, all 800 houses were provided with at least one LLIN per two persons to ensure optimum coverage of at least one of the primary vector-control interventions as per national guidelines [1]. During the entire study period, no IRS was conducted in the two catchment areas. Routine LLIN distribution continued throughout the study period.

2.5. Installation of House Screens

House screens, specifically doors, windows, and ventilation spaces (Figure 2) were installed between December 2019 and January 2020. From the list of houses, screens were installed in 400 randomly selected houses. The remaining 400 houses served as controls. Each catchment area was divided into two zones made up of villages closer to each other. Each zone contained approximately 200 households.

2.6. Adult Mosquito Collections

Adult mosquitoes were collected using three different sampling methods: indoor and outdoor Centre for Disease Control ultraviolet light traps (CDC-LTs, Model 512, John W Hock, Gainesville, FL, USA), pyrethrum spray catches (PSCs), and human landing catches (HLCs). Mosquito collections took place after the screens were installed. Mosquitoes were collected in only 20 villages spread across the two study areas. Different sampling methods were used to account for different behaviors.

2.6.1. Light Traps

For each study arm, 15 houses were randomly selected to serve as sentinel houses. Houses were replaced when either consent was withdrawn, or the sleeping structure was destroyed. In that case, the nearest neighbor was used.

On the night of collection, two CDC-LTs per house were deployed: one inside and another outside. The CDC light traps were set from 18:00 to 6:00. Indoors, the CDC-LT was suspended 1.5 m above the floor and approximately 1.5 m away from the feet of a consenting adult sleeping under an LLIN. For outdoor collections, the CDC-LT was hung nearest to where the family would sit to eat and/or spend evenings. Both indoor and outdoor CDC-LTs were not baited. These collections took place once every month between February 2020 and June 2020 and between December 2020 and June 2021, representing 12 collection months.

2.6.2. Indoor Resting Collections

PSCs were conducted using Mortein Energy ball® (Reckitt Benckiser, Alberton, South Africa) as a knockdown spray [25]. PSCs were performed once a month in a second set of 15 sentinel houses randomly selected, eight houses in Mkopeka and seven from Nyimba Urban. Indoor resting collections took place from February 2020 to June 2021, representing 17 collection months. During both CDC-LT and PSC collections, housing characteristics, such as open eaves and the type of material used for wall and floor construction, were recorded.

2.6.3. Human Landing Catches

Species-specific biting behavior and host-searching times were determined using paired indoor and outdoor HLCs in the three villages with the highest indoor mosquito densities, based on PSCs, in the Mkopeka catchment area. Collections took place during two periods: the wet season (April and May 2020) and the dry season (September and October 2020). In each month and from within the three villages, six houses were randomly selected: three control households and three intervention houses. HLCs were conducted for 5 nights, giving an overall of 30 nights per season per study arm. No HLCs were conducted between April and May 2021 due to COVID-19 restrictions in line with the COVID-19 national guidelines of Zambia's Ministry of Health.

Male volunteers were recruited from a pool of CHWs who had participated in community-based entomological surveillance during the baseline study [22], prior to the intervention installation. All volunteers underwent a 5-day training in basic entomological surveillance including practical sessions on HLCs.

To conduct HLCs, pairs of male volunteers, one indoors and the other outdoors (at least 2 m away from the house), sat with their legs exposed to attract mosquitoes. As mosquitoes attempted to bite, they were collected with a mouth aspirator. Indoor

and outdoor collections were conducted between 18:00 and 06:00 in houses occupied by a consenting adult male member of the household sleeping under a mosquito net. Mosquitoes were caught for 45 min each h, allowing a 15 min break.

2.7. Species Composition

All collected *Anopheles* mosquitoes were morphologically identified using dichotomous keys [26]. Culicine mosquitoes were only identified at the subfamily level. Members of the *An. gambiae* complex (n = 100) and *An. funestus* group (n = 141) were further identified at the sibling species level by polymerase chain reaction (PCR) [27,28].

2.8. Detection of Plasmodium falciparum Infection in Mosquitoes

Sporozoite infectivity was determined for *Anopheles* mosquitoes using sandwich enzyme-linked immunosorbent assays [29,30]. Based on the number available, randomly picked anopheline mosquitoes of the following species were tested for *P. falciparum* circumsporozoite proteins (*Pf* CSPs): *An. funestus* (n = 162), *An. gambiae s.l.* (n = 118), *An. pretoriensis* (n = 109), *An. rufipes* (n = 112), *An. maculipalpis* (n = 47), *An. gibbinsi* (n = 18), and *An. coustani* (n = 2). We heated the ELISA lysates to avoid false CSP positives common in zoophilic species [31].

2.9. Data Analysis

All data were analyzed in R version 4.1.0 software [32]. A generalized linear mixed model (GLMM) using the template model builder *(glmmTMB)* package was used to investigate the impact of house screening on indoor and outdoor malaria vector densities. A GLMM was fitted assuming a negative binomial distribution, and "floor type" and "wall type" were selected as random effects and predictor variables as fixed effects. *p* values were derived for each model.

The mean densities of mosquitoes were estimated by dividing the total number of mosquitoes collected by the total number of trapping nights per household. The risk ratio (RR) was used to estimate effect sizes associated with the differences in mosquito densities between screened and unscreened houses. The log risk ratios were transformed into risk ratios (RRs) using "predict" in the R "metafor" package. The modeled percent reduction in mosquito densities in screened houses compared to unscreened houses was calculated as $100 \times (1 - \text{RR})$. All analyses were species-specific. Anopheline mosquitoes were collected in low numbers and pooled for analysis.

To further determine the protective efficacy of the house-screening intervention, the following entomological indices were used: Human biting rate (HBR), defined as the mean number of bites per person per night by a vector species collected either indoors or outdoors. Indoor and outdoor species-specific hourly human biting rates (HBR) were calculated from HLCs. As HLCs were conducted for 45 min within each hour, average bites by mosquitoes were further divided by 0.75 (=45/60 min) to obtain the hourly catch rate. Furthermore, hourly biting rates were categorized into periods as evening (18:00 to 20:45), early night (21:00 to 23:45), midnight (00:00 to 02:45), and early morning (03:00 to 05:45).

The sporozoite infectivity rate (SIR) is defined as the proportion of *Anopheles* mosquitoes with sporozoites in their salivary glands relative to the total number of mosquitoes examined for sporozoites.

To determine the protective efficacy of the house-screening intervention, EIR was used as a measure of malaria transmission. EIR is defined as the number of infectious bites per person per unit of time, usually expressed per year or month.

Due to few mosquitoes being collected by HLCs, species-specific EIR was calculated by multiplying HBR obtained from CDC-LTs ($HBR_{CDC\text{-}LT}$) by the SIR. Species-specific HBR from CDC-LTs was calculated as the mean number of female *Anopheles* mosquitoes caught per trap/night without adjusting for room occupancy. Since the CDC-LTs were set only during the wet season (February 2020 to June 2020 and again

December 2020 to June 2021) EIR was estimated for the wet season and for an average of six months only.

3. Results

Overall, in both the intervention and control houses, we conducted 362 indoor and 287 outdoor CDC-LT collections, 473 resting collections, and 60 HLC collection nights. Less frequent outdoor trap nights for CDC-LTs were due to reduced sampling during the rainy season when heavy rains would interfere with trapping.

3.1. Anopheles Species Composition

Overall, using both indoor and outdoor collection methods, a total of 1,972 female anopheline mosquitoes were collected. There was a similar species composition in the two study arms. Nine species were identified based on morphological features: *Anopheles pretoriensis* (31.6%; n = 634), *An. funestus* group (n = 393; 19.9%), *Anopheles maculipalpis* (n = 329; 16.7%), *Anopheles rufipes* (n = 253; 12.8%), *Anopheles gambiae* s.l. (n = 232; 11.8%), *Anopheles coustani* s.l. (n = 68; 3.4%), *Anopheles gibbinsi* (n = 53; 2.7%), *Anopheles squamosus* (n = 13; 0.7%), and *Anopheles. tenebrosus* (n = 7; 0.4%). Additionally, males of the following species were collected: *An. pretoriensis* (n = 13), *An. funestus* (n = 6), *An. rufipes* (n = 5), *An. maculipalpis* (n = 2), and *An. gambiae* s.l. (n = 2). All species, except *An. tenobrosus,* were found in both unscreened houses and screened houses. *An. tenebrosus* was found only in unscreened houses. A total of 644 female culicine mosquitoes were collected.

PCR was performed on a random subsample of 141 (35.9%) collected female *An. funestus* mosquitoes, of which 21 (14.9%) did not amplify. Of the specimens that amplified (n = 120), *An. funestus* s.s. was the dominant species (n = 110; 91.7%). Other species within this group were *Anopheles parensis* (n = 6), *Anopheles leesoni* (n = 2), and *Anopheles rivolurum-like* (n = 2). As *An. funestus* s.s. is the dominant species within this taxon, the *An. funestus* group is henceforth referred to simply as *An. funestus.*

PCR was performed on a random subsample of 100 (43.1%) collected female *An. gambiae* s.l. mosquitoes, of which 15 did not amplify and four gave nonspecific amplifications when further analyzed by ITS2-PCR (n = 2, 500 base pairs; n = 1, 520 bp; n = 1, 600 bp). Of the 81 specimens that were successfully amplified, *An. arabiensis* was the dominant sibling species within the *An. gambiae* complex (n = 61; 75.3%). *Anopheles quadriannulatus* (n = 15) and *An. gambiae s.s.* (n = 5) were the two other species within this complex. As *An. arabiensis* is the dominant species within this complex, *An. gambiae* s.l. is subsequently referred to as *An. arabiensis* throughout this manuscript.

3.2. Impact of House Screening on Mosquito Densities

3.2.1. Indoor Host-Seeking

Overall, closing eaves and screening windows and doors significantly reduced the indoor host-seeking densities of *Anopheles* and culicine mosquitoes over two malaria transmission seasons. Based on modeled estimates, overall, there were 44% fewer mosquitoes in screened houses (RR = 0.56, 95% CI 0.43–073, $p < 0.05$) than in unscreened houses. There were 68% fewer *An. funestus* (RR = 0.32, 95% CI 0.20–0.51, $p < 0.05$), 63% fewer *An. arabiensis* (RR = 0.37, 95% CI 0.22–0.61, $p < 0.05$), and 37% fewer *An. pretoriensis* (RR = 0.63, 95% CI 0.46–0.87, $p < 0.05$). Further significant reductions were observed in the indoor host-seeking densities of *An. rufipes* (RR = 0.61, 95% CI 0.40–0.92, $p < 0.05$), albeit with a small effect size (Figure 3). The densities of culicines were lower in screened houses than in unscreened houses (RR = 0.53, 95% CI 0.41–0.69, $p > 0.05$), although not significantly (Figure 3). No significant reductions ($p > 0.05$) were observed due to screening and closing eaves in the species *An. coustani, An. gibbinsi*, *An. squamosus*, and *An. tenebrosus*, likely due to small sample sizes. Table 1 shows the species-specific mean densities in the control and intervention houses.

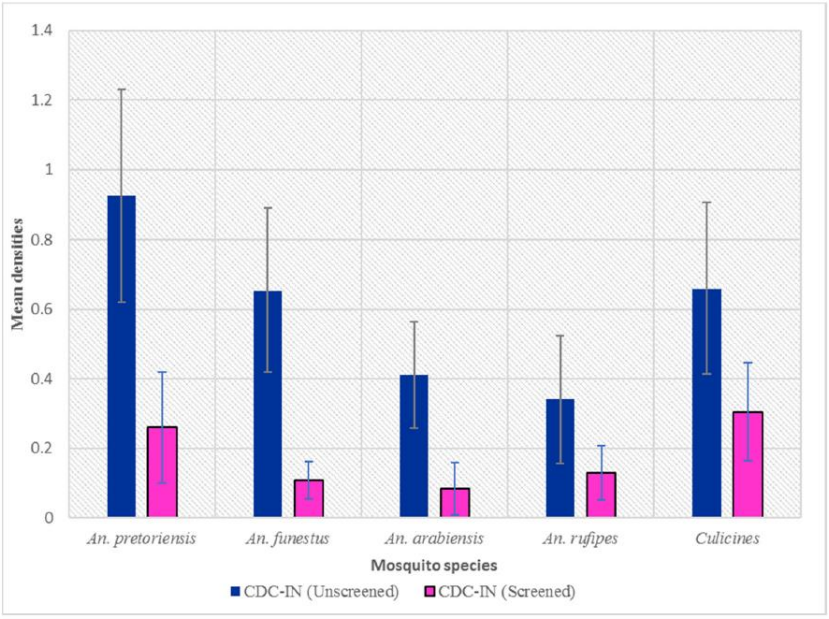

Figure 3. Mean densities of female *Anopheles* and culicine mosquitoes between unscreened (control) and screened (intervention) houses, indoors. Error bars represent 95% confidence intervals. CDC-IN, indoor Centers for Disease Control ultraviolet light traps.

Table 1. Species-specific mean densities of indoor host-seeking mosquitoes *.

Species	Unscreened Houses (Control)		Screened Houses (Intervention)	
	N	Mean (95% CI)	N	Mean (95% CI)
An. funestus	121	0.65 (0.42–0.89)	19	0.11 (0.05–0.16)
An. arabiensis	76	0.41 (0.26–0.56)	15	0.08 (0.01–0.16)
An. pretoriensis	171	0.92 (0.62–1.23)	46	0.26 (0.10–0.42)
An. rufipes	63	0.34 (0.16–0.52)	23	0.13 (0.05–0.21)
An. maculipalapis	43	0.23 (0.12–0.34)	43	0.24 (0.14–0.35)
An. coustani	27	0.15 0.03–0.26)	5	0.03 (0–0.05)
An. gibbinsi	13	0.07 (0.02–0.12)	8	0.05 (0–0.01)
An. squamosus	8	0.04 (0–0.09)	2	0.01 (0–0.03)
Total *Anopheles*	522	2.82	161	0.91
Total Culicines	111	0.6	48	0.27

* Species-specific indoor densities between unscreened houses (LLINs only) and screened houses (LLINs + house screening) based on 185 and 173 indoor CDC-LT trap nights, respectively.

3.2.2. Indoor Resting Densities

Overall, closing eaves and screening windows and doors reduced the densities of indoor resting mosquitoes by 20% (RR = 0.80, 95% CI 0.66–0.96, $p > 0.05$) although this was not statistically significant likely due to overall low collections using this method. Considering individual species, reductions in the mean indoor resting density were observed for *An. funestus* (RR = 0.56, 95% CI 0.35–0.91, $p > 0.05$), *An. arabiensis* (RR = 0.61, CI 0.39–0.96, $p > 0.05$), and culicine mosquitoes (RR = 0.65, 95% CI 0.56–0.76, $p > 0.05$) (Figure 4). The species-specific mean densities collected from unscreened and screened houses are shown in Table 2.

