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Vector control during a pilot malaria elimination project in southern Mozambique: gaps and future opportunities

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Doctoral Thesis

Vector control during a pilot malaria elimination project in southern Mozambique: gaps and future opportunities

Doctoral thesis presented by Lucia Fernandez Montoya to obtain a doctoral degree by the University of Barcelona

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That the thesis entitled

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submitted by Lucia Fernandez Montoya has been developed under their supervision and that is presented following recommendation from the University of Barcelona for the submission of a Doctoral Thesis by compendium of publications.

They also certified that this work has followed the codes of ethics and good practices and that they are not aware of any plagiarism.

Dr. Krijn Paaijmans **Dr. Quique Bassat**

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Contents

Glossary

Abbreviation	Full term
ACTs	Artemisinin Combination Therapies
An.	Anopheles
AQ+SP	amodiaquine + sulfadoxine-pyrimethamine
BHC	benzene hexachloride
BMGF	Bill and Melinda Gates Foundation
CDC	United States Centers for Disease Control and Prevention
DDT	Dichlorodiphenyltrichloroethane
DHAp	dihydroartemisinin–piperaquine
E8	Elimination 8 initiative
EIR	entomological inoculation rate
ETC	Exit trap collections
Exophagy	Mosquito habit to bite outdoor
Exophily	Mosquito habit to bite outdoor
GMEP	Global Malaria Eradication program
HLC	Human Landing Catches
HRP2	histidine-rich protein 2
IPTi	Intermittent preventive treatment of malaria in infants
IPTp	Intermittent preventive treatment of malaria in pregnancy
IPTsc	Intermittent preventive treatment of malaria in school-aged children
IRS	Indoor Residual Spraying
ITNs	Insecticide Treated Nets
LAMP	Loop-mediated isothermal amplification method
LDH	lactate dehydrogenase
LLINs	Long lasting Insecticide Treated Nets
LSDI	Lubombo Spatial Development Initiative
MALTEM	Mozambican Alliance Towards the Elimination of Malaria
MDA	Mass Drug Administration
MOSASWA	Mozambique, South African and Eswatini region
NMCP	National Malaria Control Programme
ORC	Outdoor resting collections
Pf	Plasmodium falciparum
PSC	Pyrethrum spray catches
RBM	Roll Back Malaria
RDTs	Rapid Diagnostic Test
SMC	Seasonal malaria chemoprevention
SP	sulfadoxine-pyrimethamine
WHA	World Health Assembly
WHO	World Health Organization
WHOPES	WHO Pesticide Evaluation Scheme

1. Articles included in this thesis

This thesis is presented as a collection of articles. It is composed of eight primary objectives, two secondary objectives and four articles. All four scientific articles are all accepted for publication or under review in PLoS ONE as part of the Magude project PLoS collection. PLoS ONE has an impact factor of 3.58 (SJR) and is on quartile 1 (multidisciplinary).

- **Article 1: Fernández Montoya L,** Máquina M, Marti-Soler H, Sherrard-Smith E, Alafo C, Opiyo M, et al. The realized efficacy of indoor residual spraying campaigns falls quickly below the recommended WHO threshold when coverage, pace of spraying and residual efficacy on different wall types are considered (accepted for publication)
- **Article 2**: **Fernández Montoya L**, Alafo C, Martí-Soler H, Máquina M, Malheia A, Sacoor C, et al. An evaluation of LLIN ownership, access, and use during the Magude project in southern Mozambique (under review)
- **Article 3**: **Fernández Montoya L**, Alafo C, Martí-Soler H, Máquina M, Comiche K, Cuamba I, et al. Overlaying human and mosquito behavioral data to estimate residual exposure to host-seeking mosquitoes and the protection of bednets in a malaria elimination setting where indoor residual spraying and nets were deployed together (accepted for publication)
- **Article 4**: **Fernández Montoya L,** Marti-Soler H, Maquina M, Comiche K, Cumaba I, Alafo C et al. The mosquito vectors that sustained malaria transmission during the Magude project despite the combined deployment of indoor residual spraying, insecticide-treated nets and mass-drug administration (accepted for publication)

2. Thesis summary

Executive Summary in English

Background

Malaria is a preventable vector-borne disease caused by *Plasmodioum* parasites and transmitted to humans through the bites of infected *Anopheles* mosquitoes. Despite the significant reductions in the burden of malaria achieved in the XXI century, progress has now stalled and 2021 saw 241 million clinical cases and 627 000 deaths, with most of these concentrated in sub-Saharan Africa and in children under 5 years of age. Malaria eradication was attempted in the 1950s-1980s, but although several countries eliminated malaria during that time, all attempts to eliminate malaria from sub-Saharan Africa failed, including the most comprehensive and thoroughly implemented attempt - The Garki project. The hope of malaria eradication was abandoned until 2017 when the availability of new tools, the prospective of upcoming ones and the great progress made from 2000 to 2015 raised hopes again and trigger again interested in the feasibility of eliminating malaria in Africa.

In 2015, a project to evaluate the feasibility of eliminating malaria in southern Mozambique – the Magude project- was launched. It aimed to evaluate the feasibility of interrupting malaria transmission in Magude district with a comprehensive package of interventions targeting simultaneously both the parasites (through mass drug administration, diagnosis and treatment), and the vectors (through indoor residual spraying and mass distributed long-lasting insecticide nets). The project failed to interrupt local malaria transmission. This thesis identifies the vectors that managed to sustain transmission despite the interventions package,

assess the gaps in vector control that hampered malaria elimination, identifies potential improvements to vector control to guide future efforts in the area and compares the Magude project with its closest relative – The Garki project – to shed light into the reasons why malaria elimination continues to be an unattainable goal in Sub-Saharan Africa.

Methods

Malaria incidence data were collected through a DHIS2-based rapid case reporting system. Entomological data were collected through an entomological surveillance system that collected mosquitoes indoors and outdoors on a monthly basis in six sentinel sites within the district using CDC light traps with and without bottle rotators. The entomological data was used to identify the local vectors, analyze their composition, densities, sporozoite rates, host seeking behaviors and evaluate the role of each vector in sustaining malaria transmission. Vector susceptibility to pyrethroids (used in ITNs), DDT and pirimiphos-methyl (used in IRS) was evaluated by means of WHO tube bioassays. A cross-sectional human behavior study was implemented both in the low and high transmission season to evaluate people's sleeping location (indoors/outdoors), net use and the time at which people went indoors, to bed, woke up and left their house in the morning. These data was overlapped with entomological data to evaluate the human residual exposure to host-seeking vectors of five different *Anopheles* species during the project, 2) the distribution of residual exposure in five different compartments (outdoors, indoors before bed, indoors in bed, indoors after getting up, and outdoor after getting up), 3) the personal protection that ITN conferred and could have conferred if everyone would have used a net to sleep and 4) the distribution of residual exposure that would remain if everyone used the net to sleep. ITN ownership, access, attrition and use were evaluated based on data collected

through the census of the population, the subsequent health and demographic household surveys, MDA surveys and malaria prevalence surveys. Inequalities in ITN access and use across sex and age groups, household wealth, size and location were also evaluated. IRS structure, household, and population- level coverage was analyzed from data collected during the IRS campaign and from data collected during the MDA surveys. A new method was developed to evaluate the realized residual efficacy of IRS by combining results from WHO cone bioassays conducted on different wall types, the distribution of wall types in the district, IRS coverage and the pace of spraying during the campaign. Finally, entomological results, human behavioral data and interventions data were jointly discussed to assess the amenability of each of the tentative local vector to control by IRS and ITNs. The results were used to identify ways to improve IRS and ITNs and were compared with results of the Garki project to identify persistent challenges.

Results

Twenty-one *Anopheles* species were identified during the project. *An. arabienis, An. funestus s.s., An. parensis, An. merus and An. squamosus* were found carrying *Plasmodium falciparum* sporozoites and the same vectors plus *An. quadriannulatus* and *An. rufipes* were significantly associated with malaria cases. Other *Anopheles* vector species were identified that are known to be malaria vectors in Mozambique (*An. gambiae s.s.* and *An. tenebrosus)* or elsewhere in Africa (i.e. *An. coustani*, *An. ziemanni , An. rivulorun, An. leesoni* and *An. pharoensis),* but none of these were found carrying *Pf* sporozoites.

An. arabiensis was susceptible to the insecticides used in IRS (DDT and pirimiphos-methly) and ITNs (pyrethroids), it was the most abundant species during the entire project with a large

different with the others and also responsible for the majority (74%) of the human exposure to host-seeking mosquitos both in the high (78.5%) and low transmission season (64.2%). *An. arabiensis* was found resting indoors during the whole project, it sought their host mainly indoors and during sleeping times and it presented the strongest association with malaria incidence.

An. funestus s.s. and *An. parensis* were susceptible to DDT and pirimiphos-methly but resistant to pyrethroids; after the first IRS campaign, they almost disappear from CDC trap collection and indoor resting collections and were no longer found carrying *Pf* sporozoites; they sought their host mainly outdoors and indoors before people went to bed, they accounted for 5.1% and 5.8% human exposure to host seeking mosquitoes respectively and their association with malaria incidence was weaker than that of *An. arabiensis.* The susceptibility to insecticides of *Anopheles merus* and *An. squamosus* could not be evaluated because very few *An. merus* and no *An. squamosus* were found resting indoors. *An. merus* accounted for 5.2 % of human exposure to host seeking vectors, sought its hosts mainly indoors while people were in bed and was found carrying *Pf* sporozoites during the project. *An. squamosus* accounted for 9.9% of human exposure to host seeking vectors, sought its host both outdoors and indoors in the low transmission season but mainly indoors in the high transmission season. Overall residual exposure to host seeking vectors took place mainly indoors (95.9%) and almost a third (31.4%) occurred during the traditional low transmission season. The intervention package also reduced vector sporozoite rates.

Most ITNs in the district were either ITNs impregnated in the last 12 months or LLINs. ITNs were lost at a rate of 31% per year. ITN access remained below 76.3% during the entire project.

ITN used fluctuated seasonally dropping to 40% in the low transmission and reaching a maximum of 76.4% in the high transmission season. Poorer, larger and harder to reach households, children and women below 30 had lower access to ITN than the rest of the population. ITN use was lowest in school-age children and young adults, especially among males. At the observed levels of ITNs use and considering a feeding inhibition of 81.1%, the personal protection of ITNs prevented 39.2% of residual exposure to host-seeking vectors and, if all residents would have used a net to sleep, ITNs could have prevented 63.3% of such residual exposure.

IRS structure, household and people level coverage were above 83%. Coverage was even across district administrative divisions. Although IRS residual efficacy remained above 80% for above 6 months in both the 2016 and 2017 campaigns, considering IRS coverage, pace of spraying and distribution of wall types the realized IRS residual efficacy of the 2016 campaign remained above 80% for merely 113 days, not covering the entire transmission season.

Conclusions

Anopheles arabiensis was the main vector during the project, whereas *An. funestus s.s., An. merus, An. parensis, An. squamosus*, and possibly *An. rufipes*, likely played a secondary role. ITNs access, use and personal protection was suboptimal and unequal during the project. In addition, ITN likely provided protection against *An. arabiensis* and *An. merus*, some protection against *An. squamosus* in the high transmission season but very limited protection against *An. funestus s.s.* and *An. parensis.* In the future, ITN access can improve by revising ITN allocation strategies during ITN mass campaigns and strengthening continuous distribution channels. ITN use will improve by increasing access, as access was a barrier for use during the high

transmissions season, but behavioral change campaigns will be needed to further increase ITN use, especially during the low transmission season and among school age children and males. IRS coverage was above the WHO recommended threshold but its realized residual efficacy did not cover the entire high transmission season. IRS was likely effective at controlling *An. funestus* s.s. and *An. parensis* but much less so at controlling *An. arabiensis*. Its effect on *An. arabiensis* and *An. merus* could not be evaluated. Future potential improvements to IRS include using longer lasting IRS products (when they become available) or implementing a second round of IRS per season (if possible amid the rains). Deploying IRS and ITN together likely provided greater protection compared to implementing one intervention alone. Yet, additional interventions will be needed to tackle the residual exposure to vector bites that could not be controlled by the combination of ITNs, IRS and MDA. Although there is limited evidence to strongly recommend the deployment of any specific vector control supplementary or new intervention in Magude, candidate intervention may be larviciding, house screening, lethal house lures, ATSB and the release of sterile *An. arabiensis* males.

Like the Garki project, the Magude project could not interrupt malaria transmission either despite its several advantages, namely that it was implemented in a low transmission area with free access to diagnosis and treatment, that it used ITNs in addition to IRS and MDA and that it used longer lasting residual insecticides for IRS. Both projects shared two common challenge-outdoor biting vectors and limited MDA coverage. The Magude project did not have a sufficiently long preparatory phase, baseline or control district, which jeopardized its evaluation. Projects like the Magude project should include a control district or a long-enough baseline to allow for a proper quantification of its epidemiological and entomological impact.

Resumen en Español

Contexto

La malaria es una enfermedad prevenible transmitida por vectores causada por parásitos *Plasmodium* y transmitida a los humanos a través de la picadura de mosquitos *Anopheles* infectados. A pesar de las importantes reducciones en la carga de malaria logradas en el siglo XXI, el progreso se ha estancado y en 2021 se registraron 241 millones de casos clínicos y 627 000 muertes, la mayoría de las cuales se concentraron en el África subsahariana y en niños menores de 5 años. La malaria se intentó erradicar en las décadas de 1950 y 1980, y aunque varios países eliminaron la malaria durante ese tiempo, todos los intentos de eliminar la malaria del África subsahariana fracasaron, incluido el proyecto más completo de la época: el proyecto Garki. La esperanza de erradicar la malaria se abandonó hasta 2017, cuando la disponibilidad de nuevas herramientas, aquellas en desarrollo y el gran progreso logrado desde 2000 hasta 2015, despertaron nuevamente las esperanzas de eliminar la malaria y de evaluar la viabilidad de eliminación en África.

En 2015, se lanzó un proyecto para evaluar la viabilidad de eliminar la malaria en el sur de Mozambique, el proyecto Magude. Su objetivo era interrumpir la transmisión de la malaria en el distrito de Magude con un paquete completo de intervenciones dirigidas simultáneamente tanto a controlar los parásitos [a través de la administración masiva de medicamentos (AMM), el diagnóstico y el tratamiento] como a controlar los vectores [a través del rociado residual intra-domiciliario (RRI) y la distribución masiva de mosquiteras tratados con insecticidas de larga duración (MTILD)]. El proyecto no logró interrumpir la transmisión local de la malaria. Esta tesis

identifica los vectores que lograron mantener la transmisión a pesar del paquete de intervenciones, evalúa las brechas en el control de vectores que dificultaron la eliminación de la malaria, identifica posibles mejoras en el control de vectores para guiar los esfuerzos futuros en el área y compara el proyecto Magude con su pariente más cercano, el proyecto Garki, para arrojar luz sobre las razones por las que la eliminación de la malaria sigue siendo un objetivo inalcanzable en el África subsahariana.

Métodos

Los datos de incidencia de la malaria se recopilaron a través de un sistema de notificación rápida de casos basado en DHIS2. Los datos entomológicos se recopilaron a través de un sistema de vigilancia entomológica que recolectó mosquitos en interiores y exteriores mensualmente en seis puntos centinela dentro del distrito utilizando trampas de luz del Centro de control y prevención de enfermedades de los Estados Unidos (CDC) con y sin rotadores de botellas. Los datos entomológicos se usaron para identificar los vectores locales, analizar su composición, densidades, tasas de esporozoitos, comportamientos de búsqueda de huéspedes y evaluar el papel de cada vector en el mantenimiento de la transmisión de la malaria. La susceptibilidad del vector a los piretroides (utilizados en los MTILD), DDT y pirimifós -metilo (utilizados en el RRI) se evaluó mediante bioensayos en tubos de la Organización Mundial de la Salud (OMS). Se implementó un estudio transversal del comportamiento humano tanto en la temporada de transmisión baja como alta para evaluar el lugar donde duermen las personas (interiores/exteriores), su uso de las mosquiteras y la hora a la que las personas entran, se acuestan, se despiertan y salen de su casa por la mañana. Estos datos se superpusieron a los datos entomológicos para: 1) evaluar la exposición residual humana a vectores buscadores de

huéspedes de cinco especies diferentes de *Anopheles*, 2) la distribución de la exposición residual en cinco compartimentos diferentes (exterior, interior antes de acostarse, interior durante las horas de sueño, interior después de levantarse y al aire libre después de levantarse), 3) la protección personal que los MTILD confirieron y hubiesen conferido si todos hubieran usado mosquiteras para dormir y 4) la distribución de la exposición residual que hubiese quedado si todos hubieran usado mosquiteras para dormir. La propiedad, el acceso, la pérdida y el uso de MTILD se evaluaron en función de los datos recopilados a través del censo de población, las encuestas domiciliarias demográficas y de salud posteriores, las encuestas realizadas durante la AMM y las encuestas de prevalencia de malaria. También se evaluaron las desigualdades en el acceso y uso de MTILD entre sexos y grupos de edad, y entre la riqueza, tamaño y ubicación de los hogares. La cobertura del RRI en términos de estructuras rociadas, hogares rociados y población protegida se analizaron a partir de los datos recopilados durante la campaña del RRI y de los datos recopilados durante las encuestas realizadas en las AMMs. Se desarrolló un nuevo método para evaluar la eficacia residual real del RRI mediante la combinación de resultados de bioensayos de cono de la OMS realizados en diferentes tipos de paredes, la distribución de los tipos de paredes en el distrito, la cobertura del RRI y el ritmo de rociado durante la campaña. Los resultados entomológicos, los datos del comportamiento humano y los datos de las intervenciones se discutieron conjuntamente para evaluar la capacidad de las MTILD y del RRI de controlar cada uno de los presuntos vectores locales. Los resultados se usaron para identificar formas de mejorar la distribución de MTILD y el despliegue del RRI. Finalmente, se compararon los resultados de Magude con los resultados del proyecto Garki para identificar desafíos persistentes para la eliminación de la malaria en África.