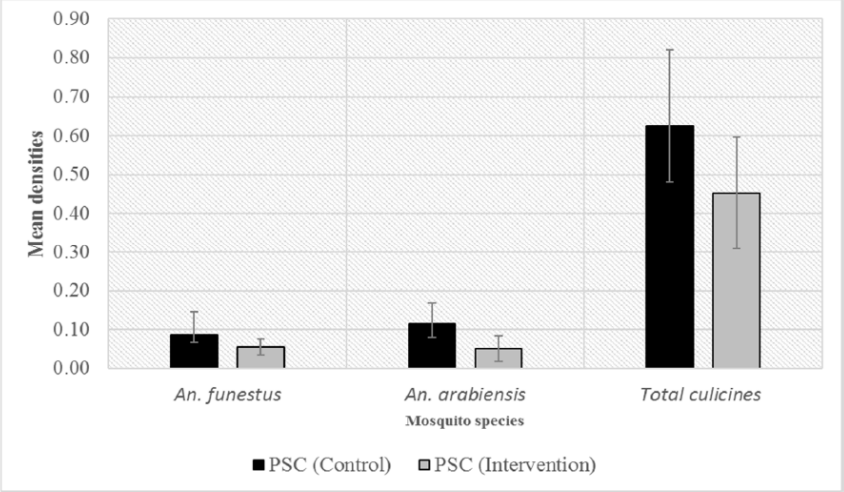

Figure 4. Mean densities of indoor resting female *Anopheles funestus*, *An. arabiensis* and culicine mosquitoes between unscreened (control) and screened (intervention) houses. Error bars represent 95% confidence intervals.

Table 2. Species-specific mean reduction in indoor resting mosquitoes *.

Species	Unscreened Houses (Control)		Screened (Intervention)	
	N	Mean (95% CI)	N	Mean (95% CI)
An. arabiensis	19	0.09 (0.04–0.13)	14	0.06 (0.02–0.09)
An. funestus	25	0.11 (0.06–0.17)	13	0.05 (0.02–0.08)
An. gibbinsi	10	0.05 (0.03–0.07)	7	0.03 (0–0.05)
An. rufipes	46	0.21 (0.12–0.30)	34	0.13 (0.07–0.20)
An. coustani	7	0.03 (0.01–0.06)	5	0.02 (0–0.04)
An. maculipalapis	56	0.26 (0.15–0.36)	49	0.19 (0.11–0.28)
An. pretoriensis	45	0.21 (0.12–0.30)	66	0.26 (0.15–0.37)
Total *Anopheles*	208	0.95	188	0.75
Total Culicines	135	0.62	48	0.19

* Species-specific indoor resting densities in unscreened (LLINs only) and screened houses (LLINs + House screening) based on 219 and 252 PSC night collections, respectively.

3.3. Outdoor Host-Seeking

Overall, outdoor host-seeking (CDC-OUT) mosquito densities were reduced by 27% (RR = 0.73, 95% CI 0.63–0.85, $p > 0.05$) in the intervention group. This reduction was not statistically different. Considering individual species, the most notable and significant reduction was observed in *An. pretoriensis* (RR = 0.60, 95% CI 0.47–0.75, $p < 0.05$). However, more outdoor host-seeking *An. rufipes* and *An. maculipalpis* mosquitoes were collected in the intervention arms than in the control arms, although this was not statistically significantly different ($p > 0.05$). Figure 5 shows the changes in the densities of outdoor host-seeking mosquitoes following house screening. The species-specific mean densities in the control and intervention arms from outdoor host-seeking mosquitoes are shown in Table 3.

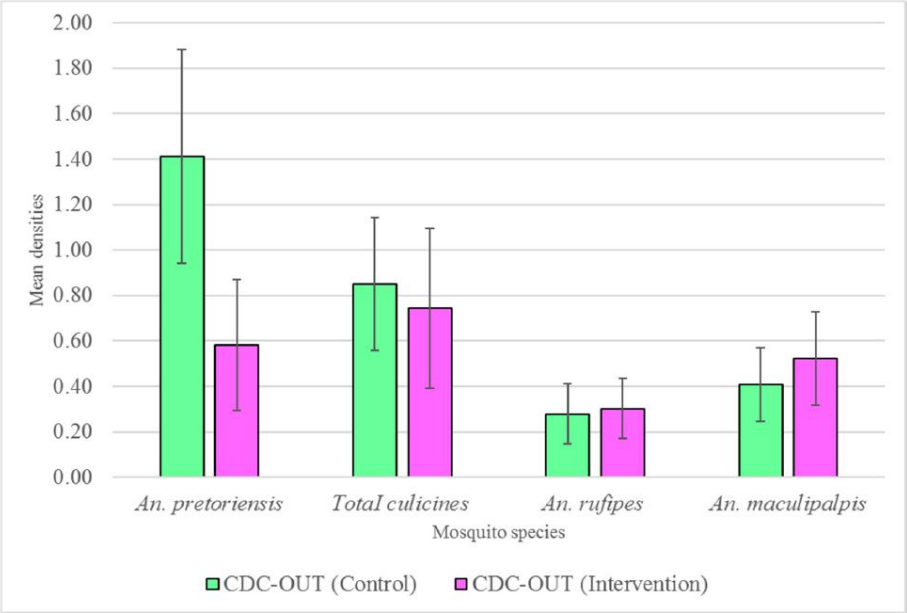

Figure 5. Mean densities of female *Anopheles* and culicine mosquitoes between unscreened (control) and screened (intervention) houses, outdoors using Centers for Disease Control ultraviolet light traps (CDC-LT) placed outdoors (CDC-OUT). Error bars represent 95% confidence intervals.

Table 3. Mosquito species-specific mean reduction in outdoor host-seeking mosquitoes (CDC-LT OUT) * in Nyimba district, Eastern province, Zambia.

Species	Unscreened (Control)		Screened (Intervention)	
	N	Mean (95% CI)	N	Mean (95% CI)
An. pretoriensis	219	1.41 (0.94–1.88)	77	0.55 (0.30–0.87)
An. funestus	141	0.91 (0.60–1.22)	54	0.41 (0.20–0.62)
An. coustani	15	0.1 (0.04–0.15)	7	0.05 (0.01–0.10)
An. arabiensis	59	0.38 (0.22–0.54)	39	0.30 (0.15–0.45)
An. gibbinsi	11	0.07 (0.01–0.13)	9	0.07 (0.02–0.12)
An. rufipes	43	0.28 (0.15–0.41)	40	0.30 (0.17–0.44)
An. maculipalapis	63	0.41 (0.25–0.57)	69	0.52 (0.32–0.73)
Total *Anopheles*	551	3.56	296	2.24
Total Culicines	132	0.85	48	0.36

* Species-specific outdoor densities between unscreened and screened study arms based on 155 and 133 outdoor CDC-LT trap nights, respectively.

3.4. Effect of House Screening on Vector Biting Behavior

A total of 51 anopheline mosquitoes were collected using the HLC method during the wet season (April and May 2020), comprising *An. funestus* (n = 25), *An. arabiensis* (n = 11), *An. maculipalpis* (n = 6), *An. rufipes* (n = 4), *An. coustani* (n = 4), and *An. pretoriensis* (n = 1). Since few mosquitoes were collected using this method, pooled results of biting times and rates for all species of anopheline mosquitoes are presented.

No anopheline mosquitoes were collected using HLC in screened houses during the dry season (September and October 2020). A total of five culicine mosquitoes were caught in unscreened houses for the entire dry season. These were discarded with no further analysis provided.

3.4.1. Indoor Biting

No mosquitoes were collected indoors in the screened houses. As such, the biting rates for screened houses were not calculated. Thus, there was a significantly higher indoor biting rate for unscreened houses (6.75 bites/person/h [b/p/h]) than for screened houses (0 b/p/h) ($\chi^2 = 6.67$, df = 1, $p < 0.05$). Pooled results show that the indoor peak biting time was early night, between 21:00 and 23:45, where biting rates were highest at 3.6 b/p/h (Figure 6).

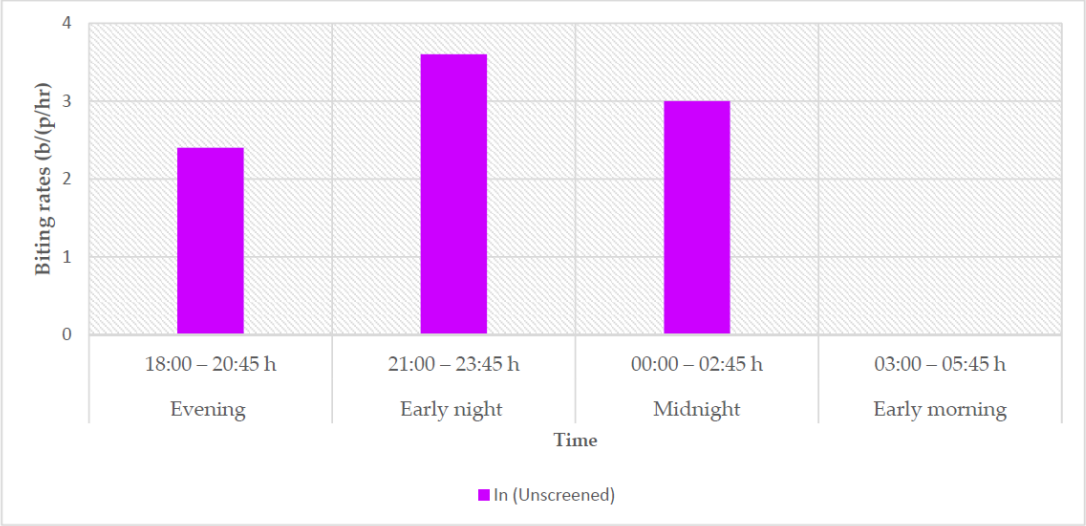

Figure 6. Pooled indoor biting rates for anopheline mosquitoes in unscreened houses for early evening, late evening, early night, and late night.

3.4.2. Outdoor Biting

Pooled results reveal a higher outdoor biting rate in control houses (9.45 b/p/h) than in intervention houses (3.31 b/p/h) (Figure 7). However, this difference was not significant ($\chi^2 = 2.95$, df = 1, $p = 0.08$).

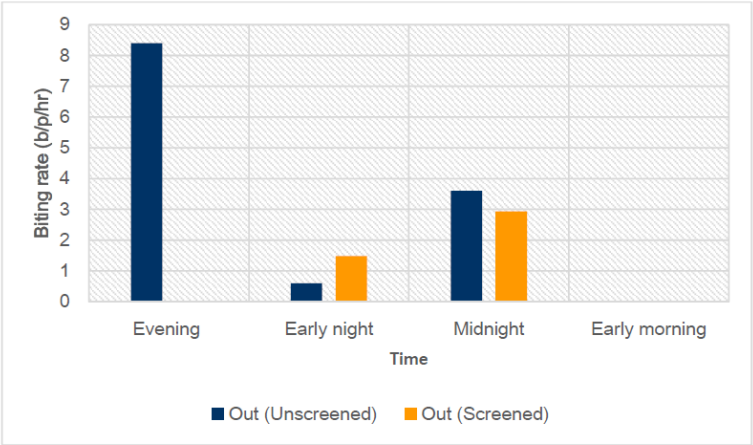

Figure 7. Outdoor biting rates for anopheline mosquitoes in unscreened houses and screened houses (evening 18:00–20:45, early night 21:00–23:45, midnight 00:00–02:45, early morning 03:00–05:45).

The peak outdoor biting time for unscreened houses was evening between 18:00 and 20:45, where biting rates were estimated at 8.4 b/p/h. In screened houses, the peak biting period was at midnight between 00:00 and 02:45.

3.5. Sporozoite Infectivity Rates

A total of 102 female *An. funestus* collected indoors were tested for *Pf* CSP. Of these, four tested positive for sporozoites, giving an overall SIR of 3.92%. Of the four sporozoite-infected mosquitoes, two were from unscreened houses, and two were from screened houses. All sporozoite-infected *An. funestus* were trapped between March and May 2020. Sixty female *An. funestus* collected outdoors were analyzed for the presence of *Pf* CSP. Of these, one tested positive, giving an overall sporozoite infectivity of 0.03. The positive *An. funestus* mosquito came from an unscreened house. In all the above, heating the ELISA lysate did not change the *Pf*-CSP positive result.

No other mosquitoes tested positive for sporozoites, giving an overall sporozoite infectivity of zero for both indoors and outdoors for the other species.

3.6. Entomological Inoculation Rates

3.6.1. Indoors

Using the indoor biting rates derived from indoor CDC-LT, the indoor EIR for *An. funestus* for unscreened houses was estimated to be 2.91 infectious bites/person/six months during the wet season.

The EIR for *An. funestus* in screened houses was estimated to be 1.88 ib/p/six months. Therefore, the overall estimated indoor EIR for unscreened houses was higher than that of screened houses. However, this was not statistically significant ($\chi^2 = 0.22$, df = 1, $p = 0.64$).

3.6.2. Outdoors

Outdoor EIR was estimated to be 4.0 ib/p/six months for *An. funestus* for unscreened houses during the wet season. Since there were no sporozoite-infected mosquitoes in the intervention houses trapped outdoors, the estimated EIR was 0 ib/p/two months. Thus, there was a significantly higher outdoor EIR in unscreened houses than in screened houses ($\chi^2 = 4.0$, df = 1, $p < 0.05$). The results are summarized in Table 4.

Table 4. Sporozoite infectivity, indoor and outdoor entomological inoculation rates (EIR) for *An. funestus* and *An. arabiensis* in intervention and control houses in the Nyimba district.

Trap Location	Treatment	Species	# Assayed	# CSP Positive	Sporozoite Rate	Human Biting Rates [1]	EIR (ib/p/y)
Indoors	Unscreened	*An. funestus*	81	2	0.02	0.65	2.91
		An. arabiensis	66	0	0.00	0.25	0.00
	Screened	*An. funestus*	21	2	0.10	0.11	1.88
		An arabiensis	19	0	0.00	0.06	0.00
Outdoors	Unscreened	*An. funestus*	40	1	0.03	0.91	4.09
		An. arabiensis	22	0	0.00	0.25	0.00
	Screened	*An. funestus*	20	0	0.00	0.42	0.00
		An arabiensis	16	0	0.00	0.30	0.00

[1] Human biting rates were derived from CDC-LTs.

4. Discussion

This study demonstrated that closing eaves and screening windows and doors with non-insecticide-treated wire mesh reduced the indoor densities of host-seeking, biting, and resting mosquitoes. On average, the densities of indoor host-seeking *Anopheles* mosquitoes were reduced by 44.4%. This reduction was observed across all species but was most notable in the major vectors: *An. funestus* and *An. arabiensis*, where densities were reduced by more than 60%. Our results are consistent with those from Ethiopia [8] and Gambia [17], where 40% and 43% reductions in the mean densities of *An. gambiae* s.l. were observed after

house screening. In Kenya, Abongo et al. [14] reported 60% and 54% fewer *An. funestus* and *An. arabiensis* densities after closing eaves and screening houses. In our study, the indoor densities of culicine mosquitoes were also reduced following the screening of eaves, windows, and doors, which is consistent with other studies [8,14,20,21,33]. Screening of houses thus reduces biting from nuisance mosquitoes and protects against viral and parasitic infections [13,20,21].

The reduced densities of mosquitoes likely explain the reduced biting activity of malaria vectors in screened houses. We also observed significantly lower indoor human biting rates in screened houses than in unscreened houses, according to HLCs. PSCs were used to estimate the densities of indoor resting mosquitoes. We collected relatively fewer mosquitoes using PSCs, which may explain the small effect sizes.

In this study, most of the mosquitoes belonged to the *An. funestus* group and *An. gambiae* complex, with most being *An. funestus* and *An. arabiensis,* respectively. This is consistent with previous reports from the area [22]. *An. funestus* is largely anthropophilic and endophilic [6]. *An. arabiensis,* on the other hand, exhibits a wider range of feeding and resting behavior and is able to feed on humans indoors and escape to rest outdoors [6]. Furthermore, in our study, only *An. funestus* tested positive for *Pf* sporozoites. This supports evidence that house screening may have the greatest impact on anthropophilic, endophagic, and/or endophilic species [7], which are also the most efficient malaria vectors [6,34].

To determine the protective efficacy of the house-screening intervention, we used EIR as a proxy measure of malaria transmission [35,36]. Although not significantly different, we estimated that people living in screened houses would receive fewer infectious bites per person (1.88 ib/p) than those living in unscreened houses (2.91 ib/p) during the wet season. These results are similar to those reported in Ethiopia [15] and Tanzania [16]. The likely explanation for the moderate efficacy of house screening experienced in this study could be that residents may have left the doors open, allowing mosquitoes to enter. The door screens installed in our study were not self-closing, an addition recommended for future studies. Second, some door screens were damaged (Saili et al. unpublished), allowing mosquitoes to enter, which was also observed in Gambian [37] and Ethiopian [15] studies.

In this study, EIR was estimated based on human biting rates that were derived from CDC-LTs. HLCs are considered the "gold standard" for collecting human-biting mosquitoes and measuring human-vector contact [38]. Other than the ethical issues [38], HLCs require close supervision and depend on the skill, motivation, and attractiveness of the volunteers collecting the mosquitoes [39]. HLCs may also introduce a mental bias due to the perception that there should be few or no mosquitoes due to an intervention. In this study, fewer mosquitoes were collected using HLCs than when using CDC-LTs, despite collections taking place during the peak malaria transmission season.

Behavioral adaptations of adult mosquitoes, such as feeding and resting outdoors, may limit the effectiveness of house screening on malaria transmission. In contrast, we observed reduced outdoor densities and EIRs in screened houses (4 ib/p/six months in unscreened houses versus 0 ib/p/six months in screened houses). These results are consistent with findings reported in Tanzania [16] and Kenya [14]. Our findings demonstrated slight density reductions for all outdoor species except for *An. maculipalpis*. While house screening primarily affects indoor, human-seeking mosquitoes [15,17], it is noteworthy that the densities of outdoor host-seeking mosquitoes were affected. We postulate that once entry into houses is denied, bloodthirsty endophilic mosquitoes simply seek alternative households or experience population decline due to limited feeding opportunities. However, other factors could have been at play in influencing the densities of mosquitoes outdoors. These may include weather (temperature, humidity, wind speed, rainfall), light levels (moonlight and artificial light), and the presence of domestic animals. Thus, house screening should not be considered in isolation.

An. pretoriensis was the most abundant species in our study. *An. pretoriensis* is known to be largely zoophilic and exophagic [40]. Its propensity to forage and rest indoors in this

study cannot be entirely explained. Although previous reports from the study area show this species to be infectious [41], no sporozoite-positive infected specimens were found in this study. This was also true for *An. rufipes, An. coustani, An. squamosus, An. maculipalpis,* and *An. gibbinsi.* Thus, despite their abundance, the role of these anopheline mosquitoes in malaria transmission appeared limited during the study period.

This study had several limitations. First, due to logistical challenges and resource limitations, the initial number of households targeted in the original study protocol [24] was not achieved. We experienced a large loss of CDC-LT batteries in the year before collections took place after the screens were installed. The batteries could not be replaced within the study period. We thus acknowledge that the frequency and geographical scope of sampling was not extensive and may explain some of the low vector densities observed in this study. The low numbers could also be attributed to seasonal effects on the productivity of mosquito breeding habitats. This warrants further research in the study area since studies on larval habitat productivity were outside the scope of the present study. A second limitation was the lack of routine (biweekly or monthly) monitoring for holes, rust, or detached screens. This would provide information on the longevity of the screens and indicate the cost-effectiveness of the intervention. This is recommended for future studies. Nonetheless, this study provides evidence that this integrated vector-control approach is effective against malaria vectors, nuisance mosquitoes, and other biting flies and may reduce malaria transmission and other mosquito-borne diseases. Currently, there is growing concern over insecticide resistance [42] and behavioral adaptations of primary malaria vectors to avoid LLINs and IRS [43]. Therefore, mainstream malaria vector-control interventions, namely, IRS and LLINs, which rely on the use of insecticides, may not achieve malaria elimination [5]. Augmenting these core vector-control interventions with supplementary vector-control tools, including house screening, is recommended [3].

5. Conclusions

Housing modifications, including closing eaves and screening doors and windows with non-insecticide-treated netting, reduced the indoor density of malaria vectors, including, *An. funestus*, *An. arabiensis,* and culicine mosquitoes. Our findings suggest that house screening has the potential to reduce malaria incidence, prevent diseases, and provide additional benefits, including fewer nuisance bites.

Author Contributions: Conceptualization, F.M., O.P.S., E.C., U.F. and C.M.M.; Data curation, K.S.; Formal analysis, K.S. and L.E.L.; Funding acquisition, F.M., E.C., U.F. and C.M.M.; Investigation, K.S., F.M., O.P.S., J.C. and B.H.; Methodology, K.S., C.d.J., O.P.S., L.E.L., J.C. and U.F.; Project administration, F.M., E.C. and C.M.M.; Resources, F.M., E.C., U.F. and C.M.M.; Supervision, C.d.J., O.P.S., U.F. and C.M.M.; Visualization, K.S.; Writing—original draft, K.S.; Writing—review & editing, K.S., C.d.J., F.M., O.P.S., T.E.N., L.E.L., J.C., B.H., E.C., U.F. and C.M.M. All authors have read and agreed to the published version of the manuscript.

Funding: The authors gratefully acknowledge the financial support for this research by the following organizations and agencies: Global Environment Facility (GEF) through the United Nations Environment Programme (UNEP) and the World Health Organization Africa Regional Office (WHO-AFRO): GEF Project ID 4668; the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Australian Centre for International Agricultural Research (ACIAR); the Norwegian Agency for Development Cooperation (Norad); the Federal Democratic Republic of Ethiopia; and the Government of the Republic of Kenya. The views expressed herein do not necessarily reflect the official opinion of the donors. Kochelani Saili was supported by a German Academic Exchange Service (DAAD) In-Region Postgraduate Scholarship and a partial University of Pretoria doctoral bursary.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. The study protocol and informed consent forms were reviewed and approved by the ERES Converges IRB Zambia (Reference: 2018-Oct-007 and 2020-Jul-018), the National Research Health Authority (Ref: NHRA00002/23/04/2021 and Health Researcher Registration #: NHRAR-

R-119/27/05/2022) and the research ethics committee of the University of Pretoria (Ref: 242/2020). Written permission to undertake the study was obtained from the Ministry of Health through the National Malaria Elimination Centre (NMEC) and Nyimba District Medical office. Local and traditional leaders were also informed about the purposes of the study. Participation in the study was voluntary, and informed consent was obtained from household heads and every participant above the age of 18 years. Verbal consent was obtained from household heads before routine mosquito collections. Each HLC collector was compensated for time spent in mosquito collection.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors are grateful to the communities in Nyimba Urban and Mkopeka where household collections were made. We thank the National Malaria Elimination Centre (NMEC), more specifically Andy Sinyolo and Japhet Chiwaula, for their help. We thank the Nyimba District Medical Office and traditional leadership in Nyimba Urban and Mkopeka for their cooperation and support. Andy Sinyolo of the National Malaria Elimination Centre (NMEC), Twig Mudenda and Limonthy Simubali of Macha Research Trust (MRT) are appreciated for their help with the initial molecular analysis. Faith I. Ebhodaghe from the University of Colorado is thanked for his advice and help with the statistical analyses. Cheryl Tosh for editing.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Chapter 6: Community perceptions, acceptability, and the durability of house screening interventions against exposure to malaria vectors in Nyimba district, Zambia

House screening remains conspicuously absent in national malaria programs despite its recognition by the World Health Organisation as a supplementary malaria vector-control intervention. This may be attributed, in part, to the knowledge gap in screen durability or longevity in local climatic conditions and community acceptance under specific cultural practices and socio-economic contexts. The objectives of this study were (1) to assess the durability of window and door wire mesh screens a year after full house screening and (2) to assess the acceptability of the house screening intervention to the participants involved. This chapter addresses objectives 4 and 5 of this thesis.

This study was conducted in Nyimba district, Zambia and used both quantitative and qualitative methods of data collection and analysis. Both direct observation and questionnaires were employed to assess the durability of the screens and the main reasons for damage. Findings on damage were summarized as percentages. Focus group discussions were used to assess people's knowledge, perceptions, and acceptability of the closing eaves and house screening intervention. Deductive coding and inductive coding were used to analyse the qualitative data.

A total of 321 out of 400 (80.3%) household owners of screened houses were interviewed. Many window screens (90.3%) were intact. In sharp contrast, most door screens were torn (n=150; 46.7%) or entirely removed (n=55; 17.1%). Most doors (n=114; 76%) had their wire mesh damaged or removed on the bottom half. Goats (25.4%), rust (17.6) and children (17.1%) were cited most as the cause of damage to door screens. The focus group discussion elicited positive experiences from after the closing eaves and screening their windows and doors that ranged from sleeping peacefully due to reduced mosquito biting and/or nuisance and having fewer insects in the house. Participants linked house screening to reduced malaria in their households and community.

This study demonstrated that in rural south-east Zambia, closing eaves and screening windows and doors was widely accepted. Participants perceived that house screening reduced human-vector contact, reduced the malaria burden and nuisance biting from other potentially disease carrying insects. However, screened doors are more likely to be damaged, mainly by children, domestic animals, rust, and termites.

The findings of this study have been published in *BMC Public Health* under the title "*Community perceptions, acceptability, and the durability of house screening interventions against exposure to malaria vectors in Nyimba district, Zambia"* and were presented at the 8th Annual Southern African Malaria Conference, organised by the South African Malaria Research Council in Pretoria, South Africa (in-person oral presentation) in August 2023.

Saili *et al. BMC Public Health* (2024) 24:285
https://doi.org/10.1186/s12889-024-17750-4

BMC Public Health

RESEARCH Open Access

Community perceptions, acceptability, and the durability of house screening interventions against exposure to malaria vectors in Nyimba district, Zambia

Kochelani Saili[1,2*], Christiaan de Jager[2], Freddie Masaninga[3], Brian Chisanga[1,4], Andy Sinyolo[5], Japhet Chiwaula[5], Jacob Chirwa[5], Busiku Hamainza[5], Emmanuel Chanda[6], Nathan N. Bakyaita[3] and Clifford Maina Mutero[1,2]

Abstract

Background House screening remains conspicuously absent in national malaria programs despite its recognition by the World Health Organization as a supplementary malaria vector-control intervention. This may be attributed, in part, to the knowledge gap in screen durability or longevity in local climatic conditions and community acceptance under specific cultural practices and socio-economic contexts. The objectives of this study were to assess the durability of window and door wire mesh screens a year after full house screening and to assess the acceptability of the house screening intervention to the participants involved.

Methods This study was conducted in Nyimba district, Zambia and used both quantitative and qualitative methods of data collection and analysis. Both direct observation and questionnaires were employed to assess the durability of the screens and the main reasons for damage. Findings on damage were summarized as percentages. Focus group discussions were used to assess people's knowledge, perceptions, and acceptability of the closing eaves and house screening intervention. Deductive coding and inductive coding were used to analyse the qualitative data.

Results A total of 321 out of 400 (80.3%) household owners of screened houses were interviewed. Many window screens (90.3%) were intact. In sharp contrast, most door screens were torn ($n=150$; 46.7%) or entirely removed ($n=55$; 17.1%). Most doors ($n=114$; 76%) had their wire mesh damaged or removed on the bottom half. Goats (25.4%), rust (17.6%) and children (17.1%) were cited most as the cause of damage to door screens. The focus group discussion elicited positive experiences from the participants following the closing of eaves and screening of their windows and doors, ranging from sleeping peacefully due to reduced mosquito biting and/or nuisance and having fewer insects in the house. Participants linked house screening to reduced malaria in their households and community.

Conclusion This study demonstrated that in rural south-east Zambia, closing eaves and screening windows and doors was widely accepted. Participants perceived that house screening reduced human-vector contact, reduced

*Correspondence:
Kochelani Saili
ksaili@icipe.org

Full list of author information is available at the end of the article

the malaria burden and nuisance biting from other potentially disease carrying insects. However, screened doors are prone to damage, mainly by children, domestic animals, rust, and termites.

Keywords Community perceptions, Acceptability, Durability, House screening, Malaria, Mosquitoes, Zambia

Background

Malaria is endemic throughout Zambia and is a major public health concern [1, 2]. To reduce the malaria burden, Zambia's National Malaria Elimination Program (NMEP) has developed a multi-pronged approach of combined vector-control interventions, mainly long-lasting insecticidal nets (LLINs) and indoor residual spraying [IRS], prompt malaria diagnosis using rapid diagnostic tests (RDTs), treatment using artemisinin-based combination therapies (ACTs) and strengthening information systems for quality and timely reporting of infections [3–6]. As a result of these interventions, the national malaria prevalence measured in children under the age of five decreased to as low as 9% by 2018 [7]. However, by 2021, the national parasite prevalence rate was reported to be 29% for children younger than five years [8].

The increased prevalence observed in the 2021 nationwide malaria indicator survey (MIS), most probably highlights the negative impact of the COVID-19 pandemic on malaria service delivery during the years 2019–2021 [9, 10]. Further, it underscores the increased need for additional innovative tools in malaria vector-control in order to achieve elimination of the disease [11]. In this connection, the WHO-recommended insecticide-based vector-control interventions used in Zambia, IRS and LLINs, are faced with serious challenges particularly, the development of insecticide resistance among the local vector populations [12–17]. Thus, insecticide resistance may undermine the continued efficacy of IRS and LLIN use against malaria vectors [18], a situation which calls for urgent introduction of new supplementary vector-control tools [11, 19].

In spite of having been recommended by WHO as a supplementary vector-control intervention [20], house screening remains conspicuously absent in the Zambia national malaria program [21, 22]. This is despite evidence showing that in rural Zambia, human-vector contact occurs primarily indoors [23] and Zambia's reported past success of malaria control with house screening as a supplementary method [24, 25]. The current omission of the intervention in the national program may be attributed to the limited evidence available on the additional benefits of house screening when used in combination with LLINs in different local malaria transmission settings [21, 22, 26]. Furthermore, knowledge on the durability or longevity of house screens when used under local climatic conditions is also limited. There is also a paucity of data on community acceptance under specific cultural practices [20].

This study was part of a larger randomized controlled study evaluating the effectiveness and impact of community-based house screening as a complementary malaria vector-control tool, conducted in rural south-east Zambia [27, 28]. As part of that trial, the intervention group consisted of 400 households provided with LLINs and fine wire mesh screens to stop mosquito entry. Eaves and smaller holes were closed with locally made bricks and mud used for house construction [28]. Wooden frames were fitted with wire mesh in front of the main door externally using hinges, while the edges of these frames were fitted onto the wall by a mixture of mud and cement. These wire gauze/mesh on the houses permitted ventilation. Community health volunteers were used to sensitize the community while the artisans (carpenters and bricklayers) were hired locally from within the study community to increase community acceptability [28].

The objectives of this study were to assess the durability of the window and door screens a year after screening; assess peoples' perception towards malaria and prevention methods and to assess the acceptability of the of house screening intervention by the participants involved.