Resultados

Durante el proyecto se identificaron veintiuna especies de *Anopheles. Anopheles arabiensis*, *An. funestus s.s.*, *An. parensis*, *An. merus y An. squamosus* fueron encontrados portando esporozoítos de *Plasmodium falciparum (Pf.) y* los mismos vectores más *An. quadriannulatus* y *An. rufipes* mostraron una asociación estadísticamente significativa con los casos de malaria. También se identificaron otras especies de vectores de *Anopheles* que se sabe que son vectores de la malaria en Mozambique (*An. gambiae s.s.* y *An. tenebrosus)* y en otros lugares de África (*An. coustani, An. ziemanni, An. rivulorun, An. leeson* y *An. pharoensis)* pero ninguno de estos se encontró portando esporozoítos de *Pf* .

Anopheles arabiensis fue susceptible a los insecticidas utilizados en RRI (DDT y pirimifós-metilo) y MTILD (piretroides), fue la especie más abundante durante todo el proyecto, con una gran diferencia con las demás, y también fue responsable de la mayoría (74%) de la exposición humana a los mosquitos buscadores de huéspedes tanto en la temporada de alta transmisión (78,5%) como en la temporada baja transmisión (64,2%). *Anopheles arabiensis* fue encontrado descansando en el interior de las casas durante todo el proyecto, buscó a su huésped principalmente en el interior y durante las horas sueño y presentó la asociación estadística más fuerte con los casos de malaria.

An. funestus s.s. y *An. parensis* fueron susceptibles al DDT y al pirimifós-metilo pero resistentes a los piretroides; después de la primera campaña del RRI, casi desaparecieron de las recogidas de mosquitos en trampas del CDC y de las colecciones de mosquitos en reposo en interiores y ya no se encontraron portando esporozoítos de *Pf;* buscaron a su huésped

principalmente en el exterior y en interiores antes de que la gente se acostara, fueron responsables del 5,1% y el 5,8% de la exposición humana a los mosquitos buscadores de huéspedes, respectivamente, y su asociación estadística con los casos de la malaria fue más débil que la de *An. arabiensis.* La susceptibilidad a los insecticidas de *An. merus* y *An. squamosus* no pudo ser evaluada porque se encontraron muy pocos *An. merus* y no *An. squamosus* descansando en interiores. *An. merus* fue responsable del 5,2 % de la exposición humana a vectores buscadores de huéspedes, buscó a sus huéspedes principalmente en interiores mientras las personas estaban en la cama y se le encontró portando esporozoítos de *Pf* durante el proyecto. *An. squamosus* fue responsable el 9,9% de la exposición humana a vectores buscadores de huéspedes, buscó a su huésped tanto en el exterior como en el interior en la temporada de baja transmisión, pero principalmente en el interior en la temporada de alta transmisión. En general, la exposición residual a vectores buscadores de huéspedes tuvo lugar principalmente en interiores (95,9 %) y casi un tercio (31,4 %) ocurrió durante la tradicional temporada de baja transmisión. El paquete de intervenciones también redujo las tasas de esporozoítos de *Pf* en los vectores.

La mayoría de las mosquiteras en el distrito eran mosquiteras tratadas con insecticidas en los últimos 12 meses o MTILD. Las mosquiteras se perdieron a una tasa del 31% por año. El acceso a las mosquiteras se mantuvo por debajo del 76,3% durante todo el proyecto. Su uso fluctuó estacionalmente cayendo al 40% en la temporada de baja transmisión y alcanzando un máximo del 76,4% en la temporada de alta transmisión. Los hogares más pobres, más grandes y más difíciles de alcanzar, los niños y las mujeres menores de 30 años tuvieron menos acceso a las mosquiteras que el resto de la población. Su uso fue más bajo en niños en edad escolar y adultos

jóvenes, especialmente entre los hombres. A los niveles observados de uso, las mosquiteras previnieron el 39,2 % de la exposición residual a los vectores buscadores de huéspedes y, si todos los residentes hubieran usado una mosquitera para dormir, éstas podrían haber evitado el 63,3 % de dicha exposición residual.

La cobertura de RRI a nivel del porcentaje de estructuras y hogares rociados, y de personas protegidas, estuvo por encima del 83%. La cobertura fue uniforme en las divisiones administrativas del distrito. La eficacia residual de IRS teniendo en cuenta los resultados de las pruebas de conos de la OMS, se mantuvo por encima del 80% durante más de 6 meses en las campañas de 2016 y 2017. Sin embargo, teniendo en cuenta la cobertura del RRI, el ritmo de rociado y la distribución de los tipos de paredes, la eficacia residual efectiva del RRI en la campaña de 2016 se mantuvo por encima del 80 % solo durante 113 días, no cubriendo toda la temporada de alta transmisión.

Conclusiones

Anopheles arabiensis fue el principal vector durante el proyecto, mientras que *An. funestus s.s., An. merus, An. parensis, An. squamosus*, y posiblemente *An. rufipes,* jugaron, probablemente, un papel secundario en la transmisión. El acceso, uso y protección personal de los MTILD fue subóptimo y desigual durante el proyecto. Además, los MTILD probablemente brindaron protección contra *An. arabiensis* y *An. merus*, cierta protección contra *An. squamosus* en la temporada alta de transmisión, pero protección muy limitada contra *An. funestus s.s.* y *An. parensis.* En el futuro, el acceso a los MTILD podría mejorar mediante la revisión de las estrategias de asignación de mosquiteras durante las campañas masivas de distribución y

mediante el fortalecimiento de los canales de distribución continuada. El uso de MTILD mejoraría al aumentar el acceso, ya que el acceso fue una barrera para el uso durante la temporada de alta transmisión, pero se necesitarán campañas de cambio de comportamiento humano para aumentar aún más el uso de MTILD en la población, especialmente durante la temporada de baja transmisión y entre los niños en edad escolar y los hombres. La cobertura del RRI estuvo por encima del umbral recomendado por la OMS, pero su eficacia residual efectiva no cubrió toda la temporada de alta transmisión. El RRI probablemente fue efectivo para controlar a los *An. funestus* s.s. y *An. parensis* pero mucho menos para controlar a los *An. arabiensis*. Su efecto sobre los *An. arabiensis* y *An. merus* no pudo ser evaluado. Las posibles mejoras futuras del RRI incluyen el uso de productos del RRI de mayor duración (cuando estén disponibles) o la implementación de una segunda ronda del IRS por temporada de alta transmisión (si es posible en medio de las lluvias). La implementación conjunta de RRI e MTILD probablemente brindó una mayor protección en comparación con la implementación de una sola intervención. Sin embargo, se necesitarán intervenciones adicionales para abordar la exposición residual a las picaduras de vectores que no pudieron controlarse con la combinación de MTILD, RRI y AMM. Aunque la evidencia científica para recomendar el despliegue de cualquier intervención de control vectorial adicional en Magude es limitada, las intervenciones que por ahora parecen potenciales candidatas son larvicidas, blindaje del hogar con mallas y pantallas protectoras, señuelos domésticos letales, cebos atrayentes de azúcares tóxicos y la liberación de machos de *An. arabiensis* estériles.

Al igual que el proyecto Garki, el proyecto Magude tampoco pudo interrumpir la transmisión de la malaria a pesar de sus varias ventajas sobre el proyecto Garki, a saber, 1) que

el proyecto Magude se implementó en un área de baja transmisión con acceso gratuito al diagnóstico y tratamiento, 2) que usó MTILD además de RRI y AMM y 3) que utilizó insecticidas residuales de mayor duración para el RRI. Ambos proyectos compartieron dos desafíos comunes: los vectores que buscaron sus huéspedes en el exterior de las casas y una cobertura incompleta de las AMMs. El proyecto Magude no contó con una fase preparatoria o línea de base suficientemente larga, ni con un distrito de control, lo que comprometió su evaluación. Proyectos como el de Magude deberían incluir un distrito de control o una línea de base lo suficientemente larga como para permitir una cuantificación adecuada de su impacto epidemiológico y entomológico.

3. Introduction

3.1 Malaria

Malaria is a vector-borne disease caused by protozoan parasite of the genus *Plasmodium* that is transmitted to humans through the bites of infected *Anopheles* mosquitoes. Malaria has affected humans for thousands of years. It is believed to have contributed to the fall of Rome, derailed military campaigns and wars, jeopardized the American occupation of Cuba after the defeat of the Spanish, slowed down efforts to colonize Africa, obstructed the construction of the Panama and Suez Canals and killed pharaons, popes and emperors (1,2). Today, in the XXI century, it continues to be a major cause of disease and deaths, especially in sub-Saharan Africa. In 2021, malaria was estimated to cause 241 million clinical cases and up to 627 000 deaths worldwide, with more than 95% of those occurring in sub-Saharan Africa and disproportionately affecting the youngest children (3). Although great reduction in malaria burden were achieved between 2000-2015, progress in malaria control has stagnated, and the malaria burden is on the rise since 2015, remaining one of the main causes of mortality and morbidity in sub-Saharan Africa.

Despite its long history, the cause and mechanisms of malaria transmission did not start to be elucidated until 1880, when Charles Louis Alphonse Laveran observed the malaria parasite in the blood of malaria patients (4). Seventeen years passed before Ronald Ross discovered that culicine mosquitoes transmitted avian malaria and described the parasite life cycle in the mosquito (5). One year later, a group of Italian scientists, of which Giovanni Battista Grassi was

the best know, demonstrated that mosquito transmitted malaria to humans, and did so by feeding *Anopheles claviger* mosquitoes on infected patients and demonstrating that a healthy individual got infected through the bites of these mosquitoes (6). In the following two years, the same group of Italian scientists proved that only female mosquitoes transmitted malaria. Since then, years of research were needed to complete the description of the biological cycle of malaria parasites in humans and mosquitoes.

Malaria transmission cycle

Nowadays, five species of *Plasmodium* are known to cause malaria in humans (*P. falciparum*, *P. vivax*, *P. ovale, P. malariae and P. knowlesi)* and more than 40 *Anopheles* species are known to transmit these parasites from human to human. *Anopheles* mosquitoes carry *Plasmodium* sporozoites in their salivary glands. During a blood meal, they inoculate the parasite into a human host. Sporozoites migrate from the skin of the human host to its liver where they invade hepatocytes. Inside of the hepatocytes, parasites undergo asexual replication during up to 10 days and produce merozoites that are released into the bloodstream. Merozoites invade red blood cells where they form immature trophozoites. In this phase, some parasites differentiate into gametocytes (sexual parasite stage) while most continue to develop into mature trophozoites and schizonts (asexual stage) in a 1-3day cycle, they break the red blood cell and release more merozoites. It is this asexual erythrocytic cycle that causes the clinical symptoms of malaria. Male and female gametocytes mature in human's peripheral blood and bone marrow for a period of between 4 and 15 days, depending on the parasite species, after which they travel to its blood capillaries. When *Anopheles* mosquitoes bite a human, they ingest the mature male and female gametocytes which mate and transform into gamete in the midgut

of the mosquito. Micro and macro-gametes fusion to form zygotes which undergo meiosis and transform into motile and elongated ookinetes. These ookinetes penetrate the midgut epithelium of the mosquito and develop into oocyst. The oocysts grow, break and release sporozoites that migrate to the mosquito salivary glands, where they remain ready to be inoculated into another human. Between 10 and 18 days are needed since a mosquito ingests plasmodium infected blood to the time the mosquito becomes infective.

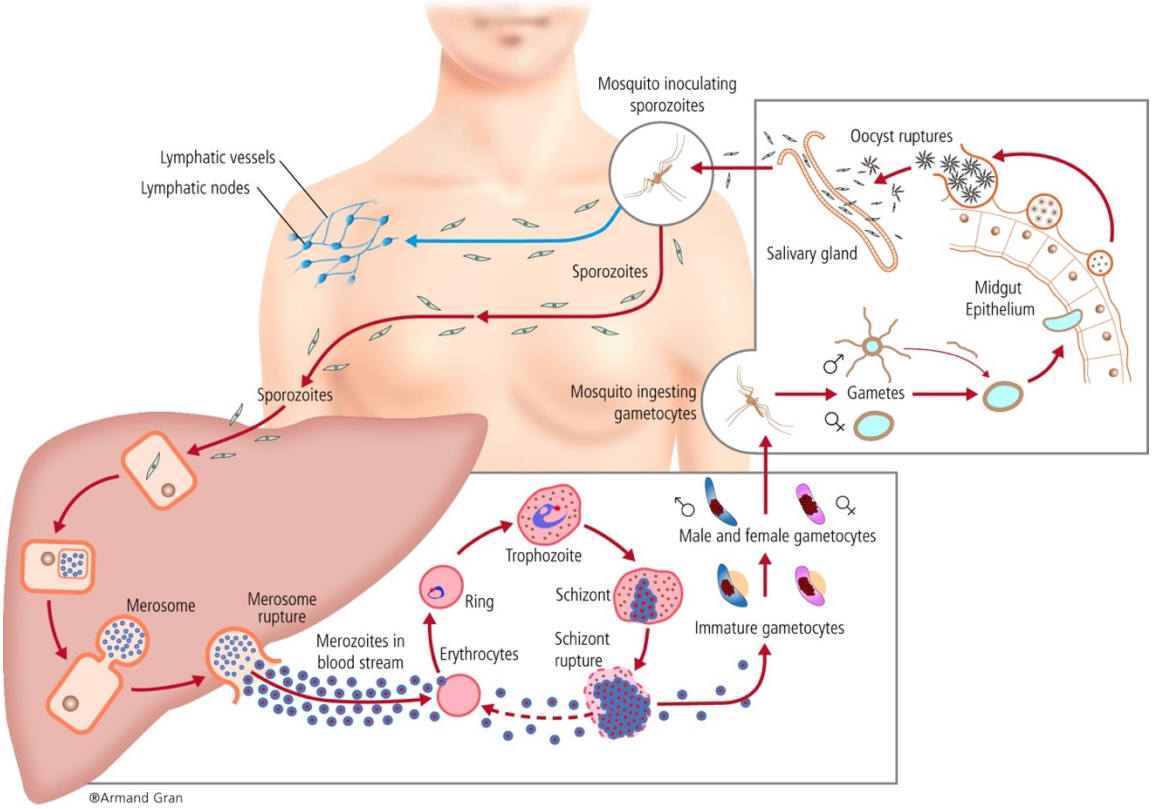


Figure 1 Malaria biological transmission cycle. Source: ISGLOBAL, @Armand Gran

Malaria control tools

The understanding of the transmission cycle allowed for the development of strategies and tools to prevent and treat malaria. Currently, malaria prevention relies on interrupting this cycle through three strategies, 1) controlling the vector so that it does not transmit malaria to

humans, 2) promptly treating malaria patients with antimalaria drugs to reduce the parasite reservoir and 3) protecting humans with a vaccine or chemoprevention therapies so that they do not get infected when bitten by an infective vector.

At present, there are four vector control interventions recommended by WHO, namely insecticide treated nets (ITNs) (pyrethroid-only and pyrethroid-PBO nets), indoor residual spraying (IRS), larviciding and house screening (7). ITNs and IRS are recommended for large scale deployment, while larviciding and house screening are regarded a supplementary intervention to be deployed after high coverage with either IRS or ITNs has been achieved. Several others vector control interventions are at different stages of development, but they have not yet demonstrated their public health value and have hence not received a WHO recommendation.

Malaria can be treated by targeting different stages of the *Plasmodium* parasites during its cycle using drugs (i.e. Aryl aminoalcohol or aminoquinolines, Antifolate compounds and Artemisinin compounds) that either clear asexual stages of the parasite (such as chloroquine or amodiaquine), kill gametocytes so that humans cannot transmit the parasites on to vectors (primaquine and the more novel tafenoquine) or kill *P.vivax* or *P.ovale* hyponozoites to prevent relapses commonly caused by these parasite species (primaquine or tafenoquine). Treatment should be provided to confirmed malaria cases (i.e. parasitologically confirmed). The type of treatment depends on the parasite that caused the infection, on patient's age and pregnancy status, and on the severity of the episode. Hence, diagnosis is key to ensure adequate treatment. At present, different methods exist to diagnose malaria, the three most important ones being: 1) light microscopy, which consists in detecting the parasite by observing Giemsa-stained thick and

thin blood smears, 2) Rapid Diagnostic Tests (RDTs) which are point-of-care devices that detect proteins of parasite's antigens (lactate dehydrogenase (LDH) or histidine-rich protein 2 (HRP2)) in a small sample of human blood and 3) molecular methods, such as polymerase chain reaction (PCR) or loop-mediated isothermal amplification method (LAMP) which identifies plasmodium DNA in blood samples. They differ on their parasite detection threshold and ease of use. Microscopy and RDT have detection thresholds around 50-100 parasites/µL and are easy to use in rural settings. PCR and LAMP have both a higher molecular sensitivity, up to 1parasite/µLm but are significantly costlier and require laboratory facilities.

Chemoprevention therapies and vaccines provide protection against malaria infection for a certain period. Chemoprophylaxis usually involves the use of a lower than treatment dose administered repeatedly during the time a person is at risk of acquiring malaria, and is the strategy typically used by naïve individuals exposed to malaria transmission (e.g. travelers). This strategy is not recommended in malaria endemic areas due to the risk of development of resistance and its poor sustainability. For settings where malaria is endemic, currently available chemoprevention interventions include intermittent preventive treatment of malaria in pregnancy (IPTp), which consist in the administration of three more doses of sulfadoxine-pyrimethamine (SP) once a month to pregnant women starting from the second trimester of pregnancy. Intermittent preventive treatment of malaria in infants (IPTi), which consists in the administration of doses of SP to children aged below 1 year of age at the time of the second and third vaccines doses against diphtheria, tetanus and pertussis (months 2 and 3) and at the time of vaccination against measles (month 9). Seasonal malaria chemoprevention (SMC), which consists in the monthly administration of amodiaquine + sulfadoxine-pyrimethamine (AQ+SP) for

all children aged < 6 years during the high transmission season (usually 3-4 months) and is used only in those countries where seasonality is very marked and little malaria transmission occurs outside of it (e.g. West African Sahel region). The only vaccine recommend so far for malaria prevention is RTS,S/AS01, a pre-erythrocytic recombinant protein vaccine recommended by WHO in 2022 for use in malaria control. WHO recommends to provide RTS,S/AS01 in a four-dose schedule to children from 5 months of age.

Table 1 WHO recommended interventions for malaria control as of May 2022, including description of intervention's mode of action, target population and geographical applicability.

Intervention	Mode of action	Target population	Applicability	WHO recommendation
Indoor Residual Spraying (IRS)	IRS kills mosquitoes that rest indoors on sprayed walls. Since mosquitoes normally rest after feeding, it reduces vector longevity and the chance that a infected mosquitoes can become infected and transmit malaria to the next host.	Entire population	Areas where the bulk of malaria vectors rest indoors after feeding. Note: this intervention is recommended for large scale deployment. It is broadly applicable for populations at risk of malaria in most epidemiological and ecological settings	WHO recommends IRS for the prevention and control of malaria in children and adults living in areas with ongoing malaria transmission. *Strong recommendation, low certainty evidence*
Pyrethroid-only nets insecticide treated nets (ITNS and LLINs)	ITNs kill pyrethroid susceptible vectors that come into contact with the net and repel mosquitoes away from entering. PBO nets kill pyrethroid susceptible and pyrethroid resistant mosquitoes that come into contact with the net. Both prevent surviving vectors from biting net users. By reducing the number of mosquito or their bites, these nets reduce the overall parasite prevalence and hence malaria cases.	Entire population	Areas where the bulk of malaria vectors feed indoors at time when people are sleeping. Note: this intervention is recommended for large scale deployment. It is broadly applicable for populations at risk of malaria in most epidemiological and ecological settings	WHO recommends deployment of pyrethroid-only long-lasting insecticidal nets (LLINs) for the prevention and control of malaria in children and adults living in areas with ongoing malaria transmission *Strong recommendation, high certainty evidence*
Pyrethroid-PBO nets (PBO nets)		Entire population	Areas where the bulk of malaria vectors feed indoors at time when people are sleeping. They should be implemented in areas where mosquitoes are resistance to pyrethroids.	WHO suggests deploying pyrethroid-PBO nets instead of pyrethroid-only LLINs for the prevention and control of malaria in children and adults in areas with ongoing malaria transmission where the principal malaria vector(s) exhibit pyrethroid resistance. *Conditional recommendation, moderate certainty evidence*
Larviciding	Larviciding kills mosquito larvae before thy become adults reducing the vector population	Entire population	Areas where the breeding sites of the main malaria vectors are relatively few fixed and findable. Larviciding is not generally recommended in rural settings. Note: this intervention should be considered for use in areas where optimal coverage with ITNS or IRS has been achieved.	WHO suggests the regular application of insecticides to water bodies (larviciding) for the prevention and control of malaria in children and adults as a supplementary intervention to ITNs or IRS in areas with ongoing malaria transmission where aquatic habitats are few, fixed and findable. *Conditional recommendation, low certainty evidence*
House screening	House screening prevents mosquito entry into houses by screening windows, ceilings, doors and/or eave spaces.	Entire population	Areas where the bulk of malaria vectors feed indoors at times when people are indoors and where houses are suitable for screening. Note: this intervention should be considered for use in areas where optimal coverage with ITNS or IRS has been achieved.	WHO suggests the use of screening of residential houses for the prevention and control of malaria in children and adults in areas with ongoing malaria transmission. *Conditional recommendation, low certainty evidence*

Seasonal Malaria Chemoprevention (SMC)	SMC prevents malaria infection in children.	Children <6 years of age	Areas of highly seasonal malaria transmission throughout the Sahel subregion where IPTi is not implemented and where amodiaquine + SP is efficacious. Note: SMC should not be given to children with severe acute illness or who are unable to take oral medication, or to HIV-positive children receiving co-trimoxazole, or children who have received a dose of either amodiaquine or SP during the past month or children with allergy to either drug	In areas with highly seasonal malaria transmission in the Sahel subregion of Africa, provide seasonal malaria chemoprevention (SMC) with monthly amodiaquine + SP for all children aged < 6 years during each transmission season. *Strong recommendation for, high certainty evidence*
Intermittent preventive treatment in infants (IPTi)	IPTi prevents malaria infection in infants. This population group bares the highest malaria burden.	Infants below 1 year of age	Areas of moderate to high transmission of *P. falciparum* where SP is still effective	In areas of moderate-to-high malaria transmission of Africa, where SP is still effective, provide intermittent preventive treatment with SP to infants (< 12 months of age) (SP-IPTi) at the time of the second and third rounds of vaccination against diphtheria, tetanus and pertussis (DTP) and vaccination against measles. *Strong recommendation for*
Intermittent preventive treatment during pregnancy (IPTp)	IPTp prevents malaria infection in pregnant women from the second trimester. Malaria during pregnancy increase the rate of miscarriage, intrauterine demise, stillbirth, low-birthweight in neonates, and neonatal death, hence beyond protected the pregnant women this intervention protects the infant.	Pregnant women	Areas with moderate-to-high malaria transmission (>250 cases per 1000 population and a prevalence of P. falciparum/P. vivax >10%). Note: SP-IPTi should not be given to infants receiving a sulfa-based medication for treatment or prophylaxis.	In malaria-endemic areas in Africa, provide intermittent preventive treatment with SP to all women in their first or second pregnancy (SP-IPTp) as part of antenatal care. Dosing should start in the second trimester and doses should be given at least 1 month apart, with the objective of ensuring that at least three doses are received. *Strong recommendation, high certainty evidence*
RTS,S/AS01 malara vaccine	RTS,S/AS01 prevents malaria infection in children.	Children from 5 months of age	Areas with moderate-to-high malaria transmission (>250 cases per 1000 population and a prevalence of P. falciparum/P. vivax >10%).	The RTS,S/AS01 malaria vaccine should be used for the prevention of P. falciparum malaria in children living in regions with moderate to high transmission as defined by WHO. *Strong recommendation for, high certainty evidence*
Case management	Case management has the double effect of curing sick individual and reducing the parasite load in the population.	Entire population	All areas with malaria transmission. The choice of diagnostic tool and anti-malaria drug should be based on information about their sensitivity and therapeutic efficacy respectively.	WHO recommends treating all patients with a parasitologically confirmed malaria infection or with suspected severe malaria. In settings where parasitological diagnosis is not possible, a decision to provide antimalarial treatment must be based on the probability that the illness is malaria. The treatment course varies based on the population age, pregnancy status, severity of clinical disease and G6PD deficiency.

3.2 Historical efforts to control and eliminate malaria with an emphasis on the role of vector control

As the understanding of malaria transmission cycle evolved, different tools and strategies to prevent malaria were developed. Vector control was one of the earliest-developed strategies to control malaria Historically, it has been the backbone of malaria control and elimination efforts and it is, nowadays, the most effective and most commonly used prevention strategy (3,8). The development of vector control tools has set landmarks in the history of malaria. From a vector control point of view, the history of malaria control can be divided into four periods: 1) the time before identification of the malaria parasite and its vector, 2) the time after the identification of the parasite and the vector but before the discovery of residual insecticides, 3) the time after the discovery of residual insecticides but before the development of insecticide treated nets and 4) the time after the development of insecticide treated nets. A new era is now starting as novel vector control tools enter the last phases of evaluation and approach WHO recommendation.

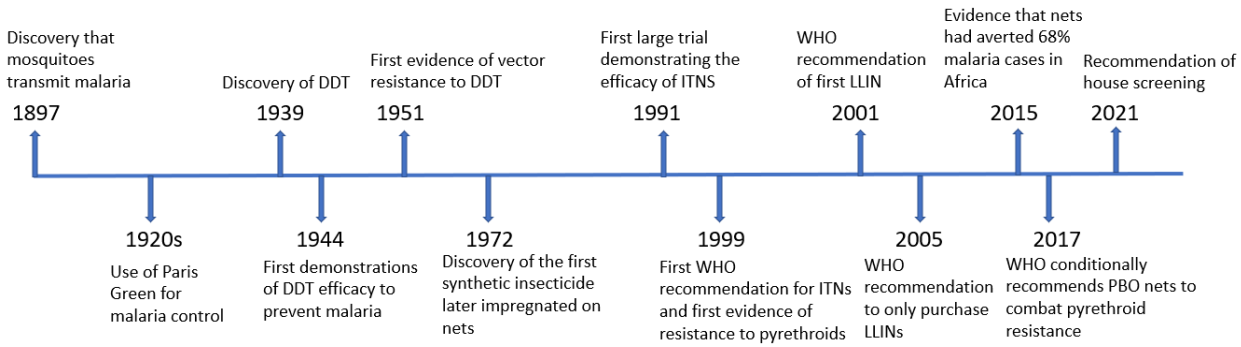


Figure 2 Key moments in the history of vector control. Source: Lucia Fernandez Montoya, prepared for this thesis.

Malaria control before identification of the malaria parasite and its vector (from BC to 1897)

Before the identification of the malaria pathogen and its vector, and since the 4th century BC, malaria was observed to occur in damp, low lying marshy areas and was associated with miasma, a Greek concept to refer to corrupt air, poisoned with noxious vapors (1,9), and to bad drinking water or food contaminated by it (10). Several strategies were observed to protect against malaria at that time, most of which are today considered strategies for preventing contact between mosquitoes and humans. One such strategies already used by the Egyptians was the use of screens or mosquito nets (11) which later became the most important form of vector control worldwide. Others were keeping the doors and windows shut between sunrise and sunset, staying in-doors after sunset, sleeping in lofty towers, making fires, removing houses or barracks from areas close to creeks, prohibiting agricultural practices from certain areas or applying substances to human skin (10,11). Although malaria prevention revolved mainly around different forms of vector control, two herbal treatments to cure malaria were discovered and used in this period by Southern American and Chinese inhabitants, namely extracts of the bark of the Cinchona tree in South America and sweet wormwood (Artemisia Annua) in China. These two herbal treatments would later lead to the isolation of quinine and artemisinin, in 1820 and 1967 respectively, two antimalaria drugs that would play a major role in the history of malaria prevention and treatment (12,13).

Malaria control strategies after the identification of the malaria parasite and its vector (from 1897 to 1939)

The discovery that mosquitoes transmitted malaria to humans in 1897 allowed for the development of rational strategies for the control of malaria. Almost immediately after this discovery, Ronald Ross suggested that if the malaria-bearing mosquitoes were exterminated malaria transmission would cease and suggested that malaria vectors could be eliminated by destructing their larval habitats (14). This immediately triggered research into chemicals that could be sprayed in water and kill mosquito larvae, leading to the development of Paris Green (and arsenic-based compound), one of the larvicides that was most commonly used until the discovery of residual insecticides (15).

Figure 3 Parish Green. Photo credit: Left: Informative poster. United States Department of the Treasury. Free for distribution. Right photo: Power Spray for Paris Green. Science Museum Group. Powder spray for Paris Green, France, 1925-1935. A630670 Science Museum Group Collection Online. Accessed July

30, 2022. https://collection.sciencemuseumgroup.org.uk/objects/co131288/powder-spray-for-paris-green-france-1925-1935-pest-sprayer. Distributed under license CC-BY-NC-SA 4.0

In parallel, Carlos Chagas demonstrated that most of the malaria infection were acquired by the bites of mosquitoes inside of houses, triggering interest in the use of physical barriers to prevent mosquito bites and giving raise to the first malaria control trials. Several trials were subsequently conducted by Angelo Celli , Patrick Manson and Giovanni Battista Grassi and demonstrated that protecting humans from mosquito bites from dawn to dusk by screening their houses prevented malaria (16,17). In 1908, to better understand the dynamics of malaria transmission, Ronald Ross started developing a series of mathematical models to quantify the number of human infections as a function of the number of mosquitoes (18–21). He concluded that malaria transmission could be controlled by bringing vector densities until a certain threshold, not necessarily having to eradicate vectors. These trials and Ross' models provided the theoretical and epidemiological evidence that malaria could be prevented through vector control.

Over the years that followed, vector control strategies such as larval control (e.g. drainage reforestation and use of oils, larvivorous fish and Paris green to kill larvae) and the protection of humans from mosquito bites (e.g. keeping high numbers of animals to divert zoophilic mosquitoes from biting on humans, locating animals sheds further away from housing, house screening and ventilation and the use of bednets) became common strategies to prevent malaria with demonstrated efficacy (1,22–24). However, the existence of areas without malaria but that had *Anopheles* mosquitoes and adequate climatic conditions for malaria transmission (e.g. in areas of Italy or the tropical colonies) casted doubts over Ross' theory and the empirical evidence

on the effectiveness of protecting humans from mosquitoes to prevent malaria (25). The paradox was named the "*Anopheles* without malaria". Attempts to explain such paradox generated different scientific theories and polarized scientific opinion for the next 30 years, dividing scientists into those that favored vector control and the use of quinine to interrupt transmission (e.g. Ross, Gorgas and Watson) and those who favored the strengthening of health systems, environmental sanitation and fostering overall socio-economic development (e.g. the Malaria Commission created by the League of nations (26) and groups of Dutch and Italian scientists) (25–30).

The World Wars and their effect on malaria control

Opinions remained polarized during the World War I, which halted the ample progress made in reducing malaria in Europe and brought about resurgences in areas that had already eliminated or almost eliminated malaria (1). It was finally resolved by demonstrating that there were two species of *An. maculipenis*, a good European malaria vector, and that although they looked morphological identical, one transmitted malaria and another one did not (25). This discovery raised interest in vector control with a focus on understanding the biology and ecology of *Anopheles* species to guide vector control efforts. Further interest in vector control arose from the evidence on the efficacy of Paris green and Gambusia fish for larval control, and of pyrethrum as an adulticide, presented at the first international congress on malaria conducted in 1925 (24,31).

World War II was a cornerstone in the malaria control history. It brought about increases in malaria incidence and mortality but it stimulated the development of new antimalarial drugs, saw the development of DDT, gave birth to the first insecticide treated nets and jungle hammocks, and witnessed the demonstration of great effectiveness of vector control campaigns (32,33).

One of the most remarkable achievements of vector control during World War II was the elimination of *An. gambiae* from Brazil after its introduction from West Africa (34). The program took place between 1930 and 1941 and was led by Frederik Soper, the man that would later push for the establishment of a Global Malaria Eradication Program (35). The successful experience in Brazil was followed to eliminate *An. gambiae* from the Nile Valley Egypt between 1942 and 1945 (36).

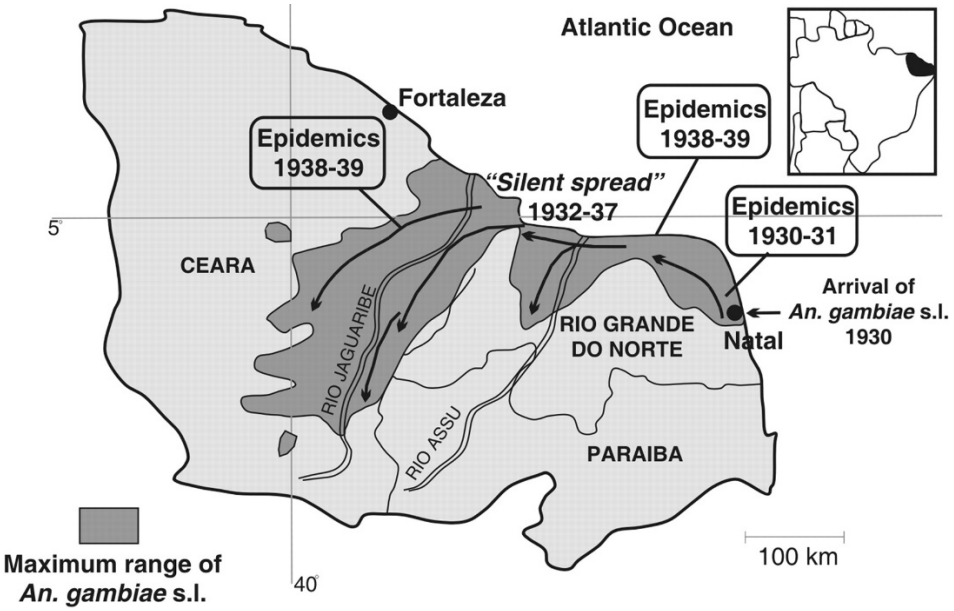


Figure 4 An. gambie s.l. invades brazil Source : Historical Analysis of Near Disaster Anopheles gambiae in Brazil (37)

The discovery of DDT and the Global Malaria Eradication Programme (1939-1980s)

The second remarkable success during the World War II, which became a turning point in the history of malaria control and set vector control as a pillar of malaria control efforts, was the re-synthetization of DDT's and discovery of its properties to kill insects by Paul Herman Muller in 1939 (38). For this discovery, he won the Nobel prize for Physiology or Medicine in 1948. Spraying houses with pyrethrums insecticide to kill indoor resting mosquitoes before they could further transmit malaria had already been used as a vector control strategy since 1930's (39), but these insecticides had a short residual life and, hence, spraying had to be repeated weekly. In contrast, DDT had a long-lasting effect, requiring only annual or semestrial application. DDT was first used in 1944 in Italy where it proved to be highly efficacious against malaria (1). Its use scaled up rapidly and large programs were implemented in 1940's and 1950's further demonstrating its efficacy. Some of these programs even showed that malaria could be eliminated using DDT and that transmission did not resume once spraying stopped (40).