Methods

Study area

The study was conducted in Nyimba district, located in the Eastern province of Zambia (4° 21′ 0″ S; 30° 35′ 0″ E) in December 2020 and January 2021 (Fig. 1). The study area has been described in detail elsewhere [27, 29]. Malaria in this area is endemic and transmission is perennial although it is highest after the end of the rain season, between March and May [7]. Malaria cases are almost entirely attributable to *Plasmodium falciparum* [7]. The major economic activity in the area is subsistence agriculture. Maize and groundnuts are the major crops grown. Other crops cultivated include sunflower, soya beans and cotton. Cattle and goats are kept as part of animal husbandry [30].

Study design

The study used a mixed qualitative and quantitative method study design. It initially involved direct observation, followed by a questionnaire to assess the durability of the wire mesh screens and main reasons for wear and tear. To enhance our understanding of the social and cultural phenomenon for the damages and/or removal, focus group discussions (FDGs) were held. FDGs were opted for because of the depth they guarantee in understanding

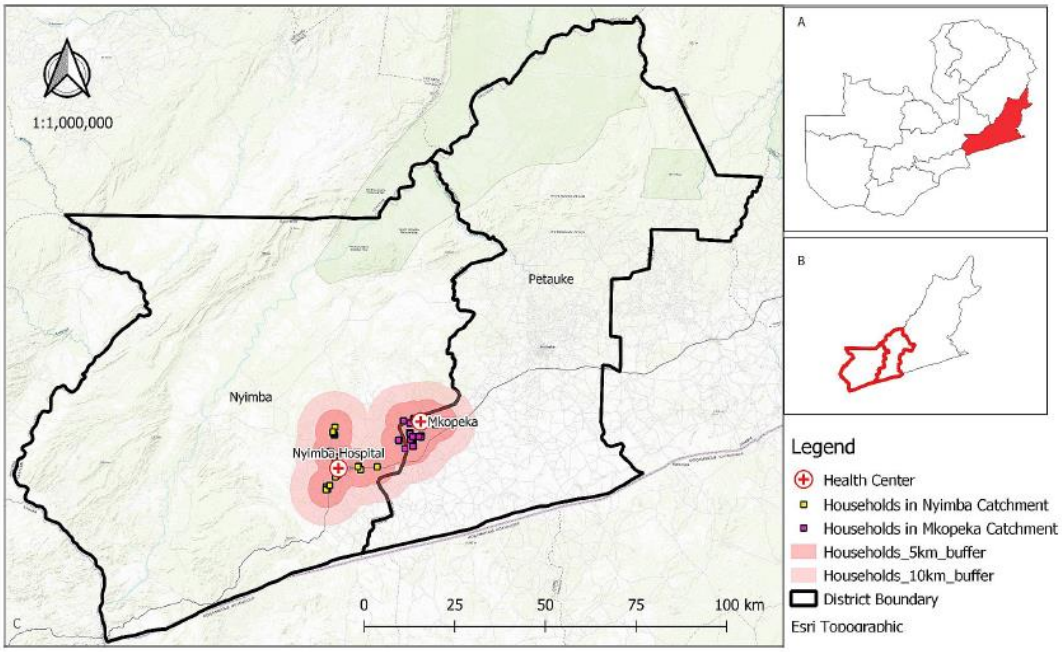

Fig. 1 Nyimba district showing the location of households that participated in the house screening. Insert: Map of Zambia showing the location of Nyimba district

a social phenomenon. The FGDs were used to first, assess people's knowledge, attitude, and perception of the local malaria situation. Second, to assess the knowledge, perceptions, and acceptability of the house screening intervention. This was important as some householders refused to respond to the question about the damage to the door screens resulting in invalid responses or missing values.

Sample size

The sample size used in this study has been described elsewhere [27]. Briefly, the sample size was derived from simulation models described in Hayes and Bennet et al. [31] for incidence rates and routine data collected from all health facilities in Nyimba district in Zambia at an estimated incidence rate of 0.312 cases per person from January to June 2019. It was estimated that to detect a reduction of 35% on malaria incidence, with 80% power at the 5% significance level, 338 houses were required per study arm [32]. A total of 400 households with one child each were recruited per treatment arm with additional households enrolled to account for households lost to follow-up.

Durability surveys

A questionnaire was used to assess the condition of the installed wire gauze on both the windows and the doors (see Additional file 1). Data was collected from 321 out of the 400 (80%) participating households, thus measuring the larger proportion of the intervention population. The questionnaire was pre-tested on 20% ($n=80$) of the screened households from the two study sites. During the pilot study, it was determined that at least one year after the installation, the wooden framework of doors and windows, the mortar holding the doors and window frames in place and the mortar that filled the eaves were still intact. This was thus, not included in the data collection tool.

To assess the condition of the doors, three broad categories were used; "intact", "torn", "removed". The screened door was considered "intact" when the wire gauze did not have any visible damage or holes or tear larger than 2 cm in diameter. The screen door was considered "torn" if the wire gauze was detached from the wooden plank or had a hole/s larger than 2 cm in diameter. If the wire gauze was removed or torn, the householder was interviewed to understand the reasons of the removal or tearing. For doors, an additional section was added to understand which part of the door was affected the most: "bottom",

"middle", "top" or "entirely removed". This is illustrated in Fig. 2.

Focus group discussion

Focus group discussions were conducted to assess participants' knowledge, perceptions, and acceptability of closing of eaves and screening windows and doors as a malaria vector-control intervention. The interviews were conducted by the research team. Before the interviews, all data collectors received a one-day training. Training included an overview of the study, review of the interview guide (Additional file 2), with an emphasis on the main objective of the focus group discussions, and qualitative interviewing techniques.

Fourteen focus group discussions were held. This corresponded to 14 out of the 20 villages that had both the house screening intervention implementation and entomological surveillance [27]. In each village, six household heads (or their proxies) that had consented to their house being screened and six that had either not given consent

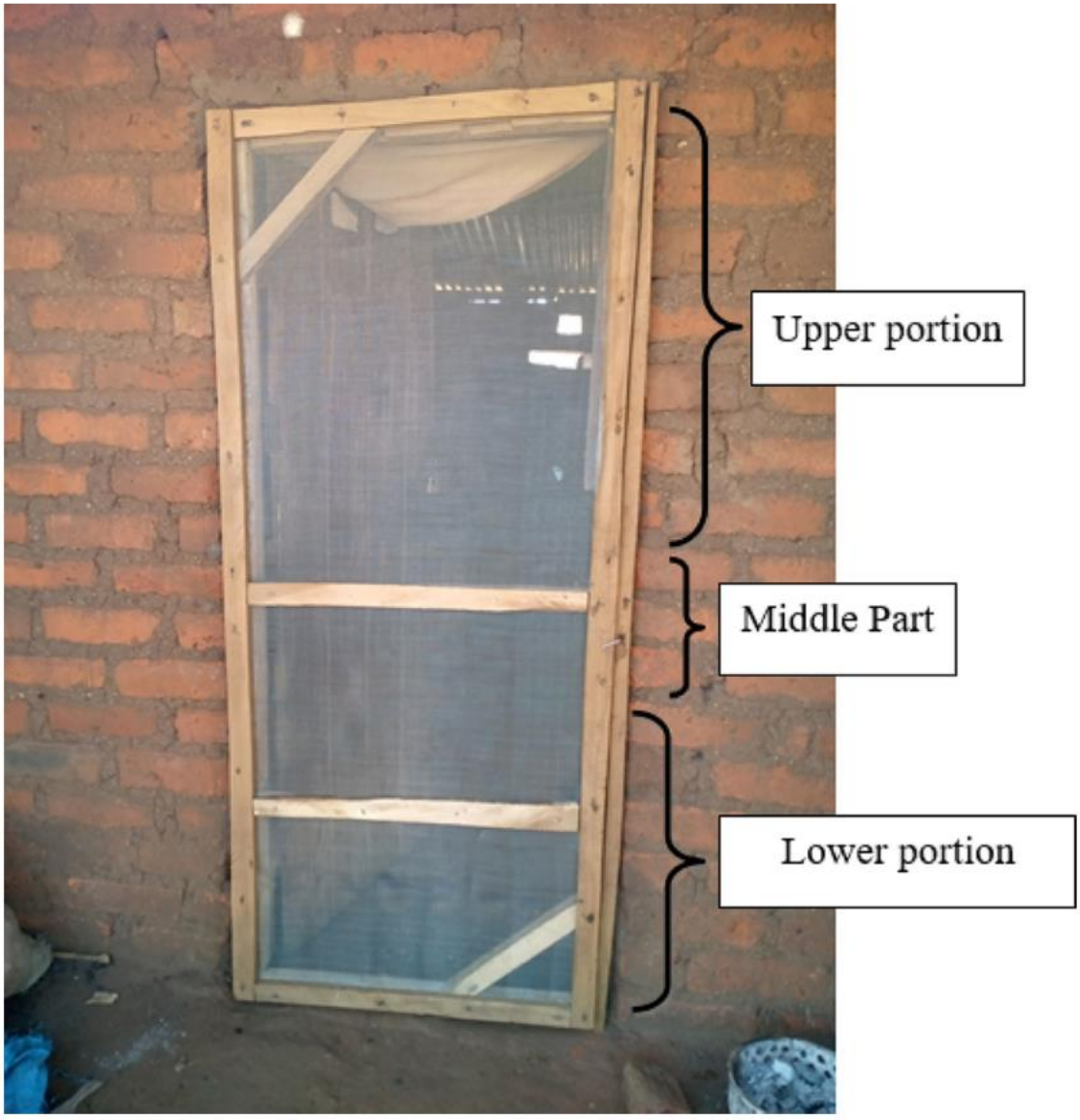

Fig. 2 A newly installed door screen showing the three portions considered in the questionnaire to assesss damage

or missed out entirely due to ineligibility or absenteeism during the screening period, were interviewed. Community health workers (CHWs) supported the selection of households. Before the interviews, all study participants were notified of the date, place, and time of the meeting for holding these FGDs. The FGDs were conducted at community centric places like schools, churches, or health facilities. All participants were 18 years and above and composed of both sexes. FGDs took between 1 and 2 h per session. All interviews were conducted in Nsenga, the most widely spoken language in the area. All data collection took place in December 2020.

During the FGDs, we used participatory rural appraisal (PRA) approaches to determine the community's perception of the malaria situation, display of symptoms among children, and confirmed malaria by RDT. Using 10 stones to represent children, we asked at least three participants to separately put stones in boxes labeled "malaria positive" and "malaria negative", as confirmed by RDT. These stones would be proportionate to the individual's perception of the number of children either malaria-positive or negative. We then asked all participants to confirm which was most accurate. This was repeated for children "displaying malaria symptoms only".

Table 1 Main themes from the qualitative study

Theme	Data supporting the theme/ sub-themes	Researchers' interpretative summary
Knowledge, perceived susceptibility, and severity of malaria in children	Basic knowledge of malaria Knowledge of symptoms of malaria Perception of the prevalence or how common malaria symptoms were and reasons for increase or decrease	• Community members theoretical understanding of the cause of malaria • Perception of malaria prevalence in comparison to previous years and • Linkage between theoretical understanding and perceived reasons for increase or decrease of malaria in children
Malaria prevention methods	Identification of core vector-control methods i.e., LLINs and IRS Identification of personal protection measures	Basic knowledge relating to malaria vector-control interventions
Knowledge and perceived benefits of house screening	What house screening entails General perceptions, experiences, and concerns Complementary role house screening plays in malaria prevention	• Community members theoretical and practical understanding of house screening as a supplementary intervention • Positive experiences
Barriers of house screening	Lack of ventilation, heat, poor lighting, termites and/or rust on screened houses	• Motivating and demotivating factors to community involvement • Negative experiences
Self-efficacy	The appropriateness of house screening as a supplementary intervention	Community member approval or disapproval of house screening
Cues to action	Considerations and challenges Community ownership	The willingness to implement house screening The willingness to maintain or repair damaged screens

Data analysis

This was a descriptive survey. All data were entered and stored into an Excel spreadsheet (Microsoft Office 2018). Findings on damage were summarized as percentages and proportional differences in the damages on the doors and windows determined by Pearson's chi- square ($\chi^{2)}$ at 0.05 significance.

At the end of each day of interviews during the data collection period, notes were taken, and discussions were held with the entire research team members as part of the preliminary data analysis. All data from the focus group discussions were audio-recorded, transcribed verbatim, and translated into English by a research assistant.

Thematic analysis was used to analyse the data. The transcripts were coded one of the authors and shared for comments and agreement on a common coding framework to the other authors. Both deductive coding and inductive coding were used. The deductive codes were derived from pre-established codes and were based on the interview guide (Additional File 2). Inductive coding was based on codes that emerged during the analysis process and were derived from the participants own words [33]. Key themes in the coding framework included the community's knowledge and perception of malaria prevalence and symptoms in children; malaria preventive methods; knowledge, perceptions, and experiences with house screening, barriers and facilitators of house screening and sustainability of the house screening intervention. These themes were framed around the Health Belief Model (HBM), a framework commonly used to explore compliance to health interventions. It can be used to interpret perceptions, acceptance, and usage of a health intervention [34, 35]. The model has six elements to explain and predict preventive health behaviours: (1) perceived susceptibility of the individual to the condition (2) perceived severity of the condition, (3) perceived benefits, (4) perceived barriers, (5) self-efficacy which is the conviction that one can successfully execute the health behaviour and (6) cues to action which trigger the readiness [34, 36]. The themes in this study were derived from these elements. This is explained in Table 1.

Results

Condition of window screens

Overall 321 (80.3%) of the 400 houses that were screened were observed and household owners interviewed.

Table 2 summarizes the findings of the condition of the window screens. There was significantly higher proportion of intact window screens than damaged (torn or removed) (χ^2=490, df=1, P<0.01) at the time of the survey. Reasons given for the torn window screens included poor workmanship, rust and children poking the screens with sticks and/or wires.

Condition of the door screens

The wooden framework and the mortar holding the doors was in place for most doors. However, we found that most of the screens were either torn (n=150; 46.7%) or removed (n=55; 17.1%) (Table 3). There was significantly higher proportion of damaged wire mesh on door screens (torn or entired removed) than intact ones (χ^2=52.1, df=1, P<0.01) at the time of the survey. For most doors (n=114; 76%), the bottom half was torn or removed. This is summarized in Table 4 and illustrated in Figs. 3 and 4.

Goats were identified most frequently (25.4%) as the cause of damage, more specifically, to the bottom half of the door screens. According to most household heads, this happened when goats attempted to enter the house to eat stored food. Rust and children *"running in and out of the house"* were the second and third most frequently cited causes of damage respectively. Destruction of the wood by termites and poor workmanship was also cited by the households as another cause of door screen damage. However, some householders refused to respond to the question about the damage to the door screens resulting in invalid responses or missing values (Table 5). This in part prompted the focus group discussions.

Focus group discussion

In total there were 162 participants spread across 14 meetings. On average, each meeting had 11 attendees. A total of 80 females and 82 males attended. Of these, 91 had houses that were not screened (control) and 71 had screened houses (intervention). The average age of the participants was 39 years. Other demographics of the participants are shown in Table 6.

Knowledge, perceived susceptibility, and severity of malaria in children

Symptoms of malaria were readily identifiable by the participants in all the 14 focus group discussions. Participants identified fever, directly translated as *"body hotness"* in the local language, as a key malaria symptom. Vomiting, chills, shivering, loss of appetite, lethargy and fatigue, blood shot eyes or *"red eyes", "pain in the body joints"* were mentioned as some common symptoms. Convulsions were also readily identified as a symptom of severe malaria due to delayed treatment.