Figure 5 Spraying interior of Italian houses with 10% DDT and kerosene for malaria control. 32nd Field Hospital, Unit B Installation. 02/26/1945. World War 2. Obtained from Wikimedia commons under license CC-BY-2.0.

The optimism built upon the astonishing results obtained by the use of DDT, which seemed an infallible tool, was further supported by McDonald's mathematical explanation of this success (41,42). MacDonald improved both the epidemiological and the entomological parts of Ross's mathematical models of malaria transmission and evaluated the relative importance of different parameters on transmission. He concluded that reducing mosquito longevity, or changing its feeding habits, had a much greater impact on transmission than merely reducing its densities, thereby justifying the impact achieved by DDT.

The success of DDT and the quantitative understanding of its effectiveness triggered momentum towards the establishment the Global Malaria Eradication Program (GMEP) (35,43). Malaria eradication was further supported with economic arguments referring to malaria's impact on local economies and on the higher long-term cost-effectiveness of eradication compared to control. In addition, the emergence of mosquito resistance to DDT in 1951 (44) urged for malaria elimination before the efficacy of DDT would be lost (27). In 1954, a continental plan to eradicate malaria from the Americas was adopted by the XIV Pan American Sanitary Conference and the second Asian Malaria Conference also concluded that eradiation should be the ultimate aim of malaria control programs (30). These regional agreements preceded the adoption of the WHO Global Malaria Eradication Programme (45).

The Global Malaria Eradication Program (1955-1969): highly reliant on IRS with DDT, dieldrin and BHC

The CMEP approved in 1955 by the 8th World Health Assembly of the World Health Organization (resolution WHO8.30). It defined eradication as "the ending of the transmission of malaria and the elimination of the reservoir of infective cases in a campaign limited in time and carried out to such a degree of perfection that when it comes to an end, there is no resumption of transmission". A theoretical eradication framework was developed in 1956 and followed by many countries around the world (46). It consisted of four phases: preparatory, attack, consolidation and maintenance described in Table 2.

WHA8.30 Malaria Eradication

The Eighth World Health Assembly,

Having considered the comprehensive report and proposal on malaria eradication submitted by the Director-General ;

Having examined the recommendations of the XIV Pan American Sanitary Conference in Santiago, Chile, in October 1954 and of the Malaria Conference for the Western Pacific and South-East Asia Regions in Baguio, Philippines, in November 1954, concerning the danger constituted by the potential development of anopheline resistance to insecticides and concerning measures to obviate that danger ;

Considering resolution EB15.R67 adopted by the Executive Board at its fifteenth session after a study of the reports available up to that time ;

Considering that the ultimate goal of malaria-control programmes should be the eradication of the disease,

I. 1. REQUESTS governments to intensify plans of nation-wide malaria control so that malaria eradication may be achieved and the regular insecticide-spraying campaigns safely terminated before the potential danger of a development of resistance to insecticides in anopheline vector species materializes ;

2. AUTHORIZES the Director-General to request those governments in whose countries malaria still exists to give priority to malaria eradication projects in their requests for assistance under the United Nations Expanded Programme of Technical Assistance, and to provide the locally available resources which are required to achieve malaria eradication ;

II. DECIDES that the World Health Organization should take the initiative, provide technical advice, and encourage research and co-ordination of resources in the implementation of a programme having as its ultimate objective the world-wide eradication of malaria ;

III. 1. AUTHORIZES the Director-General to obtain financial contributions for malaria eradication from governmental and private sources ;

2. ESTABLISHES, under Financial Regulations 6.6 and 6.7, a Malaria Eradication Special Account, which shall be subject to the following rules :

(1) The Special Account shall be credited with voluntary contributions received in any usable currency and shall also be credited with the value of contributions in kind, whether in the form of services or supplies and equipment.

(2) The resources in the Special Account shall be available for incurring obligations for the purposes set out in (3) below, the unexpended balances of the Account being carried forward from one financial year to the next.

(3) The Special Account shall be used for the purpose of meeting the costs of :

(*a*) research ;

(*b*) such supplies and equipment, apart from minimal requirements to be provided from regular and Technical Assistance funds, as are necessary for the effective implementation of the programme in individual countries ; and

(*c*) such services as may be required in individual countries and as cannot be made available by the governments of such countries.

(4) The operations planned to be financed from the Special Account shall be presented separately in the annual programme and budget estimates, this presentation to include an indication as to whether the resources required are known to be available in the Special Account or from another source.

(5) In accordance with Financial Regulations 6.6 and 11.3, the Special Account shall be maintained as a separate account, and its operations shall be presented separately in the Director-General's annual financial report.

IV. AUTHORIZES the Executive Board or a committee of the Board to which it may delegate authority to act between sessions of the Board to carry out the following functions :

(1) to accept contributions to the Special Account as provided under Article 57 of the Constitution ; and

(2) to advise the Director-General from time to time on any questions of policy relating to the administration of the Special Account or to the implementation of the programme.

Handb. Res., 2nd ed., 1.3.10 ; 7.1.5

Ninth plenary meeting, 26 May 1955 (section 1 of the third report of the Committee on Programme and Budget)

Figure 6 World Health Assembly's resolution for Malaria eradication (WHA.8.30)

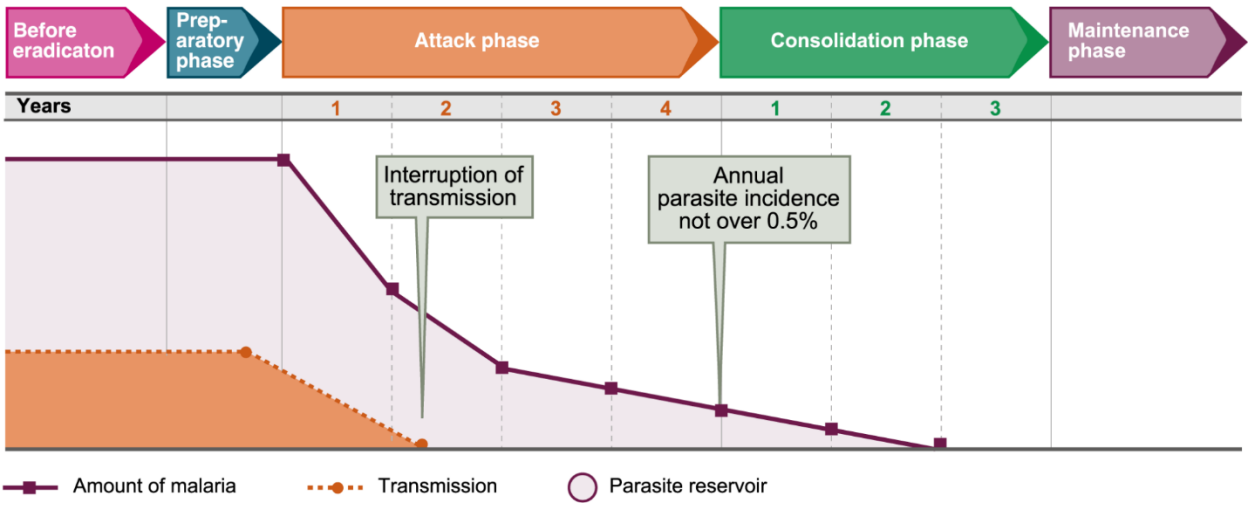


Figure 7 Phases of the malaria eradication campaigns during the Global Malaria Eradication Program. Source (27)

Table 2 Phases of the malaria eradication campaigns during the Global Malaria Eradication Program [49]

Phase	Description and sub-phases
P**reparatory**	Up to a year of duration and consisting of three core activities: **Initial survey:** to delimit the malicious areas and establish the order in which areas will be attacked. The emphasis was on malaria distribution **Planning:** schedules and estimates of operations prepared based on results survey and available facilities for the attack phase. **Preliminary operations**: recruitment and training to staff at all levels. Setting up offices, SOPs, manuals and other essentials. Enumerating houses to be sprayed, design itineraries for spraying, transportation and supply systems designed. Carry out a pilot operation to test the prepared plan and its allocated resources.
Attack	Attack on the mosquito vector based on the application of residual insecticides according to well-acknowledge techniques on a total coverage basis. This phase was supposed to be time-bound but with spraying only stopping when transmission has been stopped and the number of infective carriers reduced.
Consolidation	Aims to eradication all residual pockets of transmission and to bring the human parasite reservoir to zero, based on case finding, the use of antimalaria drugs and focal spraying when needed. Surveillance was a key component of this phase and had to be intensive and complete. This phase was meant to be carried out by public health services and to end after three years of active surveillance had shown no indigenous cases.
Maintenance	Would last as long as malaria exists in the world. Malaria should be added to the list of non-endemic disease of mandatory reporting and for which the country should be always on alert. Imported and introduced cases should be handled routinely by the national health departments.

The attack phase relied heavily on perfectly executed spraying campaigns with residual insecticides to reduce the chances that mosquitoes would survive long enough to become infective and, it was only occasionally supported by the use of anti-malarial drugs when results with residual insecticide were below expectations. It largely disregarded any other vector control measures previously used (27). Three residual insecticides, all chlorinated hydrocarbons with similar mode of action, were commonly used at the time: DDT, dieldrin and benzene hexachloride (BHC) (46) although DDT became the most commonly used insecticides due to its longer residual efficacy (i.e. 6-12 months) (46). Unfortunately, vector resistance to all these chlorinated hydrocarbons emerged rapidly and spread over all regions of the world and across several vector

species (46–48). By 1956, resistance was identified as one of the bigger challenges to malaria control (46).

The GMEP lead to the elimination of malaria in 15 countries and one territory (Bulgaria, Cyprus, Dominica, Grenada, Hungary, Italy, Jamaica, Netherlands, Poland, Romania, Saint Lucia, Spain, Taiwan, Trinidad and Tobago, United States of America, Venezuela) (49). However, the program failed in other regions and proved unsustainable overall mainly due to the following reasons: 1) the emergence and spread of vector resistance to DDT, dieldrin and BHC and of parasite resistance to chloroquine and pyrimethamine, 2) the ability of vectors to evade contact with IRS by resting outdoors, 3) the human habit of sleeping outdoors in some communities, 4) general failures to interrupt transmission in some settings despite the correct application of the attack phase, 5) resurgence and outbreaks of malaria in some areas during the consolidation phase, 6) poor ability and guidance to establish surveillance systems during the consolidation phase, 7) return from the consolidation to the attach phase in some areas and 8) weak health systems, human capacity and financial constrains to sustain expensive elimination efforts (27). As a result, the GMPE was abandoned in 1969 at the 22nd WHA and focus shifted to malaria control, although ultimate goal of eradication was maintained (50).

In the years to follow, the focus was on controlling malaria through national health systems (27). These years saw a reduction of funding for malaria control (in favor of investments in health systems strengthening) due to the wars of independence and political conflict in countries, to the perception that malaria was no longer a major problem, to natural disasters

and to the global economic crisis of the 1970s (27,51). The crisis led to increased exploitation of natural resources in some countries, which inflicted changes in the environment that led to great malaria epidemics, increasing insecticide prices that reduced the affordability of malaria control and to the trade of suboptimal malaria drugs and regimes, fostering the development of parasite resistance. Despite the abandonment of elimination as an immediate goal and reduced funds for malaria control, seven additional countries and one territory (Australia, Brunei, Cuba, Mauritius, Portugal, Réunion, Singapore, and Yugoslavia) eliminated malaria until 1987 (49). However, in general, the burden of malaria increased to the point that it reverted back to pre-GMPE levels in some countries and vector resistance to DDT and parasite resistance to chloroquine continued to spread (49,51).

The development of Insecticide Impregnated Nets (ITNs)

The problems faced during the 1970's manifested the need for new tools and strategies to control malaria. This triggered interest in different insecticides and on bednets, a form of protection against mosquito bites that had been used in different regions of the world since ancient times. At the time, the agricultural sector had realized that natural pyrethrin, chemical compounds extracted from Chrysanthemum cinerariaefolium were effective and economic insecticides and much less toxic for humans and the environment that other available options. However, they were too unstable upon exposure to light to be effectively used (52). In the 1970s, British and Japanese scientists managed to develop synthetic pyrethroids that were more photostable than organophosphates and carbamates available at the time, that were metabolized rapidly by mammals and that had limited persistence in soils and greater potency

than other insecticides (53–55). Realizing that the structure of these insecticides made them suitable for impregnation into nets, the malaria community started to investigate the use of insecticide-impregnated nets for malaria control (56,57).

In 1984, Frédéric Darriet and Pierre Carneval compiled evidence of the efficacy on intact and torn permethrin-treated nets against malaria vectors, concluding that they substantially reduced human-vector control significantly preventing malaria (58,59). Subsequently, the epidemiological impact of insecticide treated nets was demonstrated in The Gambia through a large clinical trial (60). Overwhelming evidence generated through 22 trials conducted in sub-Saharan Africa, Latin America, the Middle East and Asian countries in areas of stable and unstable transmission further confirmed the efficacy of ITNs at preventing malaria (61), setting the scene for a new era of malaria vector control. In parallel, the efficacy of artemisinin-based combination therapies started to be demonstrated in the first clinical trials too (62,63).

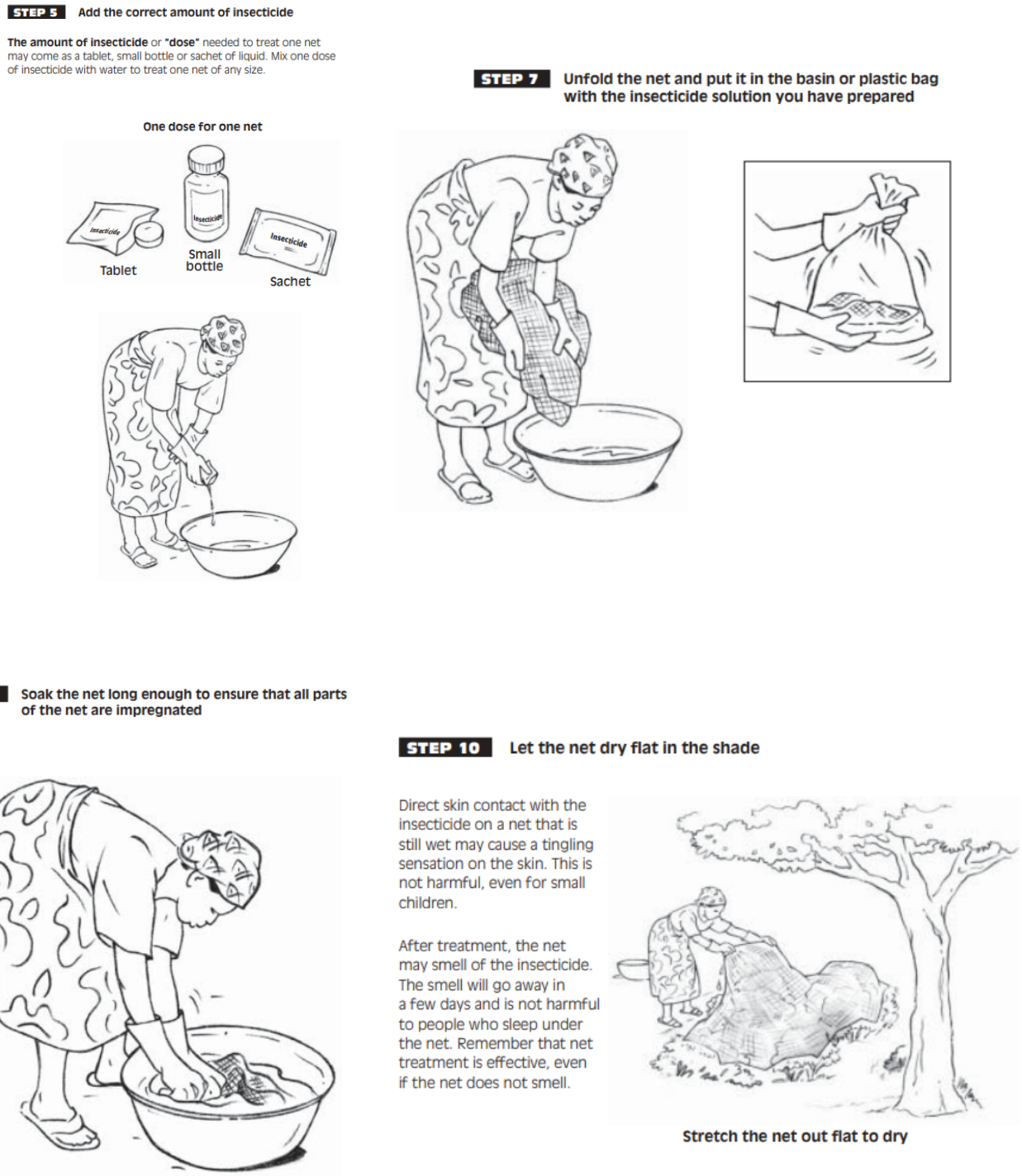


Figure 8 Instructions for treatment of mosquito nets. Taken from Instructions for treatment and use of insecticide-treated mosquito nets, WHO, 2002 (64)

The resurgence of interest in malaria control during the 1990s and early 2000s

Global momentum to reduce the burden of malaria was reignited after the Ministerial Conference on Malaria that took place in Amsterdam in 1992. The conference was convened due to the increasing gravity and complexity of malaria and the neglect of its control at the time. It emphasized 1) that there was no universally applicable strategy to control malaria, 2) that strategies had to be designed based on specific country contexts and made sustainable, 3) that only a few countries with the right conditions should aim at elimination, 4) that it was important to establish robust health and social systems, 5) that malaria programs had to be integrated into general health systems and 6) it encouraged research to develop new tools and optimize implementation of existing ones. The conference yielded a "World declaration on the control of Malaria" and endorsed a new Global Malaria Control strategy, developed based on the aforementioned principles, that was adopted by the World Health Assembly in 1993 (65). The discussion during the conference reflected a clear shift in paradigm with respect to the GMEP days that would shape malaria control efforts over the coming years, namely a change towards adapting strategies to country context and ensuring the sustainability of efforts.