Table 2 Condition of the wire mesh used in screening the windows

Condition of screened window	Frequency	Percent
Removed	1	0.3%
Torn	14	4.4%
Intact	289	90.3%
Invalid/missing values	17	5.0%
Total	**321**	

Table 3 Condition of the wire mesh used in screening the doors

Condition of door screen	Frequency	Percent
Entirely removed	55	17.1%
Torn	150	46.7%
Intact	113	35.2%
Invalid/ Missing values	3	0.9%
Total	**321**	

Table 4 Damage to screened doors

Portion of the door screen	Frequency	Percent
Bottom portion	114	76.0%
Middle part	25	16.7%
Upper portion	11	7.3%
Total	**150**	

> *"Sometimes, you cannot see any of those symptoms these ladies have mentioned. But you see your child not playing with his friends, not active.. when taken to the clinic you find that they have malaria"- male respondent, Nyakozolo village.*
> *"Sometimes a child [gets convulsions] when you delay taking them to the clinic", male respondent, Chambula village.*

Once identified, we used participatory rural appraisal (PRA) methods to determine the community's perception of the malaria symptoms and confirmed malaria in children. Using 10 stones to represent children, we asked at least three participants to proportionate the stones according to children displaying malaria symptoms. This is illustrated in Fig. 5. We then asked all participants to confirm the most accurate.

Most of the community members revealed that children showed malaria symptoms but tested negative when tested for malaria. A further probe for proportions of confirmed malaria using PRA methods had most participants placing more stones in the malaria "negative box". The ratio of positive to negative confirmed malaria as represented by the stones was generally agreed at 3 to 7.

> *".. sometimes, my child would have fever but when taken to the clinic, they would not find malaria. That leaves me wondering what caused the fever in the first place."-female respondent, Chambula village.*

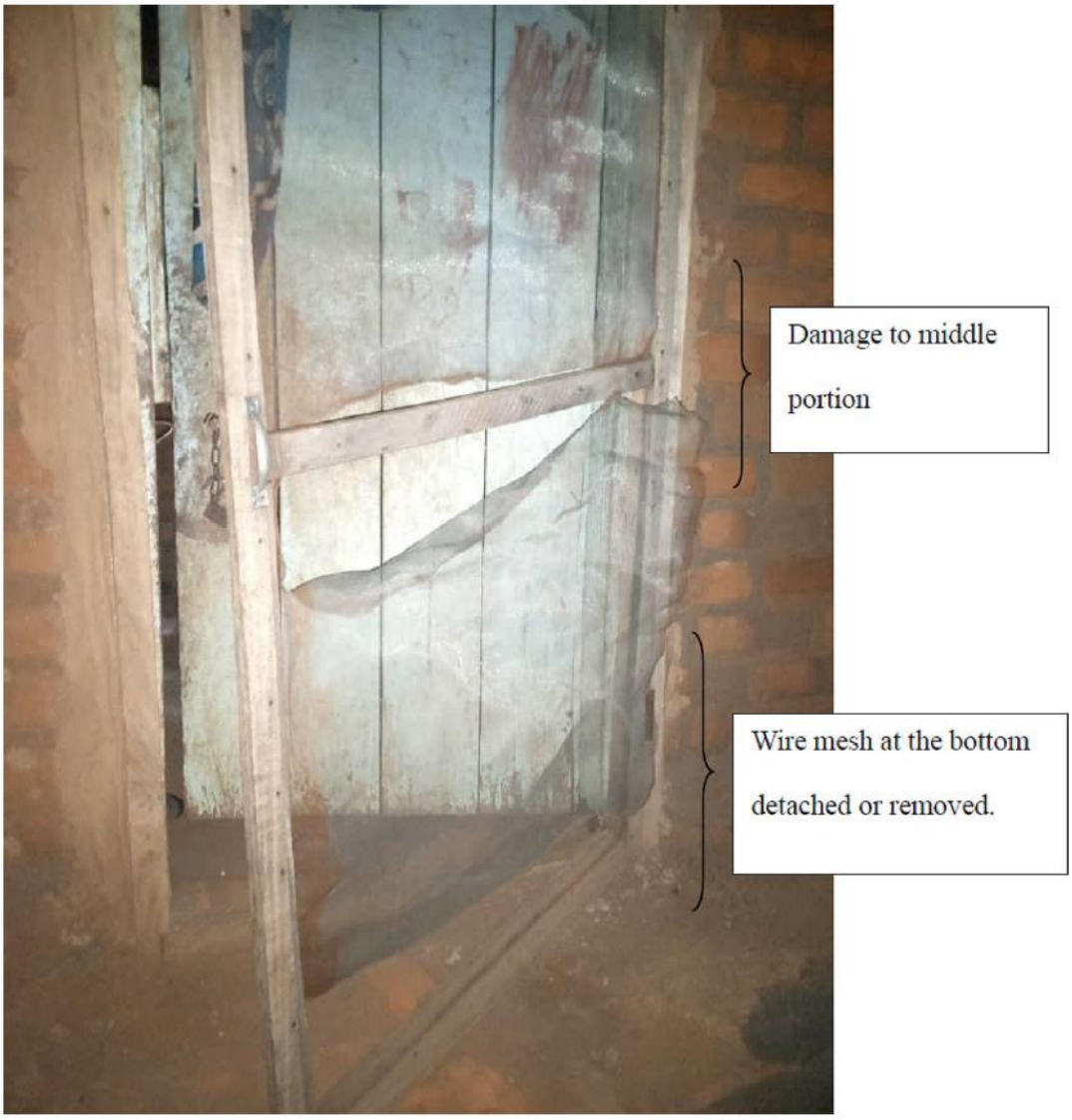

Fig. 3 Damaged door screen showing the portions that were damaged the most

"These days when you take five children to the clinic, you would find only one has malaria."- female respondent, Sikatoba village.

Participants felt that malaria cases in the community had reduced in comparison to the previous years. This was attributed to the distribution of LLINs, house screening or "*mosquito screens*", IRS and health education given to the community member through the health facilities.

"The other thing that has led to the reduction in the number of [malaria] cases is the introduction of mosquito screens. Once the mosquito screens are installed mosquitos do not enter the house."- male respondent, Kalunga village.

Malaria preventive methods

In most FGDs community members identified at least three malaria preventive methods; LLINs, IRS, and house

Fig. 4 Completely removed wire gauze on a door screen frame

screening. Burning a special type of grass/herb, traditionally known as *"mutanda imbu"*, which directly translates *'chase the mosquitoes'* was frequently mentioned. Some participants however mentioned they no longer use it.

> *"To be honest we no longer use that mutanda imbu.. not anymore. Maybe in the olden days. Now we just sleep under mosquito nets"-male respondent, Nyakazolo village.*

Many participants also mentioned *"mosquito coils"* and *"body creams to keep mosquitoes away"* i.e., spatial and body repellents respectively. Included were some personal protective measures that reduced mosquito bites such as sitting near a smoking fire or wearing long sleeved shirts and long trousers. Environmental manipulation such as getting rid of stagnant water and keeping grass short were frequently mentioned.

Table 5 Cited reasons for damage or removal of door screens

Reasons for damage	Frequency	Percent (%)
Goats	52	25.4%
Rust	36	17.6%
Children	35	17.1%
Poor workmanship	23	11.2%
Termites	4	2.0%
Cattle	2	1.0%
Other	13	6.3%
Invalid/missing values	40	19.5%
Total	205	100

Table 6 Demographics of study participants

Characteristic	Nyimba Urban	Mkopeka	Total (%)
Gender			
Male	43	39	82 (50.6)
Female	37	43	80 (49.4)
Age			
Average	38.1	39.9	
18–24	9	6	15 (9.3)
25–44	49	45	94 (58.0)
≥ 45	22	31	53 (32.7)
Education			
Informal	17	18	35 (21.6)
Primary	48	44	92 (56.8)
Secondary	15	16	31 (19.1)
Tertiary	3	1	4 (2.5)

"Burying all ditches holding still water in the yard because that is where mosquitos mostly breed from"- male participant, Mkopeka village.
"We also encourage children to wear long sleeved clothes in the evenings to avoid being bitten. Also, once they give us mosquito nets, we make sure children are nicely tucked in when they go to bed"- male participant, Lupala village.

Knowledge and perceived benefits of house screening

Throughout our discussions, participants mentioned hearing about house screening largely through the CHWs who participated in the enumeration (prior to the installation) and during installation. The participants generally referred to the wire mesh as *"ma seifa"*, a local name for the wire mesh used. Many community members acknowledged not to have heard about the house screening intervention or use of the wire mesh on windows and doors for the prevention of mosquito entry before this study. Almost all participants indicated that screening windows and doors prevents malaria by reducing mosquito entry.

"From my understanding, a mosquito has wings. The holes on the screen are so small such that even if the mosquito manages to put its head through, the wings won't be able to enter."- Male participant, Nyakozola village.
"Mosquito screens have been helpful, you will find absolutely no mosquitos in the house as long you always close your [door] screens as required."- Female participant, Malipa village.

Participants shared their positive experiences after the closing of eaves and screening their windows and doors. These ranged from sleeping peacefully due to reduced mosquito biting and/or nuisance and having fewer insects in the house. Some community members explained the intricate link between house screening, nuisance insects and potential infectious biting from other insects other than mosquitoes such as fleas.

"We now sleep like kings, peacefully. No slapping mosquitos when we are sleeping. As long as we close the screened doors nicely. It is very helpful."- male participant, Ziko village.
"Screens do not kill rats. Sometimes the rats come with fleas which do not leave the house when the rats go out. The fleas continue biting humans when rats are gone. But with the screens and closed eaves, even the rats do not enter. We want these screens, please"- female participant, Malipa village.
"Cockroaches have reduced. During this rainy season, the number of insects coming into the house [being attracted by the light] has significantly reduced."- female participant, Malipa village.

Participants linked installing gauze wire during house screening to reduced malaria infection rates in their households and community.

"I have a child who is 6 years old. Before putting the screens, I was taking him to the clinic every month, sometimes twice a month. But this time he never gets malaria ever since the screens were put. I am very thankful"- female participant, Sikatoba village.
"They put my screens last year and after some 2 to 3 months my child stopped getting sick. Even up to now!"- Female participant, Sikatoba.
"We used to go to the hospital very frequently. Now, with the screens, we don't get sick. Before the screens, each one in the family would have malaria. I tell you, malaria would make it's rounds on us. Now, none of us get malaria"- female participant, Mtausi village.
"This past year, the children used to sleep in a house without screens. They would frequently suffer bouts of malaria. But now my children sleep in a house with screens. They never get sick"-female participant, Chambula village.

Fig. 5 Identifying proportions of children displaying malaria symptoms using PRA methods

Other positive experiences related to the aesthetics i.e., the *"houses with screens looked good"*. Many praised the increased ventilation and lighting resulting from the screening. Overall, lighting and ventilation were not mentioned as a hinderance.

> *"We admire how the houses which have mosquito screens look, the windows look fancy"-female participant with a house without screens, Kapakasa village.*
> *"Before we used to block the window, with clothes and sacks. Now we allowed those installing the screens to remove some blocks and make the airspace bigger. We have fresh air all the time"-female participant, Mulira village.*

Self-efficacy

With this background, self-efficacy, the perception, or confidence of respondents towards house screening as an added intervention was measured. Respondents were asked to list in order of effectiveness house screening as a malaria intervention, against ITNs, IRS, spatial repellents, and body repellents (whichever the participants had mentioned earlier). In many cases, house screening as an intervention was second or third choice with ITNs and IRS being preferred or considered more effective. When screening was picked as the second preference, ITNs were always first choice.

We asked the participants to grade the house screening intervention on a scale of 1 to 10, with 10 being the best and one least. In many cases, the house screening received a grading of between 8 and 9 out of 10.

> *"I will give the screens 8 out of 10. They are helpful. But I have removed the 20% because they rust easily"- male participant, Mkopeka village.*
> *"I will give the screens 8 out of 10; yes 80%! Us as parents, we go out for work or at the farms. The children destroy [the screens], especially the screened doors. The 10% I have removed is because of that and rust.. we end up having big holes. "- female participant, Mkopeka village.*

Barriers to house screening

In many focus group discussions, damage, largely due to rusting was perceived as the biggest barrier to the acceptance of house screening. Similar to the durability survey, poor workmanship, goats, and children were mentioned as the top causes of damage particularly to the door screens which would then become unsightly.

> *"In my observation, the screens were not properly made. Where the screen is attached to the plank, they made it so tight that if anything bumps into it, the wires dislocate and make a hole. That is why mine is badly damaged."-male participant, Lupala village.*
> *"The people that put the door screens were in a hurry such that they did not do a good job. The door screen fell off within the first month that they installed it."- male participant, Ziko village.*
> *".. when it rains, water would splash on the screen. After the rust developed, some goats had entered the house and when the children were chasing them, the goats ran into the screen, and it got badly damaged. It does not look nice anymore"-female participant, Ziko village.*
> *"In my case, termites damaged the planks holding the screen until the screen was left unsupported."- Male participant, Chambula village.*

Light, ventilation or heat were not mentioned as inhibitors to the acceptance of the house screening even after thorough probing. Use of the local community health workers for community engagement and local artisans and bricklayers helped with the acceptability of the intervention overall.

Cues to action

In this study cues to action refers to the participant's readiness to initiate or maintain house screening. This was measured through a willingness of participants to install and/or maintain the screens in the absence of support from the Ministry of Health or its partners. Recognizing the benefits, house screening as an intervention was well received and recommended with many participants expressing the willingness to buy the material on their own. This was after realising that materials were readily available and commonly used to make locally made sieves used for mealie meal and groundnuts. However, many participants expressed hesitation to install and maintain the screens on their own. This was largely based on their experience with the wire mesh which once rusted, could barely be repaired.

> *"How can we even fix them? These are just like the household sieves we use to sieve mealie meal. It's not possible to only repair a part of it. The only way is to remove it completely and then put another one"- male participant, Mkopeka village.*
> *"He got wires and hooked them back in place. Later, it was dislocated where the screen touches the plank. After that when you try to repair it, the wires don't hold because they are rusty."- female participant, Kalunga, describing how her husband tried to fix the screening.*

In all the focus group discussions, communities requested that there should be clarity who should be maintaining the screens, i.e., either themselves, the Ministry of Health and/or project partners. There was a clear gap in the sense of ownership.

> *"If the government, I am talking about the Ministry of Health and partners, makes it clear that these things are [ours] and that [we] should be maintaining them, then we will repair them"-Lupala village, male respondent.*

Discussion

This study assessed the durability and community knowledge, perception and acceptability of the house screening intervention one year after installation. Our findings reveal that most window screens (90%) were intact or undamaged. However, 17.1% of screened doors had wire mesh entirely removed whilst about half (46.7%) had torn wire mesh. Only 35.2% were intact and fully functional. Studies in Ethiopia [37] and The Gambia [38–40] similarly reported more damage to doors than to window screens. Damage to the doors was mostly caused by domestic animals, (specifically goats), children, rust and termites, similar to the findings of Getewan et al. [37] and Kirby et al. [38]. The highest damage on the screened doors was at the bottom and middle parts as earlier defined. This created two critical barriers to acceptability of the house screening intervention. First was the negative experience resulting from the damage to the screened doors [41]. The focus group discussion echoed information recorded in the questionnaire, namely, that domestic animals, rust and childen were the biggest cause of damage to the wire mesh on the screened doors. Once rusted, the screened doors became unsightly hence householders could remove them completely. A second barrier to acceptability was the inability to repair broken screens. This may in turn affect long term sustainability of house screening by the householders [21]. The inability to repair was due to the rusting of the metallic or wire mesh. Once rusted, this material was practically irreparable.

The above findings revealed impediments to the acceptance of house screening as a supplementary vector-contol intervention. From the results of this study, we therefore suggest the following improvements on the design of the screened doors to prevent entry of mosquitoes inside the house in the rural areas of Zambia. First to replace the wire mesh with polyvinyl chloride (PVC) fibre glass which may be readily available locally. This may increase durability and in the long run reduce the costs associated with damaged screens [21, 42]. Percieved high costs and inability to repair, thus, low sustainability, ranked highly among concerns associated with housing improvement as a supplementary malaria vector-control intervention [21, 26, 43]. The replacement of wire mesh with PVC fibre glass may provide a solution to this. Second recommendation is a hard material for the bottom-half of the door, peharps made of locally available plywood or hardwood. The bottom part of the door was more likely to be damaged from domestic animals and small children running inside and outside of the house. Third, the upper part of the door should be reinforced with larger sized wire (chicken wire) or plank. (see Additional file 3). And fourth, it is recommended that all wood to be treated with anti-termite. Whilst initial costs may be higher, these changes may reduce damages and the need for replacement. This may prove more cost-effective in the long run. The prototype described in The Gambia study [44] could provide further alternatives to the above modifications.

The FGDs revealed universal knowledge of house screening. This could be attributed in part to working closely with CHWs, masons (brick layers) and carpenters from the participating villages within the study area. Involving a local community member in delivering malaria interventions breaks the power differences that may exist between the researchers and the community [45]. This built trust and thus, increased awareness and promoted acceptance [41, 45–47]. House screening was associated with reduced mosquito densities and as a consquence, reduced biting and malaria infections. These findings corroborate with the findings of a parrallel study by Chisanga et al. [47] who showed that house screening significantly reduced self-reported malaria in the study area. Individuals in screened houses reported over 40% less self-reported malaria, 25% less number of sick days and 17.5% episodes of suspected malaria [47].

Further, house screening was readily associated with reduced nuisance from other pests. Participants told of how screening reduced entry of rats, cockroaches, snakes and other insects particularly during the rainy season. Our findings are consistent with those from The Gambia [38] and Malawi [41]. One participant intricately highlighted the added health benefits of house screening with reduced exposure to plague, a flea-borne rodent-associated disease. Nyimba recorded fatal cases of plague in 2015 [48, 49]. This underpins the added benefit of improved housing as a developmental intervention in further reducing the burden of other arthropod-borne diseases such as diarrhoea, plague, lyphatic filariaisis and *Aedes*-transmitted diseases [22, 50, 51]. Other participants, felt their houses *"looked beautiful"* with the screens. These experiences are similar to those described in The Gambia [38] and may highlight yet another motivation to having houses screened with wire mesh.

Another key finding of this study was that house screening was indeed viewed as a supplementary method of preventing malaria by the participants. Community members always ranked ITNs and at times, IRS to be better than house screening. This may imply that house screening would not interfere with use of ITNs and IRS. This is an important finding. The WHO recommends universal access to vector-control, either ITNs or IRS at optimal coverage levels for all populations at risk of malaria in most epidemiological and ecological settings [20]. House screening as an intervention remains supplementary and should not be viewed as a replacement for the core malaria interventions [20].

Light and ventilation were not mentioned as barriers to acceptance. This is similar to findings by Getawen et al. [37] who showed that screening doors and windows did not interfere with either air flow nor lighting. Our findings however, contrast observations from The Gambia [38, 39] and Malawi [41] where some participants complained about poor lighting as a result of the closed eaves and screened doors and windows. Choice, type and design of the mesh on the screens must take into consideration the householders thermal comfort, ventilation and airflow [41, 44, 51, 52]. In this study, householders at times requested typically small air spaces to be increased by the removal of a layer of bricks or the clothes and sacs used to block theses spaces. This allowed more light and greater airflow. This added step by the householder may have further resulted in the co-benefit of reduced acute respiratory diseases [22] and increased acceptabilitity [46]. With increased air flow, adequate lighting and the absence of mosquitoes or other disturbing insects, indeed many could *"sleep like kings, peacefully"*. This information could be included in community engagement key messaging about house screening to increase acceptability [41, 46].

This study had limitations. Our discusions were limited to the end-user of the house screening. In this study, we did not interveiw or formally obtain the perceptions and experiences of the community leaders, policy makers such as the Zambia's Ministry of Health and the facilitators of the house screening, namely the CHWs, carpenters and masons [41]. Future studies should obtain the views of these key stakeholders. Further, we do not rule

out any influence that could have been excerted on the participants by the presence of the team of investigators from the study [41, 53].

Conclusion

This study demonstrated that in rural south-east Zambia, closing eaves and screening windows and doors was a widely accepted intervention. Participants perceived that house screening reduced human-vector contact, reduced the malaria burden and nuisance biting from other potentially disease carrying insects. This adds to the growing body of evidence that house screening can be an effective and accepted supplementary vector-control tool. However, screened doors are more likely to be damaged, mainly by children, domestic animals, rust, and termites and largely on the bottom half. Based on these findings, we recommend PVC fibre glass for the screening material and a hard material for the bottom half of the screened door to increase durability.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12889-024-17750-4.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements

The authors are grateful to the communities in Nyimba Urban and Mkopeka in who participated in this sudy. We thank the Ministry of Health through the National Malaria Elimination Centre (NMEC). We are grateful to the Nyimba District Medical Office and traditional leadership in Nyimba Urban and Mkopeka for their co-operation and support. We thank Winard Mutoka Jr. for his help with the map. Lucas Mulauzi Jr. is thanked for his help transcribing the verbatim.

Author contributions

CMM, EC, FM and KS conceived the study and wrote the main study protocol. KS, FM, and BC designed this study. KS, FM, BC, AS, JC, and JC collected the data. KS, BC and FM performed the analysis. KS wrote the initial draft of the manuscript, which was revised by FM, CdJ, AS, JC, JC, BH, EC, NNB and CMM. All authors read and approved the final manuscript.

Funding

The authors gratefully acknowledge the financial support for this research by the following organizations and agencies: Global Environmental Facility (GEF) through United Nations Environment Programme (UNEP) and the WHO-Africa Regional Office (WHO-AFRO) (GEF project ID: 4668); the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Australian Centre for International Agricultural Research (ACIAR); the Federal Democratic Republic of Ethiopia; and the Government of the Republic of Kenya. The views expressed herein do not necessarily reflect the official opinion of the donors. Kochelani Saili was supported by a German Academic Exchange Service (DAAD) In-Region Postgraduate Scholarship and a University of Pretoria doctoral bursary.

Data availability

The datasets used and/or analysed during the current study have been made available as supplementary material. Further information can be obtained from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate

The study protocol and informed consent forms were reviewed and approved by the ERES Converges IRB Zambia (Reference: 2018-Oct-007 and 2020-Jul-018), the National Research Health Authority (Ref: NHRA00002/23/04/2021 and Health Researcher Registration #: NHRAR-R-119/27/05/2022). The PhD protocol was approved by the research ethics committee of the University of Pretoria (Ref: 242/2020). Written permission to undertake the study was obtained from the Ministry of Health through the National Malaria Elimination Centre (NMEC) and Nyimba District Medical office. Local and traditional leadership were also informed about the purposes of the study. Participation in the study was voluntary, and informed consent was obtained from household heads and every participant above the age of 18 years. The authors confirm that all methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]International Centre of Insect Physiology and Ecology (icipe), NairobiP.O. Box 30772-00100, Kenya
[2]School of Health Systems & Public Health, University of Pretoria Institute for Sustainable Malaria Control, University of Pretoria, Pretoria, South Africa
[3]World Health Organization, Lusaka, Zambia
[4]Development Economics Group, Wageningen University and Research, Wageningen, Netherlands
[5]National Malaria Elimination Centre, Lusaka, Zambia
[6]World Health Organization, Regional Office, Brazzaville, Congo

Received: 22 July 2023 / Accepted: 11 January 2024
Published online: 24 January 2024

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Chapter 7: General Discussion and Conclusion

Introduction

This chapter provides a summary and discussion of the findings obtained through various activities conducted as part of this thesis. It presents the strengths and limitations of the entire thesis followed by a general conclusion. It concludes with recommendations for future research and/or policy formation in malaria.

1. Background

There is an urgent need to augment the current core malaria-vector tools, LLINs and IRS, with "supplementary" vector-control tools.[1] Included in the supplementary interventions are "housing modifications". House modifications are defined as "structural changes, pre- or post-construction of a house that prevents the entry of mosquitoes and/or decreases the exposure of inhabitants to vectors with the aim of preventing or reducing the transmission of malaria".[1] This thesis has focussed on two aspects of housing modifications, namely, closing eaves and screening windows and doors collectively referred to simply as house screening.

2. Summary of key findings

This thesis has successfully informed the effectiveness of house screening i.e., closing eaves and screening windows and doors on indoor host-seeking and resting mosquito densities and its potential to reduce malaria based on the entomological inoculation rate (EIR). Appendix D summarizes the main approach to address each objective and the findings of this study.

3. Discussion

Chapter 2 of this thesis was literature review with a focus on house screening in Zambia. The literature search shows that other than a recent study (and parallel to this one) on the economic impact of house screening,[2] a prospective trial on house screening and/or the additive value on malaria vector densities had not been conducted in Zambia. This overall study was therefore important.

This chapter further argued for steps that may need to be taken if house screening of residential houses should be a larger part of integrated vector management (IVM) in

Zambia. First, determine acceptability; how well the intervention will be received by the target population. A recent report from Mtwara, Tanzania[3] highlights the risk of researchers and implementors face when they fail to adequately address the fears and concerns of participants prior to intervention implementation. Second, intersectoral collaboration and transdisciplinary research.[1,4] And third, engaging traditional leaders who by virtual of their position can be the 'make or break' of a community accepting a health intervention.[5] These must be engaged and informed in a formal and culturally sensitive manner to garner their support.

The protective efficacy of an intervention is largely a function of the bionomics of the local mosquito populations.[6] Hence, local knowledge of the species composition of malaria vectors, entry and exit behaviour into human dwellings and their blood-feeding and resting behaviour is fundamental for the design of interventions specific to the local ecological and epidemiological situation. To fill this need, this study provided information on vector species composition, biting and resting behaviour and vector entry and exit behaviour into houses occupied by humans. This information is presented in *Chapters 3 and 4,* conducted as part of baseline study prior to the house screening implementation.

Chapter 3 provides information gathered on species composition of potential malaria vectors and their relative abundance in Nyimba district, and their sporozoite infectivity and entomological inoculation rates (EIR). Our findings show that *Anopheles funestus* was identified as the main driver of both indoor and outdoor malaria transmission in Nyimba district.[7] *Anopheles funestus* is a long- lived species, highly anthropophilic with strong endophagic and endophilic behaviour.[8] Thus, in the absence of insecticide resistance and/or improved formulations of current insecticides, this species may be controlled by LLINs and IRS. The low EIR found this study[7] compared to previous years[9] may highlight suppression of sporozoite infectivity following increased vector-control interventions, namely LLINs and IRS with the organophosphate pirimiphos-methyl.[10-11] However, that malaria transmission persists, albeit at low levels, shows that these core interventions cannot be deployed solely. In addition, the bionomics of this species also makes it amenable to house screening.[8]

In the same study, *Anopheles rufipes* was implicated in both indoor and outdoor malaria transmission in rural south-east Zambia.[7] Whilst for long, the species has been considered of secondary importance in Zambia due to its largely zoophilic, exophilic and exophagic tendencies,[12] this study showed that this mosquito species is gaining prominence in malaria transmission.

The study presented in *Chapter 4,* was an attempt to the determine the entry and exit behaviour of anopheline mosquitoes using a relatively simple sampling tool herein referred to as the Glue Net Trap (GNT) and window entry and exit traps. We also set out to determine the insecticide susceptibility status of malaria vectors in the study area by using mosquitoes collected at larval stage. The results of this study were largely disappointing as negligible mosquitoes were caught using this method in field surveys on rural houses in rural southeastern Zambia. Further, mosquitoes removed from the GNT, both during the cage experiments and field collections, were in damaged state and morphological identifications impossible[13] Thus, the Glue Net trap may not be an effective mosquito sampling tool in Zambia's rural areas.

Anopheles pretoriensis, a potential secondary vector,[14] dominated mosquitoes from larval collections and as such, insecticide resistance tests were carried out only on this species. Findings from this study show that populations of *An. pretoriensis* were susceptible to DDT and pyrethroids in the study area and within the study period. This may be expected as *An. pretoriensis* is largely exophilic and exophagic hence may have minimal contact with insecticides used on nets and/or sprayed on walls during IRS.[15-17]

After conducting the baseline studies, the house screening intervention was then implemented followed by mosquito collections in both screened and unscreened houses. *Chapter 5* represents the results of this study. The study demonstrated that closing eaves and screening windows and doors with non-insecticide treated wire mesh reduced the indoor densities of host-seeking and resting mosquitoes. On average indoor host-seeking densities of *Anopheles* mosquitoes, measured by CDC-LTs reduced by 44.4%. This reduction was observed across all species, but most notable in the major vectors *An. funestus* and *An. arabiensis w*here above 60%

reductions were observed. Further, results of this study showed that screening eaves, windows, and doors, reduced the indoor densities of culicine mosquitoes. Thus, house screening provides the additional benefits of reduced biting from nuisance mosquitoes and protection against viral and parasitic infections.[18-20] It was also estimated that people living in screened houses would receive lower infectious bites per person (1.88ib/p) than those living in unscreened houses (2.91ib/p) during the wet season. Of slight interest, was that *An. pretoriensis* was the dominant species caught in CDC-LTs in the second and third year of sampling. None were sporozoite infected, highlighting the negligible role that this species has in malaria transmission.[12]

The WHO recommends for national programs wishing to implement house screening as supplementary vector-control intervention to consider the level of community buy-in i.e., acceptability and/or willingness to implement the intervention.[1] *Chapter 6* addressed this need in rural Nyimba district. The qualitative studies revealed that house screening was largely accepted by the communities in Nyimba district. This was because house screening was associated with reduced mosquito densities and as a result, reduced biting, and malaria infections.[21] These findings corroborate with the findings of a parallel study by Chisanga *et al.*[22] who showed that house screening significantly reduced self-reported malaria in the study area. Individuals in screened houses reported over 40% less self-reported malaria, 25% less number of sick days and 17.5% episodes of suspected malaria.[2] Further, house screening was readily associated with reduced nuisance from other pests; house fly, rats, fleas, cockroaches, snakes and other insects particularly during the rainy season.[21,23] This underpins the added benefit of improved housing as a developmental intervention in further reducing the burden of other arthropod-borne diseases such as diarrhoea, plague, lymphatic filariasis and *Aedes*-transmitted diseases.[18,20,24]

Another key finding of this study was that house screening was indeed viewed as a supplementary method of preventing malaria by the participants. Communities always ranked ITNs and at times, IRS to be better than house screening. This may imply that house screening would not interfere with use of ITNs and IRS contrary to other reports.[25] The WHO recommends universal access to vector-control, either