Since then, several declarations, conferences and partnerships were convened and created, which increased funding and interest in malaria control. In 1996, the World Health Organization established a special program on malaria. In 1997, amid economic recovery in Africa and acknowledging the social and economic burden of the disease, African countries adopted the Harare declaration for Malaria Prevention and Control and a five-years African Plan of Action (66), which made emphasis on the importance of sustainability of malaria prevention and control

efforts and called for conducting inter-disciplinary research to improve malaria control. With regards to vector control, it promoted encouraging populations to use preventive measure such as house screening, personal protection measures and mosquito nets and make a selective use of vector control measures. Shortly after, and to address the need to improve research efforts for malaria control, the Multilateral Initiative on Malaria (MIM) was launched in Dakar in 1997 (67).

The 1990s demonstrated the potential for achieving significant reduction in malaria through strategies available at the time such as early treatment of suspected cases with effective drugs, use of insecticide-treated nets to prevent infection and ability to predict malaria epidemics to trigger early response. In 1998, the WHO launched the Roll Back Malaria initiative, which was created as a partnership between the World Bank, United Nations International Children's Emergency Fund (UNICEF), United Nations Development Program (UNDP) and WHO to halve malaria burden by year 2020 and reduce it further in subsequent years (68). In 2000, the Abuja declaration on Roll Back Malaria in Africa, set yet more ambitious and specific goals committing to halve the malaria mortality in Africa by 2010, by 1) providing adequate treatment for 60% of those suffering with malaria within 8 hour of symptoms onset, 2) covering 60% of people at risk of malaria with personal and community protective measures such as ITNs and 3) provide to at least 60% of pregnant women with access to chemoprophylaxis and presumptive intermittent treatment (69). The 1990's ended with a recommendation by the WHO Pesticide Evaluation Scheme (WHOPES) for pyrethroid-impregnated nets (70). Paradoxically, the same year when the

WHOPES recommended the use of pyrethroid-impregnated nets (1999), the first report of vector resistance to pyrethroids was published (71).

The years 2000s to 2010s was the decade when many of the institutions, initiatives and partnerships that nowadays support global malaria control and elimination efforts were created and, as a result, when the scale up of interventions started and malaria eradication was put back on the global agenda. This decade saw the formation of new entities that are nowadays key donors for malaria control, such as the Bill and Melinda Gates foundation (2000); the Global Fund to Fight AIDS, Tuberculosis and Malaria (2002) and the United States President's Malaria Initiative (2005); and of key accelerators for the development and testing of new drugs, diagnostics and insecticides, such as Medicines for Malaria Venture (1999), the Program for accelerated action on HIV/AIDS, malaria and tuberculosis in the context of poverty reduction; the European And Developing Countries Clinical Trials Partnership (EDCTP), the Foundation for Innovative Diagnosis (FIND), Unitaid (2006) and the Innovative Vector Control Consortium (IVCC) (2005). In addition, UNICEF expanded its investment in improving access to ACTs, ITNS and LLINs (72). The 2000's also saw the first global malaria report (2005). WHO recommendation for ACTs, triggered by the reduced efficacy of chloroquine (CQ) and sulfadoxine pyrimethamine (SP) and the WHO recommendation to use artesunate and artemisinin suppositories for pre-referral treatment of severe malaria (2005)(72).

The development of Long-Lasting insecticide treated nets

In the vector control space, the use of DDT was banned for all purposes expect for malaria disease control, in 2001 in the Stockholm convention, due to its demonstrated toxicity to humans (73). The insecticide impregnated nets recommended by WHOPES in 1999 were mainly made of polyester and deteriorated fairly fast. They remained effective at killing mosquitos for less than a year without washing but lost most part of their efficacy within two washes (70). Hence, to remain effective, these nets had to be manually treated at least once yearly or after two washes. At that time, it was recommended to distribute ITNs to vulnerable groups, such as children under five and pregnant women (74). Although ITNs had demonstrated to be highly effective, and despite RBM efforts to scale up net coverage, high coverage could not be achieved because of the high cost of ITN and the need for frequent insecticide retreatments (75). Research and development efforts were intensified to create an improved product, leading to the development of the first Long Lasting Insecticide Treated bednet (LLIN), Olyset®, and to its subsequent recommendation by WHOPES in 2001. The second LLIN, Permanent®, was recommended shortly after (76,77). These new nets were made of polymers, were more resistant and had long lasting insecticide impregnation. Over the following years, nets were further improved by introducing new yarns and kitting patterns and new insecticide impregnation technologies that made the insecticide impregnation last longer (78).

Until the year 2003, most nets in countries were still untreated nets, but the use of ITN was already considered one of the most effective malaria prevention methods and some countries had established national impregnation campaigns or programs (75). By 2007, due to

the weak health systems and poverty in the population at risk of malaria, only 15% of children under 5 and pregnant women were sleeping under the net. On the same year, and to scale up coverage, WHO issued a position statement recommending programs to "purchase only long-lasting insecticide nets", to aim for "full coverage of all people at risk" and stated that "the best opportunity for rapidly scaling-up malaria prevention was the free or highly subsidized distribution of LLINs through existing public health services" (78). In the following years coverage increased dramatically. By 2015, 55% of people were sleeping under a net and, on the same year, it was demonstrated that ITN were accountable for 68% of the malaria cases averted in sub-Saharan Africa since 2000 (8).

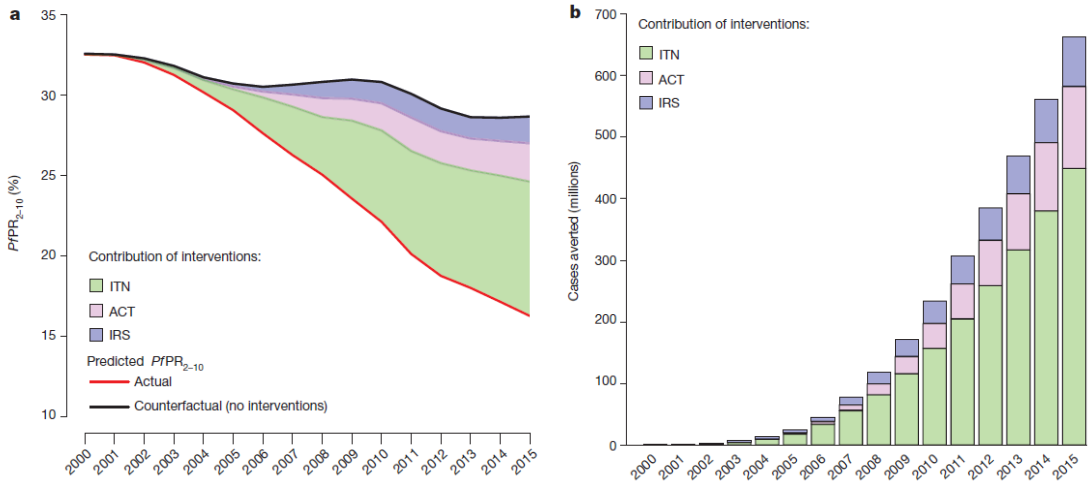


Figure 9 Effect of ITN of malaria control between 200o and 2015. a, Predicted time series of population-weighted mean PfPR2–10 across endemic Africa. The red line shows the actual prediction and the black line a 'counterfactual' prediction in a scenario without coverage by ITNs, ACTs or IRS. The coloured regions indicate the relative contribution of each intervention in reducing PfPR2–10 throughout the period. b, The predicted cumulative number of clinical cases averted by interventions at the end of each year, with the specific contribution of each intervention distinguished. Results shown in both panels are derived from a Bayesian geostatistical model fitted to 527,573 PfPR survey points; n524,868 ITN survey points; n596 national survey reports of ACT coverage; n5688 country-year reports on ITN, ACT and IRS distribution by national programs; and n520 environmental and socioeconomic covariate grids. Panel b

additionally incorporates data from n530 active-case detection studies reporting P. falciparum clinical incidence. Source: Bhatt el at. (8)

A new call for malaria eradication: late 2000's until today

After the Global Malaria Eradication Program failed to achieve global malaria eradication, eradication disappeared from the global agenda until the year 2007. In October of that year, encouraged by the increasing global commitment and funding for malaria, the progress in developing a malaria vaccine, new malaria drug and insecticides, the rapid increase in ITN and ACTs coverage, the Bill & Melinda Gates Foundation (BMGF) called for malaria eradication (79) and funded the creation of a research agenda for malaria eradication (MalERA) (80).

The BMGF's call for eradication, triggered extensive discussions within the malaria community about the actual feasibility of eradication and the potential chronogram and deadline for such an ambitious aim. It divided expert opinion between those that believed that malaria could indeed be eradicated in the foreseeable future and those that believed that the call was still unrealistic. WHO created the Strategic Advisory Group on Malaria Eradication (SAGME) to evaluate the feasibly of malaria eradication and their report concluded that "it is impossible to set a target date for malaria eradication, to formulate a reliable operational plan for malaria eradication or to give it a price tag" because they predicted that there will be still 11 million cases annually by 2050 (81). In contrast, as stated in a Lancet Commission "Malaria eradication within a generation: ambitious, achievable and necessary" published in 2018, other researchers believed that malaria "can and should be eradicated before the middle of the 21st century". Nonetheless, since the call by the BMGF, the world malaria community agrees that the ultimate

goal should continue to be “a world free of malaria”, as reflected in the “Global malaria action plan for a malaria free world” established by the RBM in 2008 (82) and the more recent WHO Global Technical Strategy for Malaria 2016-2030 (83). But concerns remain about whether malaria elimination is feasible in sub-Saharan Africa, a region where no country has yet managed to eliminate it and where attempts to eliminate malaria have repeatedly failed.

3.3 History or malaria elimination in Africa, feasibility, attempts and lessons learnt for vector control

Interest to control malaria in the African continent was triggered by the need to protect European settlers during the colonization era. The first attempts to control malaria were restricted to urban areas and industrial enterprises and based on larval control (drainage or larviciding) and on personal protection with house screening or with chemoproxylaxis (84). After the first world war, and due to the influence of Sir Ronald Ross on African public health policies in the territories administrated by European countries, larval source management became a common practice in the rapidly growing cities and was used together with distribution of quinine for personal protection or through mass drug administration (85). The interest in controlling malaria in Africa increased after World War II when Africa became geopolitically a strategic region due its high food production and high yield of natural resources that were essential for the reconstruction of European countries after the war (86).

The feasibility of malaria elimination in Africa was a subject of active discussion among malariologists of the time. Compared to large areas of Europe, malaria transmission in Africa was more intense, stable, perennial, and driven by vectors with longer lifespan, highly anthropophilic and who bite humans very regularly, making them much more likely to transit malaria than vectors in Europe. This was expected to make elimination a greater challenge in Africa than in Europe (28,41,87).

The discovery of residual insecticides brought the possibility to expand malaria control in Africa from urban to rural areas and several countries started using IRS to control malaria. At the time *An. gambiae* and *An. funestus*, known to be the two main vector of transmission were believed to be highly anthropophilic. The first successful IRS campaign took place in KwaZulu-Natal, South Africa, in 1931, and used short-lasting pyrethrums. After longer-lasting residual insecticides were discovered in 1940s, several large-scale IRS operations with DDT or BHC were conducted in South Africa, Southern Rhodesia (Zimbabwe), Swaziland, the central high plateau of Madagascar, Mauritius and Liberia and some smaller scale ones in the Kipsigis reserve in western Kenya and the mountains of Rwanda-Urundi (currently Rwanda and Burundi). Some of these efforts were complemented by chemoprevention, although chemoprevention was regarded at the time as a complementary method to residual insecticides. The results were very positive. There were great reductions in the burden of malaria and in the risk of epidemics almost everywhere. In southern KwaZulu-Natal, the densely populated residential plateau of Mauritius and Monrovia area (Libera) malaria seemed eliminated and in some places vectors were

practically eradicated, e.g. *An. funestus* was eradicated in Mauritius and *An. gambiae* reduced by 98% (39,88).

The hope for eliminating malaria in sub-Saharan Africa was elicited by the encouraging results of these projects but it was noted that these successful projects had been implemented in areas with favorable conditions (e.g. areas of low transmission of South Africa or the mountainous regions of Uganda-Urundi) and that there was a need to demonstrate feasibility of elimination in more challenging rural settings of Africa presenting stable malaria transmission (89). Doubts existed on the feasibility of eliminating malaria in mainland rural Africa. In a report to the first Conference for malaria in Africa (Nigeria, 1950), MacDonald pointed that there was no reason to think that residual insecticides would not lead to malaria elimination in areas of stable transmission in Africa, but that, given the nature of African vectors, higher mosquito mortalities (up to 85% in some areas) would be needed to achieve elimination in Africa implying higher costs than in Europe (41). Once more, expert opinion was divided. This time into those that favored large-scale deployment of IRS in all African countries based on the aforementioned successes and those that feared that large IRS programs may reduce the acquired immunity of the population living in highly endemic areas and cause severe epidemics. The conference concluded with a recommendation to governments responsible for the administration of African territories that "malaria should be controlled by modern methods as soon as possible, whatever the degree of endemicity, and without awaiting the outcome of further experiments". Modern methods referred largely to IRS with residual insecticides (90).

Following that conference, several malaria elimination pilot projects started in different ecological, mainly rural, settings of Africa with the aim to identify cost-effective strategies to eliminate malaria that could be scaled up to achieve elimination in the continent. A review of these projects for the second African malaria conference concluded that elimination could be achieved with residual insecticides. However, projects faced multiple challenges and it was noted that although there were good examples of successful control with residual insecticides in African mainland regions and islands, full and sustained control had not yet been attained in any large mainland area (91). In view of the challenges in some settings, the value of chemoprevention as a complement to IRS was also discussed and a call was made for the implementation of pilot projects to provide evidence on the effect of combining IRS and chemoprevention or of implementing chemoprevention alone (91).

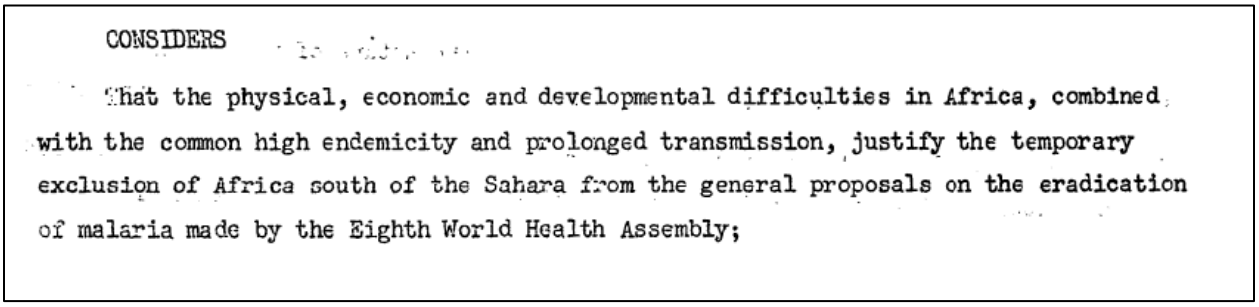
Common challenges faced by the projects conducted up to the second African malaria conference were 1) that achieving full coverage with IRS was challenging because remote areas were hard to reach due to the poor infrastructure, absence of maps, limited and poorly trained IRS staff and lack of supervisors; 2) that the effect of residual insecticides was neutralized upon contact with certain wall materials, forcing researchers to often vary insecticide doses and formulations to find the right fit for local housing; 3) that applying good quality IRS was challenging due to the rudimentary and often roughly handheld equipment; 4) uncertainty in the duration of insecticide's residual efficacy and the fact that short duration sometimes required frequent rounds of IRS but the rainy season hampered access to some areas, impeding the needed frequent spraying cycles; 5) difficulties to reach the high levels of mosquito mortalities,

required in high transmission settings to bring the basic reproduction rate (R0) below 1, as a result of poor IRS coverage, vector exophily and the emergence of vector resistance to the used insecticides (39,91).

At the time, entomological knowledge was limited and considered insufficient to guide vector control campaigns. *An. funestus* and *An. gambiae* were considered the two main vector of transmission but they were not yet considered species complex as they are today. The feeding and resting behaviors of *An. gambiae* varied greatly from location to location posing challenges for control by IRS, which targeted solely indoor resting vectors. Such diversity of behaviors led entomologist to suspect the existence of different species of this vector but molecular techniques for their distinction were yet under development. Techniques were also lacking for studying the mechanisms of resistance and why some vector populations were able to develop resistance to all dieldrin, DDT and HBC and others just to one or to none of these insecticides. Likewise, although some vectors were known to be anthropophilic, techniques were lacking to determine the type of blood they fed upon.

The second African Malaria conference concluded that some of the problems encountered by the pilot projects, especially those related to poorly developed infrastructure and administration and the high endemicity compared to other regions of the world, were intrinsic to Africa and recommended the temporary exclusion of sub-Saharan Africa from the Global Eradication Program, and to focus efforts on rapidly expanding malaria control to attain national protection while conducting research in parallel to inform elimination efforts (91). The Expert

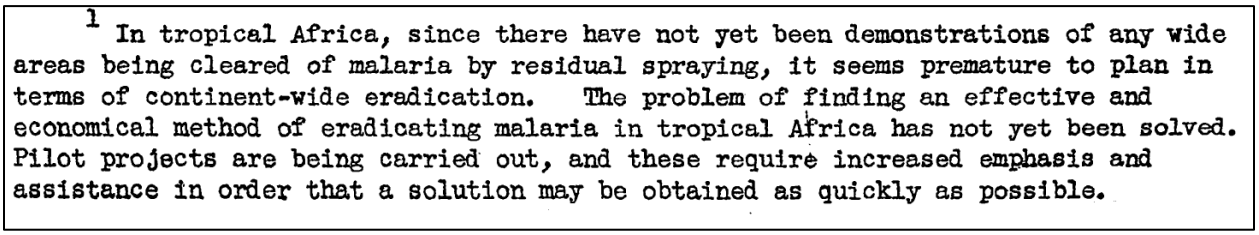
Committee on Malaria subsequently indicated that it was premature to plan a continent-wide eradication campaign in Africa and by recommending that WHO should provide support to the ongoing elimination pilot projects in the continent so as to find a solution for Africa as soon as possible (46).



CONSIDERS

That the physical, economic and developmental difficulties in Africa, combined with the common high endemicity and prolonged transmission, justify the temporary exclusion of Africa south of the Sahara from the general proposals on the eradication of malaria made by the Eighth World Health Assembly;

Figure 10 Conclusion from the second African malaria conference regarding the feasibility of malaria elimination in Africa. Source: Report of the second African malaria conference conducted in Lago, Nigeria in 1956 (91)



1 In tropical Africa, since there have not yet been demonstrations of any wide areas being cleared of malaria by residual spraying, it seems premature to plan in terms of continent-wide eradication. The problem of finding an effective and economical method of eradicating malaria in tropical Africa has not yet been solved. Pilot projects are being carried out, and these require increased emphasis and assistance in order that a solution may be obtained as quickly as possible.

Figure 11 Conclusion from the sixth meeting of the Expert Committee on Malaria regarding the feasibility of malaria elimination in Africa. Source: Sixth meeting of the Expert Committee on Malaria (46)

Pilot projects continued to be conducted in Africa after the second African malaria conference, some solely based on IRS, some combining IRS and chemoprevention and some testing chemoprevention alone through different distribution strategies (direct administration during IRS campaigns or salt-based administration) (89,92). Malaria transmission was only interrupted in some west African forest areas of Liberia and Cameroon (93) and in high latitude

areas of Uganda (94) but pilots conducted in lowland holoendemic savannah areas of Tanzania (Pare Taveta and Tanganika), Nigeria (Kankiya and Sokoto), Cameroon (norther regions) or Burkina Faso (Bobo-Dioulasso) failed to interrupt malaria transmission (95–98). These projects demonstrated that in most areas, IRS alone or in combination with chemoprevention, or chemoprevention alone whether neither sufficient to interrupt transmission, even in cases where very good intervention coverage was achieved such as in northern Cameroon (92). They also demonstrated that in areas where malaria was interrupted or almost interrupted, the gains could not be sustained over time due to weak public health services and infrastructure (89,91,92).

The challenges faced by the unsuccessful pilot projects implemented after the second African malaria conference (1956-1962) included, among others: 1) impossibility of achieving and sustaining total coverage with either IRS and/or chemoprevention due to logistical challenges or lack of population acceptance of the interventions; 2) the exophilic and exophagy of *An. gambiae* that allowed them to evade IRS; 3) expanding vector resistance to dieldrin and DDT (specially in western Africa); 4) population misuse of salts treated with anti-malarial drugs; 5) fast emerging parasite resistance to pyrimethamine; 6) population habits to sleep outdoors; 7) population movements from highly endemic areas into the pilot project areas. In addition, in the areas where malaria transmission was interrupted, gains could not be sustained due to the weak and ill-trained public health services that could not successfully manage the consolidation phase (30,92)

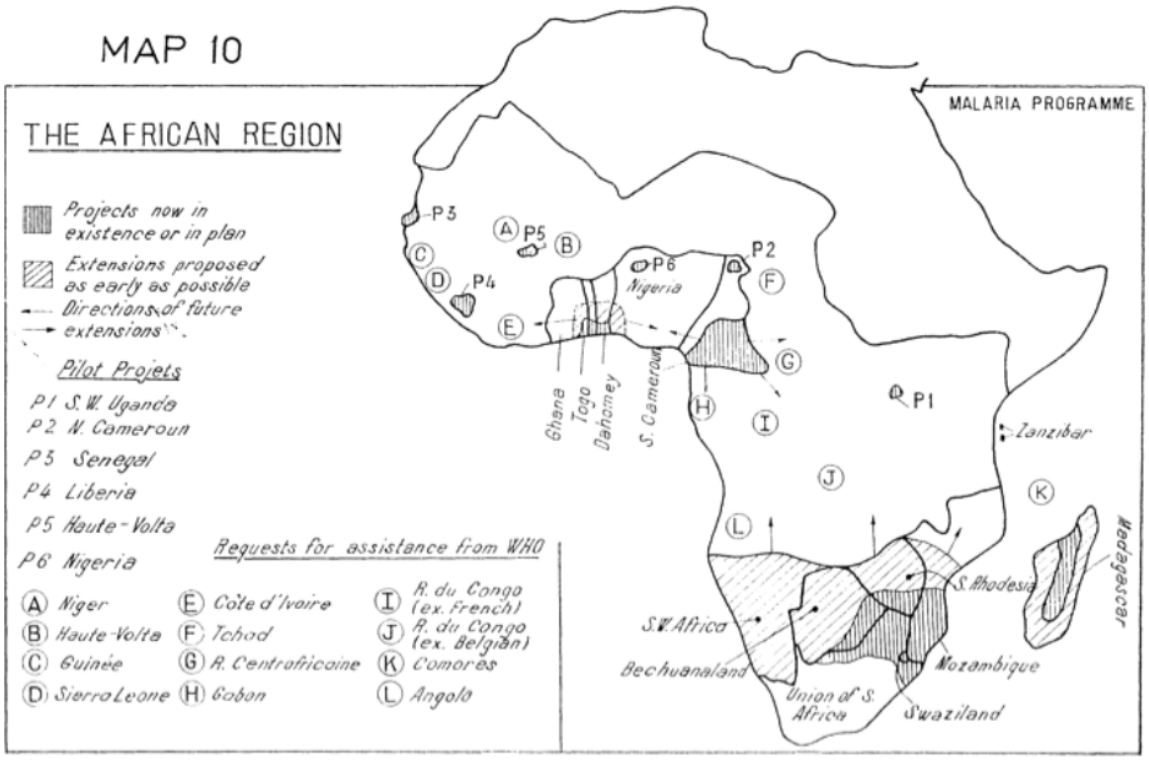


Figure 12 WHO malaria eradication pilot projects in 1960. Source: AFRO Malaria Year Book No. 2, Regional Office for Africa, WHO, 1960. Obtained from a reproduction in: The Global Challenges of Malaria: Past lessons and Future prospects (39)

The experience of the 1950's and 1960's led to the following conclusions: 1) IRS in combination with chemoprevention achieved high reduction in malaria transmission, but 2) the interruption of malaria transmission with IRS or IRS and chemoprevention was not feasible in all areas of Africa and, 3) in those that were, the available infrastructure and national health services were not sufficiently developed to sustain it over time. As a result, several countries move towards the establishment of nation-wide pre-eradication programs. These programs aimed at building the national health services and public health infrastructure to the level that they could support elimination (92). However, upon independence, many African countries canceled their malaria pre-eradication programs as they did not consider malaria as big of a problem as their

western administrators did (39). The idea of achieving malaria elimination in Africa in the short-term was finally abandoned and focus shifted mainly towards control as an integral part of national health systems of the newly independent countries (30,92). Nonetheless, the failure of the pilot projects implemented in the 1950s and 1960s were not fully understood. The epidemiological and entomological data collected during the 1950s and the 1960s left behind gaps in knowledge about the dynamics of malaria transmission in Africa and the performance of IRS and chemoprevention that WHO would aim to fill in 1969 through the Garki Project.

The Garki project, Nigeria (1969-1976)

The Garki project (99) was conducted in a highly endemic area of Nigeria (the Garki district) between 1969 and 1976. It aimed to improve the quantitate understanding of the dynamics of malaria transmission, the impact of IRS and MDA in such dynamics and to generate a mathematical model that will "identify and quantify factors of significance in the control of malaria". The project was a cluster trial that implemented the following three different control strategies in three different clusters of villages:

1) **IRS with propoxur 50% water-dispersible at 2g/m2 powder (area B)** sprayed three of four times before and during the high transmission season, with spraying rounds separated by 2 months from each other.
2) **IRS with propoxur +low frequency MDA with sulfalene-pyrimethamine (area A1)** distributed every 10 weeks to the entire population excluding infants.
3) **IRS with propoxur + high frequency MDA with sulfalene-pyrimethamine** (distributed every 2 weeks during the wet season, every 10 weeks in the dry season to the entire

population excluding infants) + **limited larviciding with temephos** during the transition from the wet to the dry season **(area A2)**

4) **Control (area C).**

Fig. 1. The Garki project study area, showing the follow-up villages and the treatment areas

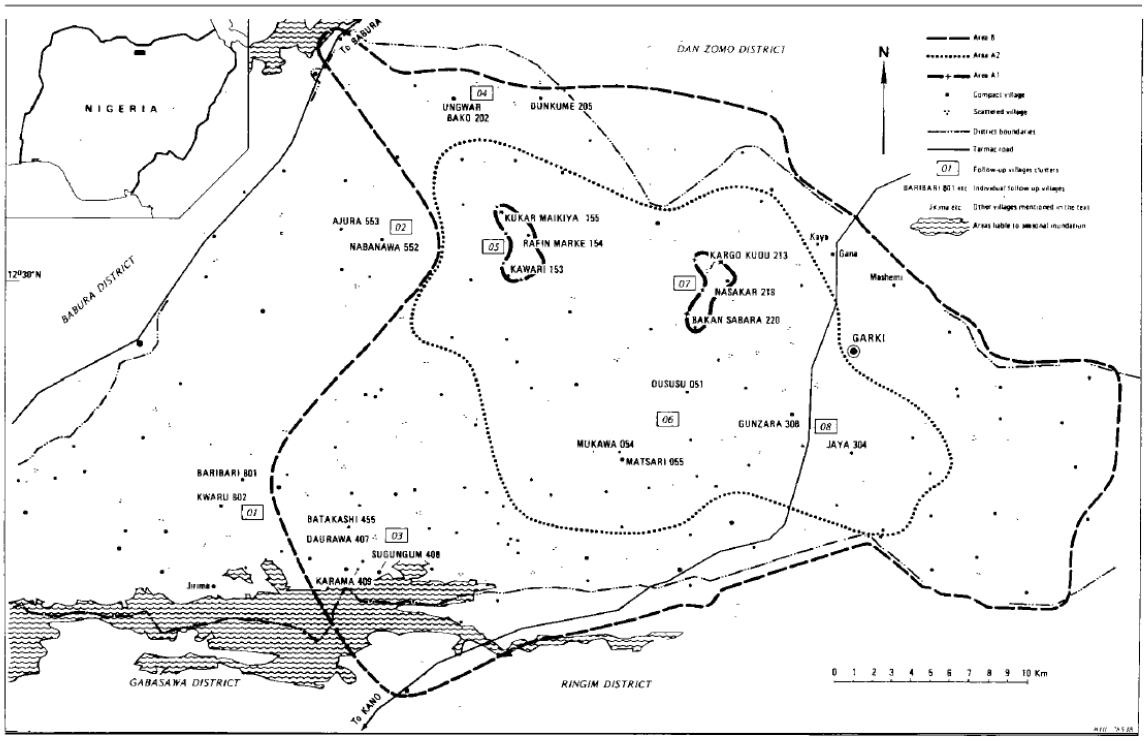


Figure 13 Intervention and control areas during the Garki project. Source: "The Garki project" (99)

The project was divided into four phases:

1) **Preparatory phase** (one year form September 1969) to conduct preliminary entomological and epidemiological surveys, selecting study areas, designing study protocol, data collection forms and SOPs, etc.

2) **Baseline phase** (1.5 years, October 1970 to March 1972): collection of baseline entomological and epidemiological data and implementation of preliminary studies.

3) **Intervention phase** (1.5. years, April 1972 to October 1973): implementation of the interventions and continuation of entomological and epidemiological data collection.
4) **Post-intervention phase** (1973 to 1976): implementation of active and passive drug administration in the villages previously covered by MDA and continuation of entomological and epidemiological data collection.

Entomological surveillance was conducted using human landing catches (HLC), Pyrethrum spray catches (PSC), Exit trap collections (ETC) and Outdoor resting collections (ORC).

Intervention coverage

The IRS campaigns achieved high coverage (as measured immediately after spraying) ranging from 96.6% to 99% of structures sprayed of those existing at the time of spraying and varied from 74% to 100% across villages. MDA coverage was on average 85% and oscillated by round and village between 69% and 99%, being greater in the wet season than in the dry season due to higher absenteeism during the dry season.

Main findings:

The evaluation of IRS impact on vector densities in each village was conducted using entomological data form the baseline, pre-spraying period, for the same village and the concurrent changes on vector densities in neighboring untreated villages. The key findings of the Garki project regarding vector composition, densities, infection and EIR; entomological sampling methods, entomological and epidemiological impact of interventions are listed below.

Vector composition and bionomics

- Eleven *Anopheles* species were identified, *An. gambiae* s.s., *An. arabiensis, An. funestus, An. rufipes, An. pharoensis, An. wellcomei, An. squamosus, An. coustani, An. maculipalpis, An. nili and An.preforiensis.* The main vectors were *An. gambiae s.s. An. arabiensis and An. funestus* with *An. pharoensis* likely being a secondary vector.
- The abundance of *An. gambiae s.l.* was much higher than that of *An. funestus,* specially during the implementation period. *An. gambie s.l.* species could not be systematically identified however, *An. arabiensis* was found to be the dominant species everywhere.
- Both *An. funestus* and *An. gambiae* bite in the second half of the night, with biting later than *An. gambiae* and that biting occurred earlier outdoor than indoor.
- Only 20 *Anopheles* (all *An. gambiae* s.l.) were found carrying sporozoites during the project, 2 collected in outdoor pit shelters and 18 in Human Landing Catches.
- *An. gambiae* s.s. was found to be more anthropophilic and to have higher sporozoite rates than *An. arabiensis.*
- Transmission continued during the dry season despite vectoral capacity dropping below the at the time critical levels for endemic transmission.
- The cumulative inoculation rate ranged from 18 to 145 infective bites per person per year.
- There were differences in vector density and composition across close villages. The vector population of different villages appeared genetically isolated from each other.

Entomological impact of propoxur

- The residual efficacy of propoxur was between 2 and 4 months.

- Despite the high coverage of IRS and good bioassay results, the effect of Propoxur on local vectors was mediocre. The poor effect was attributed to the high baseline vectorial capacity and EIR and the exophilic of a proportion of the *An. gambiae s.l.* population.
- The effect of propoxur was greater in *An. funestus* than in *An. gambiae s.l.*
- With the limited numbers of mosquitoes collected, there was no apparent effect of propoxur on the relative abundance of *An. gambiae s.s.* and *An. arabiensis.*
- The annual wet season sporozoite rates did not suggest a large effect of IRS, nor any increase in effect by the addition of MDA.
- The effect of propoxur varied significantly accross villages between 71% and 96.1% because of the degree of exophily of *An. gambie s.l.* (predominantly *An. arabiensis*). There variations were associated with the frequency of certain chromosomal inversion in both species and with the overall pre-spraying ratio between mosquitoes collected through HLC and mosquitoes collected in PSC.
- There was no significant difference in the effect of propoxur between 1972 and 1973.
- The pre-spraying ration between the number of man-biting mosquitoes and indoor resting mosquitoes predicted the impact of propoxur to a certain extent.
- The proportion of mosquitoes collected outdoor by HLC increase during intervention period. So did the ratio of mosquitos collected in HLC versus those collected by PSC, and such changes could be observed for at least 2 years after the project.
- The effect of propoxur persisted for at least 3 years after the project.
- Using another insecticide different from Propoxur would not have improved results.