ITNs or IRS at optimal coverage levels for all populations at risk of malaria in most epidemiological and ecological settings.[1]

4. Strengths of the study

Cluster randomization. In this study, villages served as clusters randomly assigned to either control (unscreened) or intervention (screened).[2] Further, households selected for entomological collections within these clusters were randomly selected[7] (see *Chapters 3 and 5)* Randomized control trials are considered the 'gold standard' for evaluating the effectiveness of an intervention since they have low risk selection bias.[26]

Sampling methods targeting different mosquito behaviour and/or the presence or absence of mosquito species. The entomological surveillance used in this study was robust, using different sampling methods targeting different bionomics of malaria vectors. Sampling methods included indoor and outdoor Centre for Disease Light Traps (CDC-LTs) to determine foraging behaviour, pyrethrum spray catches (PSC) for indoor resting densities; indoor and outdoor human landing catches (HLCs)- the 'gold standard'[9,15] for biting behaviour and larval collections to determine the presence or absence of mosquito species and insecticide resistance testing[15] (see *Chapters 3 and 5*). This study also experimented with Window Traps and a relatively novel method, the Glue net Trap (GNT),[27] to determine the exit and entry behaviour of malaria vectors *(Chapter 4)*. Results of this chapter were, however, rather disappointing and the GNT and window traps are not recommended for sampling mosquitoes in rural houses. Nonetheless, using different sampling methods ensured that mosquito collections were not biased towards indoor collections thereby capturing the true species composition of the study area.[15,28]

Molecular analyses to determine species composition and sporozoite infectivity. Considerable efforts were made to determining species composition within *An. gambiae* complex and *An. funestus* groups. Other anophelines, generally considered to be secondary vectors, were molecularly identified using the Internal transcribed spacer-2 ribosomal-DNA (ITS-2) polymerase chain reaction (or ITS2 PCR). Sporozoite detections were also made not only the primary vectors, but also

included the secondary vectors (See *Chapter 3 and 5).* This resulted in the incrimination of *An. rufipes* as a malaria vector.[7]

Implementation of the house screening intervention. In considering house screening as a malaria vector-control intervention, the WHO recommends practical consideration of how the intervention will be implemented.[29] This study partially starts to fill in that knowledge gap. *Chapter 5 and 6* provides the model worth considering. First, use of health facility-affiliated community health workers for community sensitisation. Second, was the involvement of masons (brick layers) and carpenters from the participating villages within the study area. Involving a local community member in delivering malaria interventions breaks the power differences that may exist between the researchers and the community.[30] Since the artisans were from the local area, they set up central workshops and thus reduced transport associated costs.

This study did not focus only on the 'science' and the data collection. Engaging the community for their perspectives allowed the research team to obtain feedback on house screening that may serve other studies well.

5. Limitations of the study

While our study contributed to evidence of the additive impact of house screening to the use of LLINs, it is important to acknowledge various methodological limitations. These are discussed below as well as the various chapters in which they appear.

Chapter 3: During the baseline study, mosquitoes were sampled for less than a year and in only two catchment areas of Nyimba district. The period and geographical scope of sampling was not extensive and may explain some of the low vector densities observed in this study. Future studies must consider adding more sampling sites to establish malaria transmission potential of all malaria vectors.[7]

Chapter 5: Due to a loss of CDC-LT batteries, compounded by a complicated procurement system, the initial number of households per study arm initially targeted for entomological collections set out in the original study protocol[31] was not achieved.

The batteries could not logistically be replaced within the study period. The smaller sample size of the number of collections may have reduced the power to detect change between screened and unscreened densities.

The delayed procurement also compounded the timing of the screening. Screening of houses took place in December 2020 and January 2021, during the rainy season. This may have increased the rate of deterioration of the screens due to rusting. In a largely rural area with few tarred roads, transportation of materials to the central workshops within the study areas thus became problematic.

This study did not include an aspect of routine (weekly, biweekly, or monthly) screen monitoring for holes, rust, or detachments. This would provide information on the longevity of the screens and thus, determining the cost-effectiveness of the intervention. This is recommended for future similar studies.[21]

Due to restrictions that came with the Covid-19 pandemic, HLCs to determine vector biting behaviour, were restricted to only two seasons of collection, the second of which no *Anopheles* mosquitoes were trapped. The sample size for HLCs was small and could not be used for determination of human biting rates. Similar studies must include a wider time and sampling frame for HLCs.

Chapter 6: The qualitative study to determine the acceptabilty of the house screening intervention was limited to the end-user of the house screening.[21] We did not interveiw or formally obtain the perceptions and experiences of the community leaders, policy makers such as the Zambia's Ministry of Health and the facilitators of the house screening, namely the CHWs, carpenters and masons.

During the study period, a proportion of door screens were torn or entirely removed. This no doubt may have negatively affected the results. The objective was to determine the additive impact of house screening when combined with LLINs over two malaria transmission seasons in realistic settings. Whilst we did our best to replace houses with torn door screens, in some villages, this was not entirely possible.

Finally, house screening highlighted the question of equity.[1] Only semi-modern houses (defined in this study as houses with a metalic roof) could be screened due to the higher costs and non-feasibilty of screening grasss thatched houses (see also Chisanga *et al.*[2]). Metalic roofs may be associated more with a higher wealth index.[1] Equity in intervention implementation is thus a ethical right that this study may have failed to address.[26]

6. Conclusions

Anopheles funestus is the main driver of malaria in Nyimba district whilst *An. arabiensis* and *An. rufipes* may play secondary roles. This is a long-lived species with a high affinity to feed on humans indoors and rest indoors. This behaviour makes it a more efficient malaria vector as evidenced by the high sporozoite rates found in the study. Thus, in this local setting, where the dominant vector is anthropophilic and endophagic, house screening remains an appropriate supplementary intervention. This was demonstrated in this study after the house screening intervention. House screening has potential to reduce indoor host-seeking and resting mosquitoes. In turn, the reduced indoor exposure of inhabitants to malaria vectors has high potential to reduce malaria transmission.

The wire (metallic) mesh used in this study lasted slightly over a year. Whilst the window screens remained intact, most doors were damaged especially on the bottom half. Damage resulted from children running in and out of the house, goats, and rust that caused detaching or tearing. It is recommended that stronger material be used for the screened material (e.g., polyvinyl chloride PVC material) which is reinforced with chicken wire. House screening was a largely accepted supplementary vector-control intervention that did not interfere with the use of core vector-control tools, namely LLINs.

7. Recommendations

a. Recommendations for practice

- House screening requires extensive coordination of vehicles that transport of material and workers to the community to support intervention installation and maintenance.[1] We recommend adequate investments into operations to mitigate the challenges that were experienced in this research project such as transportation, procurements, and employment of dedicated personnel at central level for smooth management of procurement and logistics.
- At the community level, we recommend that health facility affiliated CHWs be trained and/or oriented in basic enumeration techniques and given key messages on house screening for the purpose of community sensitization. CHWs are trusted members of the community, selected by the community and bridge the gap between the researcher and the community.
- This study recommends use of local artisans from the community to implement such as an intervention. Involving a local community member in delivering malaria interventions breaks the power differences that may exist between the researchers and the community.

b. Recommendations for research

- Human behaviour is a critical factor that should be included in assessments of malaria interventions. These include whether people are awake or asleep, outdoor sleeping practices, ITN usage (or non-usage) and screened door usage. These are important to determine whether there is an overlap between vectors and humans in both space and time.[15]
- Biweekly or monthly monitoring. To assess the durability of the screens, regular monitoring of the intervention are recommended. During the time of monitoring, the data collector may collect additional information about household behaviour including the door closing behavior.

- One *Achilles heel* of this study was a lack of insecticides resistance status of the primary vectors. Future studies should consider this. It is also worth noting that before and after intervention malaria prevalence rates were collected and will be reported elsewhere (Sangoro *et al.*, unpublished). Future studies should however consider a cohort study to be conducted parallel to the main intervention study.[26]

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Appendices

Appendix A-C show the protocols used for molecular identification of the *Anopheles gambiae* complex*, Anopheles funestus* group and the internal transcribed spacer-2 ribosomal-DNA polymerase chain reaction (ITS2 PCR) protocol for other species.

Appendix A: Differentiation of the *Anopheles gambiae* complex by PCR

This PCR uses 4 primers that in combination produce three differentially sized amplicons of the ribosomal DNA spacer region of *An. gambiae* complex mosquitoes. The expected product sizes are as follows: *An. gambiae* s.s (~390 bp), *An. arabiensis* (~315 bp), and *An. quadriannulatus* (~150 bp)[1].

Primers:

UN: 5'- GTG TGC CCC TTC CTC GAT GT -3'

GA: 5'- CTG GTT TGG TCG GCA CGT TT -3'

AR: 5'- AAG TGT CCT TCT CCA TCC TA -3'

QD: 5'- CAG ACC AAG ATG GTT AGT AT -3'

PCR Program: (SCOTT)

1.	94ºC	2 min	
2.	94ºC	30 sec	
3.	50ºC	30 sec	
4.	72ºC	30 sec	
5.	Go to step 2		29x
6.	72ºC	7 min	
7.	4ºC		∞

Reaction Mixture:	**25 μL**	**20 μL**	**12 μL**
10X	2.5 μL	2.0 μL	1.25 μL
dNTPs 2.5 mM	2.0 μL	1.6 μL	1.0 μL (final conc. 200 μM each)
AR	3.0 μL	2.4 μL	1.5 μL (150 pmol)
QD	3.0 μL	2.4 μL	1.5 μL (150 pmol)
GA	0.5 μL	0.4 μL	0.25 μL (25 pmol)
UN	1.0 μL	0.8 μL	0.5 μL (50 pmol)
Taq	1.5 U	1.2 U	0.9 U
dH_20	fill to total reaction mix volume.		

Use between 0.5 and 1 μL of template DNA.

Reference

1. Scott JA, Brogdon WG, Collins FH. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. American Journal of Tropical Medicine and Hygiene. 1993; 49.

Appendix B: Differentiation of the *Anopheles funestus* group by PCR

This PCR differentiates species of the *An. funestus* complex based on variation in the ITS2 region of nuclear rDNA. There is a universal forward primer and seven species-specific primers. The expected product sizes are as follows: *An. funestus* (505 bp)*, An. leesoni* (146 bp), *An. vaneedeni* (587 bp)*, An. parensis* (252 bp), *An. rivulorum* (411 bp), *An. rivulorum*-like (313 bp), and *An. funestus*-like (390 bp). Because the expected amplicons from *An. rivulorum* and *An. funestus*-like are too close in size to be effectively visualized on an agarose gel, only one of these primers should be used at a time in the reaction mixture.[1-3]

Primers:

UV: 5'- TGT GAA CTG CAG GAC ACA T -3'
FUN: 5'- GCA TCG ATG GGT TAA TCA TG -3'
VAN: 5'- TGT CGA CTT GGT AGC CGA AC -3'
RIV: 5'- CAA GCC GTT CGA CCC TGA TT -3'
PAR: 5'- TGC GGT CCC AAG CTA GGT TC -3'
LEES: 5'- TAC ACG GGC GCC ATG TAG TT -3'
RIVLIKE: 5'- CCG CCT CCC GTG GAG TGG GGG -3'
FUNLIKE (MalaFB) 5′- GTT TTC AAT TGA ATT CAC CAT T -3′

PCR Program: (FUNESTUS)

1.	94ºC	2 min	
2.	94ºC	30 sec	
3.	45ºC	30 sec	
4.	72ºC	40 sec	
5.	Go to step 2		29x
6.	72ºC	5 min	
7.	4ºC	∞	

Reaction Mixture: **25 µL**

10X 2.5 µL

dNTPs 2.5 mM	2.0 µL (final conc. 200 µM each)
UV	0.3 µL (33 pmol each primer)
FUN	0.3 µL
VAN	0.3 µL
RIV (or FUNLIKE)	0.3 µL
PAR	0.3 µL
LEES	0.3 µL
RIVLIKE	0.3 µL
Taq	1.6 U
dH_20	fill to 25 µl

Use 1 µL of template DNA.

References:

1. Cohuet ASFTJCKPCM, Fontennile D. Species identification within the *Anopheles funestus* group of malaria vectors in Cameroun and evidence of new species. American Journal of Tropical Medicine and Hygiene. 2003; 69(2):200-5.
2. Koekemoer L Hunt R CMKL. A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. American Journal of Tropical Medecine and Hygiene. 2003; 66:804(11):8-.
3. Spillings BL, Brooke BD, Koekemoer LL, Chiphwanya J, Coetzee M, Hunt RH. A new species concealed by *Anopheles funestus G*iles, a major malaria vector in Africa. The American Journal of Tropical Medicine and Hygiene. 2009; 81(3):510-5.

Appendix C: Internal transcribed spacer-2 ribosomal-DNA polymerase chain reaction (ITS2 PCR) protocol

This PCR is very robust and therefore can be used to check the quality of DNA extractions. It targets the ITS2 region of nuclear rDNA and produces amplicons of varying sizes depending on mosquito species. It can be used in tandem with the Funestus PCR to identify ambiguous samples. Because ITS2 binds to the conserved 5.8S rDNA and ITS2B binds to the 28S rDNA, this PCR can be used to sequence samples from almost any anopheline mosquito for species identification. ITS2B1, a novel, alternate primer, binds slightly downstream from ITS2B and produces a slightly larger amplicon that can be used to sequence through the entire ITS2.[1]

Expected product sizes for different mosquito species:

Funestus group:

An. leesoni ~520 bp

An. parensis ~ 620 bp

An. vaneedeni ~ 830 bp

An. rivulorum and *rivulorum*-like ~520 bp

An. longipalpis ~620 bp and ~900 bp

An. funestus and *funestus*-like ~850 bp

Other species:

An. rufipes, maculipalpis, and *pretoriensis* ~500 bp

An. theileri ~ 520 bp

An. coustani ~620 bp

An. gambiae complex ~600 bp

An. squamosus ~330 bp

Primers:

ITS2A: 5'- TGT GAA CTG CAG GAC ACA T -3'

ITS2B: 5'- TAT GCT TAA ATT CAG GGG GT -3'

ITS2B1: 5'- GTC CCT ACG TGC TGA GCT TC -3'

SQFor405 5'- CCA TTT CCA TTA TGT CCT ATC TAT AGG -3'

SQRev707 5'- GGG AAA GCA GGA GTT CGT TGA G -3'

Note: Only the ITS2B and ITS2B1 primers work well for sequencing.

PCR Program: (ITS2)

1. 94ºC 2 min

2.	94ºC	30 sec
3.	50ºC	30 sec
4.	72ºC	40 sec
5.	Go to step 2	39x
6.	72ºC	10 min
7.	4ºC	∞

Reaction Mixture:	**25 μL**
10X	2.5 μL
dNTPs 2.5 mM	2.0 μL (final conc. 200 μM each)
ITS2A	0.3 μL (30 pmol)
ITS2B	0.3 μL (30 pmol)
SQFor405	0.3 μL (30 pmol)
SQRev707	0.3 μL (30 pmol)
Taq	2.0 U
dH_20	fill to 25 μL

Use 1 μL of template DNA.

Reference

1. Koekemoer L Hunt R CMKL. A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. American Journal of Tropical Medicine and Hygiene. 2003; 66:804(11):8-.

Appendix D: Durability survey questionnaire

This appendix appears as an additional file in a published manuscript as shown in Chapter 6.