Fig. 11. Effect of propoxur on the man-biting rates of *A. gambiae* s.l. and *A. funestus*, estimated from the night-bite collections in Sugungum, the village with the highest prespraying densities

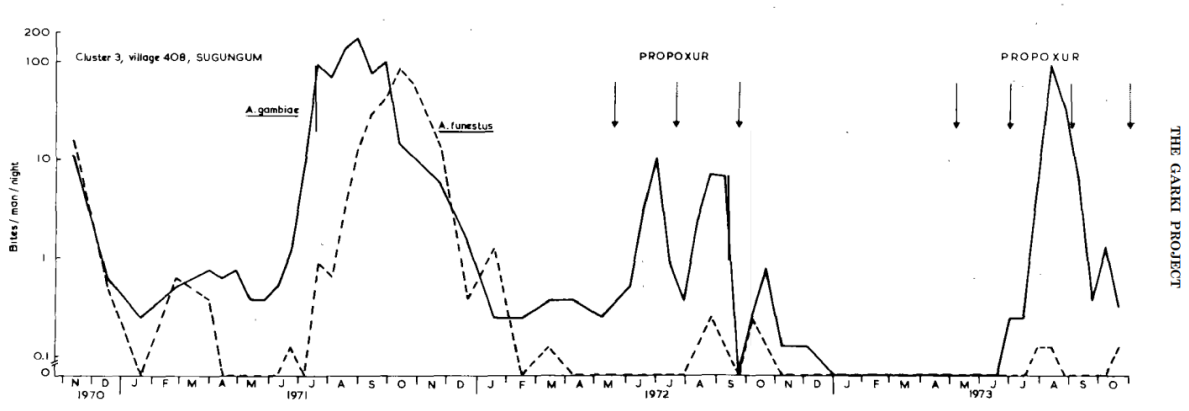


Figure 14 Impact of propoxur on vector man biting rates during the Garki project. Source: "The Garki project" (99).

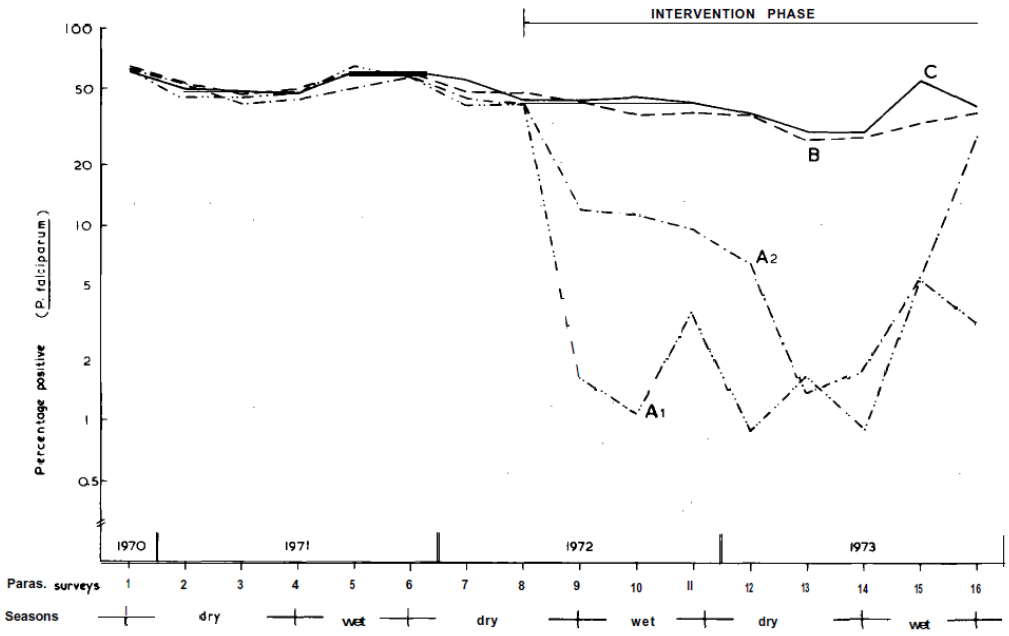
Epidemiological impacts

- The impact of interventions on parasite prevalence (calculated by microscopical examination of 200 fields of blood thick films) was:
    - **In the untreated control villages (Area C)** *P. falciparum* prevalence decreased from 60.4% in the rainy season of 1971, to 43.3% in 1972 and 47.5% in 1973.
    - **In the area with propoxur only (area B):** *P. falciparum* prevalence decreased from 60.1% in the rainy season of 1971, to 36.8% in 1972 and 35% in 1973. Considering the prevalence changes in the control area, propoxur reduced parasite prevalence by 15% in 1972 and by 26% in 1973. Propoxur decreased the duration of parasitemia. A new transmission equilibrium was reached after 2 years.
    - **In the area with propoxur and low frequency MDA (areas A1):** *P. falciparum* prevalence decreased to 10.4% in 1972 and 16.5% in 1973.

- **In the area with propoxur and high frequency MDA (areas A2):** plus larviciding with temephos (areas A1): *P. falciparum* prevalence decreased to 2.4% in 1972 and 4.2% in 1973.

- The effect of MDA was greater than that of propoxur.
- There were variation in epidemiological impact of interventions across villages, but they were unrelated to the differences in MDA or IRS coverage. In the control and propoxur areas, variations were related to entomological differences.
- Some unexpected increase in prevalence and *Pf* density was observed in the wet season of the second year despite the fact that MDA coverage did not drop, and it was associated with an increase in vector density due to the favorable conditions for vector breeding.
- Malaria transmission in the area of higher impact (IRS+high frequency MDA) went back to former levels two years after the project.

Fig. 36. Crude percentage positive for *P. falciparum*, by survey and treatment throughout the baseline and intervention periods a



a Interventions: propoxur in AI, A2, B; mass administration of sulfalene-pyrimethamine in A2 every 10 weeks, and in AI every 2 weeks in the wet season and every 10 in the dry season.

Figure 15 Epidemiological impact of the Garki Project. Source: "The Garki project" (99).

The Garki project concluded that IRS should not be recommended for malaria control in the Sudan Savannh and that the combination of propoxur and MDA was too expensive to be recommended for large scale deployment.

It further concluded that all mosquito collection methods (PSC, HLC and ETC) were essential for the entomological investigations. CDC light traps were not considered a good substitute for HLC. PSC and ETC were considered insufficient to understand the entomological impact of residual spraying because the numbers of mosquitoes collected by PSC and ETC post spraying where very small. PSC and ETC therefore needed to be supplemented by HLC. The Garki project concluded

that an adequate baseline and control areas were necessary to evaluate the impact of malaria control measures.

The Garki project was more thoroughly implemented than any of the previous malaria elimination pilot projects conducted in Africa between the 1950s and the 1960s, yet, it did not interrupt local malaria transmission. Failure to interrupt malaria transmission was attributed to 1) the high levels of baseline vectorial capacity, 2) the fact that that total effective intervention coverage was unachievable and 3) the fact that a significant proportion of *An. gambie s.s.* and *An. arabiensis* rested outdoor, avoiding IRS. The outdoor resting places of *An. gambiae s.l.* were not well known at the time. Hence, the control of outdoor resting *An. gambiae s.l* was considered unfeasible with the tools and knowledge available then. The Garki project was therefore taken as a definite sign that malaria could not be eradicated in Africa using IRS and chemoprevention alone (note that ITNs were not yet available at this time). An assumption that was not re-considered until the Bill and Melinda Gates foundation made a call for malaria eradication in 2007. Such call reignited interest in the feasibility of malaria elimination in Africa and, consequently, some projects were designed to test such feasibly with the more modern tools available in the 2000s.

3.4 History of malaria control and elimination in Southern Africa

If a continental area of sub-Saharan Africa is ever to achieve elimination, it will likely be southern Africa (i.e. South Africa, Swaziland, Botswana, Namibia). South Africa, Eswatini and

Botswana were considered by WHO as candidate countries to eliminate malaria by 2020 (100) and, although they did not eliminate malaria by that year, they presented the lowest case load of all countries in mainland Africa by the end of 2020 (3). Nowadays, malaria transmission in South African and eSwatini is concentrated in areas bordering Mozambique (3) and largely attributed to cases imported from this country (101). Hence, Mozambique plays a pivotal role in the elimination of malaria in the region.

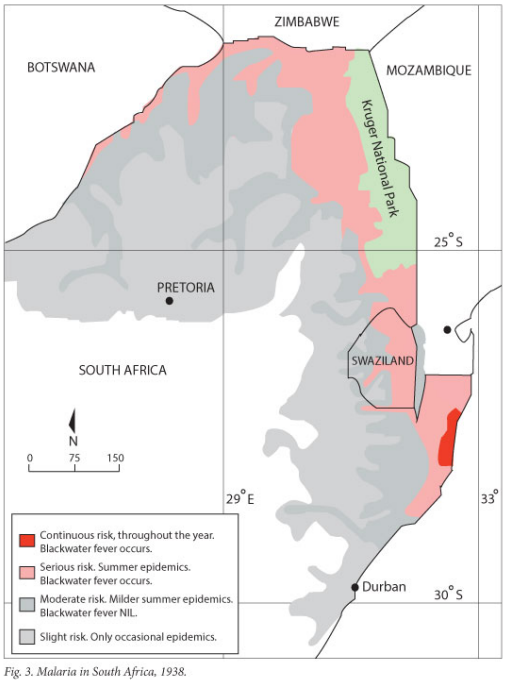


Fig. 3. Malaria in South Africa, 1938.

Figure 16 Malaria transmission in South Africa in 1938. Source: Coetzee et al. (102)

Before the arrival of residual insecticides, malaria transmission in southern Africa was hyper-endemic, exhibited intense seasonal patterns and was driven by *An. gambiae* s.l. and *An. funestus* mosquitoes. Malaria control was based on the use of quinine treatment and prophylaxis, larviciding using oil and Paris green and environmental sanitation, but these strategies had only

limited success. The first great success in the control malaria in the region was achieved in 1932 in South Africa with the implementation of IRS with Pyagra (liquid pyrethrum and kerosene). Subsequently several large-scale malaria control projects based on IRS with DDT and BHC were implemented in the region leading to great reduction in malaria parasite prevalence and incidence and going as far as eliminating malaria transmission in some areas (e.g. Kwazulu-Natal in South Africa). In addition, they inflicted significant changes in vector composition. They significantly reduced the populations of *An. gambiae* s.l. and *A. funestus* s.s. and caused the virtual disappearance of *An. funestus s.s* in some areas for several years, but allowed other more zoophagic and zoophilic species, such as *An. arabiensis* and *An. quadrimaculatus,* to survived and sustained transmission, becoming major vectors of concern over time (102).

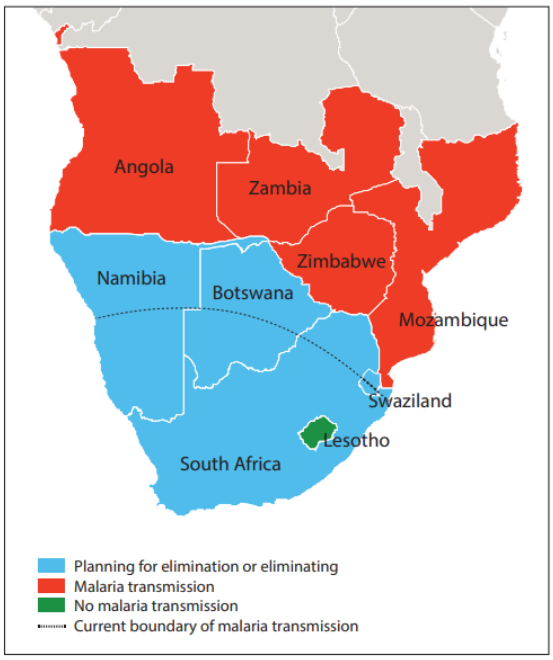
IRS was sustained from the 1940's up to the present in most of the countries with great success. Along with the development public health systems and socio-economic development, the large IRS programs kept transmission at relatively low levels during the 1960's, the 1970's and the 1980's, albeit with some epidemics often related to climatic events (88,103). However, the situation deteriorated in the 1990's, especially in South Africa where the number of malaria cases tripled between 1995 and 1996 and stayed high until 1998. This increase coinciding with the relaxation of the immigration restrictions for Mozambicans to enter south African and with particularly good rainy season and trigger interest in strengthening collaboration across countries (102).

Urged by such deteriorated epidemiological situation, in 1999, Mozambique, South Africa and Eswatini enrolled in the Lubombo Spatial Development Initiative (LSDI). An initiative established with the aim to reduce malaria transmission in Maputo province and bordering areas of South Africa and eSwatini (formerly known as Swaziland). LSDI implemented annual rounds of IRS with bendiocarb along with treatment with artemisinin-based combination therapies (ACTs) (104,105). Like previous large-scale IRS implementations, LSDI achieved great reductions in malaria transmission in the intervention areas. Between 1999 and 2005 malaria prevalence went from 65% to 4% (102,104). In Mozambique, and although entomological surveillance was limited to window exit traps and resistance monitoring, LSDI showed that *An. funestus s.s* was the primary vector pre and post indoor residual spraying and that, although the vector had started to show resistance to carbamates (104), IRS successfully reduced it densities and sporozoite rates. *An. arabiensis* and *An. merus* were identified as vectors too. IRS was successful at reducing densities and sporozoite rates of *An. arabiensis* and *An. merus.* It also reduce the relative proportion of *An. arabiensis* and increased that of *An. merus* (104). After the initiative ended in 2011, a large rebound of malaria cases was observed in all three countries (104,105).

In 2009, the Ministries of health of Bostwana, Namibia, South Africa, Eswatini, Angola, Mozambique, Zambia and Zimbabwe created the Elimination 8 (E8) initiative under the auspices of the Southern Africa Development Community (SADC) to strengthen coordination and cross-border collaboration to achieve malaria elimination in the region. Encourage by the great reduction in malaria transmission achieved in the 2000s and 2010s and in line with global elimination goals (100), in 2015, the E8 created an strategic plan aimed at achieving malaria

elimination in the four countries with the lowest transmission levels (i.e. Bostwana, Namibia, South Africa, Eswatini) by 2020 and then pursue the same goal in the other four countries (i.e. Angola, Mozambique, Zambia and Zimbabwe) (106). Despite the efforts, none of the countries achieved elimination by 2020. Several of the challenges identified were of entomological nature, namely 1) low coverage and poor quality and timing of IRS, 2) changes in vector composition and behavior, 3) inadequate knowledge of the vector species composition abundance, distribution and susceptibility to insecticides, 4) climate factors that altered vector density and receptivity in some areas. Other challenges included delayed detection and response to outbreaks, malaria importation due to population displacement and migration caused by natural disasters or economic activities.

Figure 1: Elimination 8 Regional Map



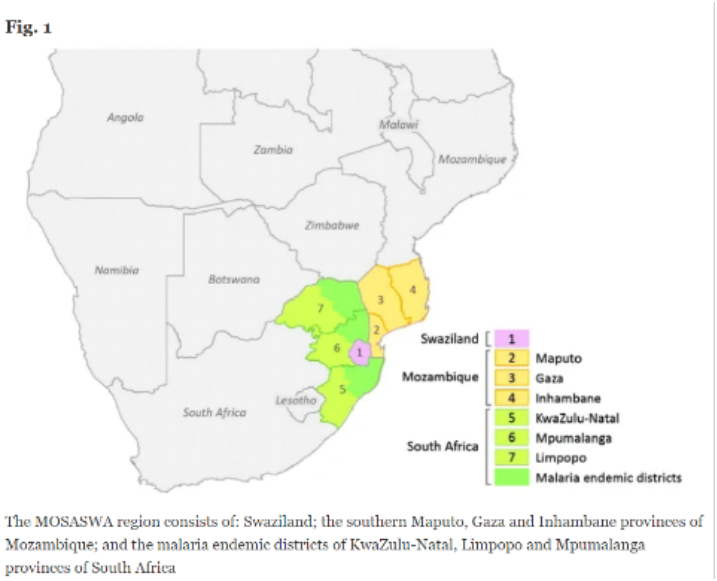


Figure 17 Map of the E-8 countries (left) and the MOSASWA region (right) Sources: left image Elimination 8 Strategic Plan 2015–2020 (106) and right image Moonasar et al. (101)

In 2015, a second regional initiative was created, the Mozambique, South Africa and eSwatini regional initiative (MOSASWA) as part of the E8. It was built based on the Lubombo Spatial Development Initiative (LSDI) and aimed to achieve malaria elimination in Swaziland and South Africa, accelerate the transition from malaria control to pre-elimination by 2020 in southern Mozambique to support elimination in Swaziland and South Africa, and subsequently achieve pre-elimination status in Gaza and Inhambane provinces of Mozambique by 2025. To reduce the burden of malaria in southern Mozambique, it planned to support the implementation of a robust and efficiently executed IRS programmed in Maputo Province, scale it up to Gaza and Inhambane Provinces, strengthen entomological surveillance and scale-up drug-based parasite clearance strategies To strengthen malaria control in the region it aimed to harmonize vector control strategies and entomologic surveillance practices and to support operational research to inform the development of regional strategies. To control malaria transmission at borders, it planned to increase migrant access to diagnosis and treatments by implementing border health posts and strengthening malaria surveillance to identify transmission foci. To make efforts sustainable in the long term, it aimed to strengthen regional leadership, increase domestic funding, mobilize and advocate for increased and sustainable financing, explore innovative financing mechanisms and to promote the engagement of the private sector in malaria control (101).