Purpose of questionnaire: To assess the physical condition of the window and house screens and understand the main causes for tear.

Location: Nyimba district, Zambia

Date of interview: …… . **Household ID:** …… ….

Village: …… **Zone:** ……

1. When was the house screened? (Mark the appropriate box)

☐ December 2019 ☐ January 2020

2. What is the condition of the windows? (Observe and mark the appropriate box. See definitions below)

☐ Intact ☐ Torn and/or has some holes ☐ Removed entirely.

i. **Intact:** the wire gauze does not have any visible damage or holes or tear larger than 2cm in diameter.

ii. **Torn and/or has some holes:** if the wire gauze is detached from the wooden plank or has a hole/s larger than 2cm in diameter.

iii. **Removed entirely:** The wire gauze is removed. If entirely removed, interview householder to determine the reasons.

Reasons for removal and/or tearing……

3. **Condition of the doors**

☐ Removed entirely ☐ Torn or has some holes ☐ Intact

*Definitions as shown above in 2.

4**. If torn, which part of the door screen is most torn? (Observe and mark the appropriate box)**

☐ Top

☐ Middle

☐ Bottom

☐ Wire mesh of the whole door is removed.

If removed entirely, interview the householder to determine the reasons behind removal.

Cause/Reasons for tear or removal……

Name of collector: …… . …….

Signature: …….

Appendix E: Focus Group Discussion Interview Guide

This appendix appears as an additional file in the published manuscript as shown in Chapter 6.

FOCUS GROUP DISCUSSIONS INTERVIEW GUIDES

INSTRUCTIONS

COMPOSITION: The Focus Group Discussion (FGD) is to be administered to a group of up to 12 consenting respondents drawn from selected villages. These are villages where the AFRO II project conducted surveys previously and where some households had their houses screened. The FGDs will consist of 50% of respondents whose houses were screened and the other 50% whose houses were not screened. As much as possible there should be equal gender representation and fair different age group distribution in each group.

During each interview, there will be an interviewer and a note-taker. Interviews will also be recorded for further transcription. Respondents should be informed that their identity will remain anonymous and that they are free to participate or NOT. Also inform the group that the interviews will be recorded. The interview should on average take **about an hour**.

THE PURPOSE: Before the interview, make sure to introduce the team and the purposes of the discussion namely to get in-depth understanding from households that consented to their houses being screened and eaves closed as a malaria preventing method. Thank participants for their availability for the interview. The discussion should also assess knowledge, attitudes and practices on malaria control using house screening. Be warm and allow a free flow of the conversations without interruption.

Date of Interviews: ……(*dd/mm/yy*): **Catchment Area**: Mkopeka/Nyimba Urban)……

Village:…….. ……..

Interviewer details: Full Name……Designation (write in full)……...Tel/mobile……

LIST OF PARTICIPANTS

	Full Name	**Age**	**Gender**	**Was the house screened? Y/N**	**When was the house screened?** *Month/Year*
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					

QUESTIONS

MALARIA PREVALENCE

1. How is the malaria situation in the village this year? How does this situation compare with other years in general?

2. How does this year compare with other years in terms of mosquito numbers in the houses?

3. What would you say is the proportion of children in the village who have had (confirmed) malaria this year? (using objects such as stones guide, the participants to estimate proportions in percentages)

4. What do you think are the reasons for the increase or decrease in the confirmed malaria cases in children?

    a. Optional /probing question: What proportion of children in the village have had malaria symptoms such as fever this year? (using objects such as stones guide the participants to estimate proportions in percentages)

    b. What are the reasons for the increase or decrease in the malaria symptoms such as fever in children this year?

MALARIA CONTROL

5. What methods are people in this village using to prevent mosquitoes entering their houses and to prevent malaria? (List all that are mentioned)

6. How effective are these methods? Have you faced any challenges in using these methods?

a. **Optional/probing question**: What do you think are the challenges in using these methods?

KNOWLEDGE, ATTITUDES AND PRACTICES TOWARDS HOUSE SCREENING

***If house screening was mentioned as a method of malaria control in the previous section, allow for a natural flow of questions.

7. Who can tell me (more) about house screening? Have you heard about house screening? What about closing eaves?

8. Where did you hear about house screening? [determine source of information]

9. When did you hear about house screening? [determine time frame of the source of information.]

10. Do you think house screening would help us prevent malaria? If so, how?

11. For those whose houses are screened, do you see any benefits of closing the eaves and house screening? What are the advantages of using the screens?

12. Are there any disadvantages of closing the eaves and screening the windows and the doors like we did? What are these disadvantages, if any?

13. For those whose houses were screened, what has been your experience with the house screens?

14. On a scale of 1 to10, how would you rate the effectiveness of house screening in preventing mosquito entry in the houses? (Allow the respondents to agree as a group)

15. On a scale of 1 to10, how would you rate the effectiveness of house screening in preventing malaria in children? (Allow the respondents to agree as a group)

DURABILITY OF THE SCREENS

16. What is the current condition of the screens on your windows and doors? (*Record number of respondents who say good out of total respondents available).*

17. Where does the damage to the house screens occur mostly?

18. What are the causes of the damage to the screens?

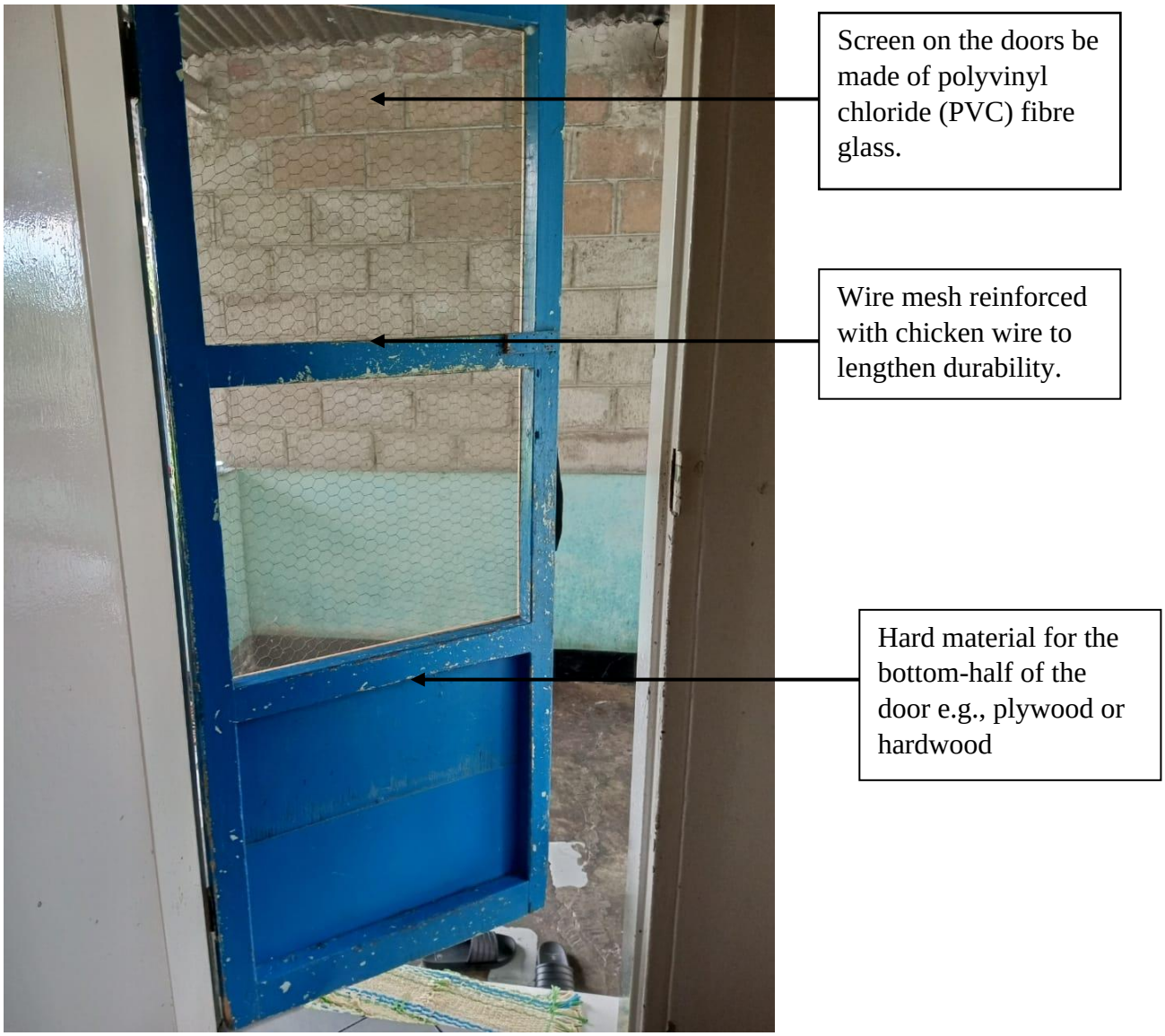
SUSTAINABILITY & WILLINGNESS TO PAY

19. Do people repair or replace the screens? If not, why are people NOT repairing the screens?
20. Question for those whose houses were SCREENED. Are people willing to maintain the screens in the absence of support from Government of Republic of Zambia (GRZ)/Donors/the AFRO II project? [ask with tact]
21. Would you recommend house screening to someone whose house was not screened?
22. *Question for those whose houses were NOT screened.* Are you willing to adopt house screening as a method of malaria prevention in your house?
23. *Question for those whose houses were NOT screened.* Would you be willing to adopt house screening in the absence of GRZ/Donor/AFRO II support.

Thank the participant. **THE END**

Appendix F: Suggested improved door

This appears as an additional file in the published manuscript as shown in Chapter 6. Suggested changes to screened doors to increase durability.



Appendix G: Zambia National Research Health Authority approval letter

NATIONAL HEALTH RESEARCH AUTHORITY
Paediatric Centre of Excellence, University Teaching Hospital, P.O. Box 30075, LUSAKA
Tell: +260211 250309 | Email: znhrasec@gmail.com | www.nhra.org.zm

Ref No: NHRA00002/23/04/2021 **Date: 23nd April, 2021**

The Principal Investigator,
Mr. Kochelani Saili
National Malaria Elimination Centre,
Box 32509,
Lusaka, Zambia.

Dear Mr. Saili,

Re: Request for Authority to Conduct Research

The National Health Research Authority is in receipt of your request for authority to conduct research titled **"THE VALUE OF HOUSE SCREENING AS AN ADDITION TO LONG-LASTING INSECTICIDAL NETS IN PROTECTING AGAINST MALARIA IN ZAMBIA."** I wish to inform you that following submission of your request to the Authority, our review of the same and in view of the ethical clearance, this study has been **approved** on condition that:

1. The relevant Provincial and District Medical Officers where the study is being conducted are fully appraised;
2. Progress updates are provided to NHRA quarterly from the date of commencement of the study;
3. The final study report is cleared by the NHRA before any publication or dissemination within or outside the country;
4. After clearance for publication or dissemination by the NHRA, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted, University leadership, and all key respondents.

Yours sincerely,

Prof. Godfrey Biemba
Director/CEO
National Health Research Authority

All correspondences should be addressed to the Director/CEO National Health Research Authority

Appendix H: University of Pretoria Research Health Ethics, Faculty of Health Sciences (2023)

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567. Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0278 Approved for use through August 31, 2023.

Faculty of Health Sciences **Research Ethics Committee**

13 April 2023

Approval Certificate
Annual Renewal

Dear Mr K Saili,

Ethics Reference No.: 242/2020 – Line 3
Title: The value of house screening as an addition to long-lasting insecticidal nets in protecting against malaria in Zambia

The **Annual Renewal** as supported by documents received between 2023-03-28 and 2023-04-12 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2023-04-12 as resolved by its quorate meeting.

Please note the following about your ethics approval:
- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2024-04-13.
- Please remember to use your protocol number (242/2020) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
***Deputy Chairperson** of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepika.bohani@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Appendix I: Initial ERES Converge ethics approval letter for PhD study

Plot No. 1, Cnr Joseph Mwilwa & Great East Road
Rhodes Park, Lusaka - Zambia
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 977 493220
Email: eresconvergeltd@gmail.com

I.R.B. No. 00005948
F.W.A. No. 00011697

20th October, 2020.

Ref. No. 2020-Jul-018

The Principal Investigator
Mr. Kochelani Saili,
C/O the National Malaria Elimination Centre,
P. O. Box 32509,
Chianama Hills Hospital Gounds, Great EAST Rd.
LUSAKA.

Dear Mr. Saili,

RE: THE VALUE OF HOUSES SCREENING AS AN ADDITION TO LONG-LASTING INSECTICIDAL NETS IN PROTECTING AGAINST MALARIA IN ZAMBIA.

Reference is made to your protocol resubmission dated 12th October, 2020. The IRB resolved to approve this study and your participation as Principal Investigator for a period of one year.

Review Type	**Ordinary**	Approval No. **2020-JUL-018**
Approval and Expiry Date	Approval Date: 20th October, 2020.	Expiry Date: 19th October, 2021
Protocol Version and Date	Version - Nil.	19th October, 2021
Information Sheet, Consent Forms and Dates	• English, Nyanja.	19th October, 2021
Consent form ID and Date	Version - Nil	19th October, 2021
Recruitment Materials	Nil	19th October, 2021
Other Study Documents	Data Collection Sheet, Questionnaire.	19th October, 2021
Number of participants approved for study	-	19th October, 2021

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- A reprint of this letter shall be done at a fee.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Dr. Jason Mwanza
Dip. Clin. Med. Sc., BA., M.Soc., PhD
CHAIRPERSON

Appendix J: ERES Converge ethics approval letter

Ethics clearance for the larger study in which this PhD was embedded.

Plot No. 1, Cnr Joseph Mwilwa & Great East Road
Rhodes Park, Lusaka - Zambia
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 977 493220
Email: eresconvergeltd@gmail.com

I.R.B. No. 00005948
EW.A. No. 00011697

19th February, 2020

Ref. No. 2018-Oct-007

The Principal Investigator
Dr. Elizabeth Chizema Kawesha
Ministry of Health
National Malaria Elimination Centre
P.O. Box 30205,
LUSAKA.

Dear Dr. Kawesha,

RE: "EVALUATING THE FEASIBILITY AND IMPACT ON MALARIA TRANSMISSION OF COMMUNITY BASED HOUSE SCREENING AS AN ADDITIONAL VECTOR CONTROL INTERVENTION IN ZAMBIA COMMITTED TO MALARIA ELIMINATION."

Reference is made to your protocol submission dated 2nd February, 2020. The IRB resolved to approve this study and your participation as Principal Investigator for a period of one year.

Review Type	**Fast Track**	Approval No. **2018-Oct-007**
Approval and Expiry Date	Approval Date: 19th February, 2020	Expiry Date: 18th February,2021
Protocol Version and Date	Version - Nil.	18th Februrary,2021
Information Sheet, Consent Forms and Dates	• English, Nyanja, Bemba, Tonga.	18th Februrary,2021
Consent form ID and Date	Version - Nil	18th February,2021
Recruitment Materials	Nil	18th Februrary,2021
Other Study Documents	Questionnaires.	18th Februrary,2021
Number of participants approved for study	-	18th Februrary,2021

Where Research Ethics and Science Converge

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- A reprint of this letter shall be done at a fee.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Dr. Jason Mwanza
Dip. Clin. Med. Sc., BA., M.Soc., PhD
CHAIRPERSON