On the same year 2015 multilateral agencies, bilateral agencies, research institutions, private foundations and donors operating in Mozambique joined forces to create the

Mozambican Alliance Towards the Elimination of Malaria (MALTEM). The aim of the initiative was to generate scientific evidence to inform the development and implementation of a malaria strategy in Mozambique, increase the capacity of the National Malaria Control Program (NMCP) for malaria control and elimination, align political interest and build synergies among NMCP and partners to ensure the complementarity of efforts and to raise funds (domestic and external) for malaria elimination. MALTEM designed and led the implementation of the malaria elimination pilot project analyzed in the present thesis, namely the Magude project implemented in the Magude district (107).

3.5 The Magude Project: attempting malaria elimination in Magude district, southern Mozambique

The Magude district

Magude is a rural district with an area of 6961 km^2 with 48 448 (92.1%) residents that borders on the west with South African's National Kruger Park. Its vegetation is dominated by open forests and savannahs and surrounded on the east by privately owned sugar cane fields. The majority of population relies on subsistence agriculture, fishing or working as sugar cane cutters in the sugar plantations within Magude, or the neighboring district of Manhiça. Fifty nine percent of the population does not receive formal education. Houses are traditional round-shaped or rectangular-shaped huts constructed using cane (32.5%), cement (26%), mud brick (21.6%) or reeds covered by adobe (15.6%) (108). There are a total of 1603 pigs owned by 107 households and 50 997 cows owned by 3182 households.

Two distinct climatological seasons are observed in the district, a rainy season expanding from October to March and a dry season from April to September. Although the district presented low malaria transmission before the start of the Magude project, malaria has traditionally accounted for the highest disease burden in the district. Malaria incidence fluctuates seasonal following rains, and the high transmission season spans from November to April (108).

The Magude project

The Magude project aimed to evaluate the feasibility of interrupting malaria transmission in the Magude district with a comprehensive package of interventions that target the parasite and the vector simultaneously. It was implemented between 2015 and 2018.

To control parasites, the Magude project implemented two annual rounds of mass drug administration (MDA) during the high transmission seasons of 2016 and 2017 using a 3 days course of dihydroartemisinin–piperaquine (DHAp), an ACT combination different from the one used for treatment purposes in Mozambique (aertemether-lumefantrine, AL). MDA was implemented on top of standard diagnosis and treatment delivered by the national health system using HRP2-based and artemether–lumefantrine. In the first high transmission season (2015-2016), the two MDAs were conducted in November 2015 and January 2016 covered 72% and 58%, respectively. In the second high transmission season (2016-2017), they were conducted in December 2016 and February 2017 covering 67% and 65% respectively.

To control vectors, it combined annual rounds of IRS with LLINs distributed through two mass campaigns conducted by the NMCP, one right before the project (May 2014) and the second one in December 2017. The first round of IRS was conducted between August 2015 and October 2015 using dichlorodiphenyltrichloroethane (DDT) for thatched or mud walls (47% of houses), and pirimiphos-methyl (Actellic 300 CS®, Syngenta Crop Protection AG, Basel, Switzerland) for concrete walls (53% of houses). The second and the third rounds were conducted between September and November in 2016 and 2017 respectively using solely Actellic 300 CS ®. The LLINs mass distribution campaign of May 2014 distributed 35432 LLINs (Olyset®, Sumitomo Chemical Ltd, Japan; Permanet 2.0®, Vestergaard Frandsen, Switzerland). The second LLINs mass distribution campaign, conducted in December 2017, distributed 44 400 LLINs (Dawa Plus 2.0®, Tana Netting, United Arab Emirates). Nets were continuously distributed through expanded programs of immunization (EPI) and antenatal care services (ANC) during the Magude project.

Finally, community mobilizations campaigns were conducted to increase acceptability, uptake and use of the implemented interventions.

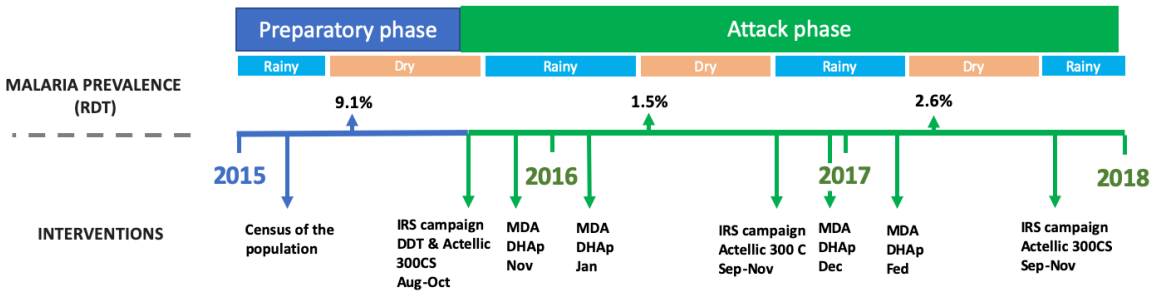


Figure 18 Phases of the Magude project.

The expectation was that IRS and ITNs would reduce vector densities, after which the prophylactic effect of MDAs and ITNs would protect individuals from acquiring infection from the few surviving infective vectors. Due to the low parasite circulation post MDA, the new generation of mosquitoes would not become infected, interrupting once and for all malaria transmission in the district (107).

To monitor the epidemiological outcomes of the project, a DHIS2-based rapid case reporting system was established in January 2015, before the project intervention started. It captured weekly malaria case diagnose by microscopy or RDTs and stratified by >5 and <5 years of age (including malaria cases among pregnant women tested in the outpatient ward). In addition, cross-sectional malaria prevalence surveys were conducted every May, right after the end of the high transmissions season, from 2015 to 2018.

Unfortunately, and despite using the most comprehensive package of interventions ever used to attempt malaria elimination in Sub-Saharan Africa, the project failed to interrupt local malaria transmission (109). Considering transmissions years from July to June, malaria incidence declined from 195 per 1 000 at baseline (2015-2016) to 75 per 1 000 in the first year of intervention implementation (2016-2017) and to 67 cases per 1000 in the second intervention year (2017-2018). Malaria prevalence as measured by RDT declined from 9.1% in May 2015 to 1.5% in 2016, increased to 2.6% during the second intervention year (2017) and declined again to 1.4% during the third intervention year (2018) (109).

Understanding the reasons why transmission was not interrupted during the Magude project is crucial to guide future malaria elimination efforts in the region as well as to inform the development of new interventions that may be needed to attain such goal.

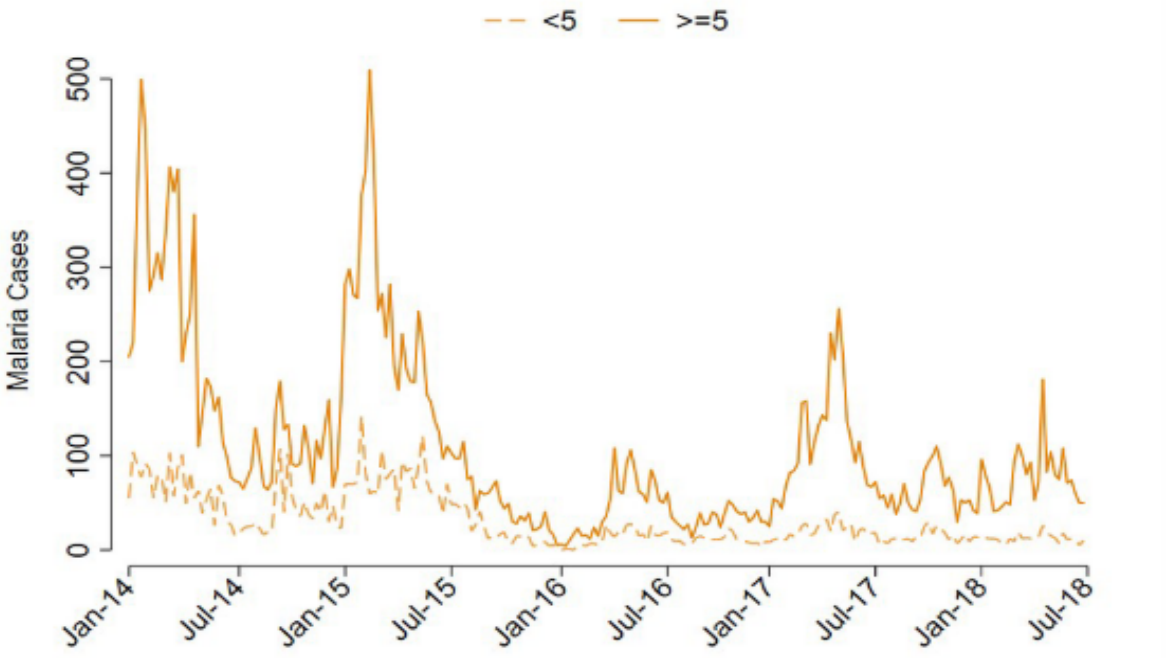


Figure 19 Malaria cases before and during the Magude project. Source: Galatas et al [115]

4. Research questions and hypotheses

The fact that malaria transmission continued during the Magude project indicates that sufficient local malaria vectors survived and could acquire and transmit malaria between humans despite the comprehensive intervention package implemented. Therefore, one of the issues that may have hampered the interruption of transmission is sub-optimal vector control, namely: 1) that the implemented vector control interventions did not manage to reduce vector densities as expected and 2) that ITNs did not manage to prevent transmission by the surviving vectors. This could be due to either suboptimal implementation of vector control interventions or to the fact that local vectors were not amenable to the implemented interventions.

Vector control could have been suboptimal due to poor ITN access, use, physical integrity or reduced bio-efficacy of the nets, or to poor IRS coverage and residual efficacy. Vector resistance to the insecticide used in IRS or LLINs, and vector behaviors that differ from those targeted by LLIN or IRS- such as vectors that bite outdoor, early in the evening when people are not under the net or that rest outdoors- could have also jeopardized the ability of these two interventions to control local vectors. Each of these hypotheses needs to be investigated to understand shortcomings in vector control during the Magude project and to identify improvements that could be implemented in future similar projects.

If some of the hypotheses assuming deficiencies in vector control interventions are confirmed, the question would remain whether improving such intervention would have been

sufficient to achieve optimal vector control or whether other tools would be needed, and, if so, what type of tools. Finally, it is important to compare the factors that prevented the interruption of transmission during the Magude project with those identified during former malaria elimination efforts in Africa, to understand how challenges have evolved over time. The present doctoral research is therefore underpinned by the following research questions (RQ) and hypothesis (H):

Research questions and hypothesis:

From an entomological perspective:

RQ1: Which vectors continued to transmit malaria during the Magude project?

RQ2: Were local vectors amenable to control by the implemented vector control interventions?

H1: Local malaria vectors were resistant to the insecticides used in ITNs (pyrethroids) or IRS (DDT and pirimiphos-methyl).

H2: Local malaria vectors were able to avoid contact with ITNs or IRS due to exophagic and exophilic behaviors, respectively, or by feeding indoors at times when people were not under an ITN.

RQ3: Where and when did residual exposure to vector bites occur?

From a vector control perspective.:

RQ4: Did the implemented vector control intervention leave gaps in protection? If so, which gaps?

H3: There was poor ITN access during the implementation of the project.

H4: There was low LLIN use during the implementation of the project.

H5: LLINs could not protect against certain, or all vectors, due to exophagic vector behaviours.

H6: The IRS campaigns achieved poor coverage, as defined by the proportion of structures sprayed.

H7: The residual efficacy of IRS fell below the optimal level (80% mosquito mortality 24h post-exposure in WHO cone bioassays) before the end of the high transmission season.

RQ5: How could IRS implementations and ITNs distributions improve in the future?

RQ6: Shall IRS and ITNs be deployed together in the future, or shall Magude proceed with one intervention alone?

RQ7: What would have been the added value of implementing additional vector control interventions and which intervention could be suitable for Magude?

To understand the difference in current challenges compared to historical efforts to eliminate malaria from sub-Saharan Africa

RQ8: Were the factors that prevented the interruption of malaria transmission during the Magude project similar to those that prevented interruption during the Garki project?

5. Objectives

The present doctoral thesis aims to identify the vectors that transmitted malaria during the project and to evaluate their amenability to control by the implemented vector control interventions, to understand whether there was adequate vector control during the Magude project, and if not, what could have been done to improve vector control and, finally, to compare the Magude project with its closest relative, the Garki Project. The ultimate goal is to guide future malaria elimination efforts in southern Africa. The general and specific objectives of the present research are listed below.

Main objectives (addressed in the scientific articles)

Objective 1: To identify and describe the vectors that transmitted malaria during the Magude project and their relative important in malaria transmission.

1.1. To estimate *Anopheles* species composition and densities

1.2. To identity *Anopheles* species that carried *P. falciparum* sporozoites

1.3. To calculate infection rates per species and during the project period

1.4. To characterize vector host seeking behavior

1.5. To estimate the association between vector densities and malaria incidence

Objective 2: To evaluate whether local malaria vectors were amenable to control by the implemented vector control tools during the project

2.1. To assess resistance of local malaria vectors to the insecticides used in ITNs (pyrethroids) and IRS (DDT and pirimiphos-methyl)

2.2. To assess vector host-seeking and resting behaviors.

<u>Objective 3</u>: To evaluate where and when residual human-vector contact occurred during the project

3.1. To assess vector host seeking times

3.2. To assess human behaviors during vector host-seeking times

3.3. To estimate the proportion of human exposure to vector bites that occurs in different compartments during the day, namely: outdoors, before people go indoors, indoors, before people go to bed, indoors, while people are in bed, indoors, after people have gotten up, and outdoors, after people got up and left the house

<u>Objective 4:</u> To identify limitations in the implementation of and protection conferred by LLIN against malaria vectors during the project

4.1. To estimate LLIN access

4.2. To estimate LLIN use

4.3. To estimate the proportion of exposure to host-seeking mosquitoes prevented by ITNs and difference across population subgroups

4.4. To estimate the maximum personal protection that LLIN would have conferred if all residents would have used them to sleep

<u>Objective 5:</u> To identify limitations in the implementation of and protection conferred by IRS against malaria vectors during the project

5.1. To estimate IRS acceptability

5.2. To estimate the coverage of IRS campaigns

5.3. To estimate the duration of the optimal residual efficacy of IRS campaign and compare it with the duration of the high malaria transmission

Objective 6: To identify possible improvements in IRS to guide future campaigns.

Objective 7: To identify possible improvements in ITN distributions to guide future campaigns.

Objective 8: To assess, from an entomological perspective, whether the deployment of IRS and ITN together added value compared to the deployment of one intervention alone.

Secondary objectives (addressed in the discussion based on results of all articles and additional published scientific evidence)

Objective 9: Identify suitable additional new interventions to deploy in Magude.

Objective 10: To compare factors that impeded the interruption of malaria transmission during the Garki project with those of the Magude project

Table 3 Summary of research questions, hypothesis and objectives, the studies conducted to respond to them and articles where the results were published

Research Question (RQ) and Hypothesis (H)	Objective	Studies conducted to respond to the objective	Article(s)
RQ1: Which vectors continued to transmit malaria during the Magude project?	**Objective 1: To identify and describe the vectors that transmitted malaria during the Magude project and their relative important in malaria transmission.** 1.1. To estimate *Anopheles* species composition and densities 1.2. To identity *Anopheles* species that carried *P. falciparum* sporozoites 1.3. To calculate infection rates per species and during the project period 1.4. To characterize vector host seeking behavior 1.5. To estimate the association between vector densities and malaria incidence	• Entomological surveillance	Article 4
RQ2: Where local vectors amenable to control by the implemented vector control interventions?	**Objective 2: To evaluate whether local malaria vectors were amenable to control by the implemented vector control tools during the project** 2.1. To assess resistance of local malaria vectors to the insecticide used in ITNs (pyrethroids) and IRS (DDT and pirimiphos-methyl) 2.2. To assess vector host-seeking and resting behaviors.	• Entomological surveillance • Insecticide resistance monitoring	Article 1 and Article 4

RQ2: Where and when did residual exposure to vector bites occurred?	**<u>Objective 3</u>: To evaluate where and when residual human-vector contact occurred during the project** 3.1 To assess vector host seeking times 3.2 To assess human behaviors during vector host-seeking times 3.3 To estimate the proportion of human exposure to vector bites that occurs in different compartments during the day, namely: outdoors, before people go indoors, indoors, before people go to bed, indoors, while people are in bed, indoors, after people have gotten up, and outdoors, after people got up and left the house	• Entomological surveillance • Human sleeping patterns	Article 3
RQ4: Did the implemented vector control intervention leave gaps in protection? If so, which gaps? **H3:** There was poor ITN access during the implementation of the project. **H4:** There was poor LLIN use during the implementation of the project. **H5:** LLINs could not protect against certain, or all vectors, due to exophagic vector behaviours.	**<u>Objective 4:</u> To identify limitations in the implementation of and protection conferred by LLIN against malaria vectors during the project** 4.1. To estimate LLIN access 4.2. To estimate LLIN use 4.3. To estimate the proportion of exposure to host-seeking mosquitoes prevented by ITNs and difference across population subgroups 4.4. To estimate the maximum personal protection that LLIN would have conferred if all residents would have used them to sleep 4.5. To estimate the exposure to vector bites left unprevented and its distribution over time and location (indoor/outdoor)	• MDA surveys • Population census a • Demographic and health annual surveys • Cross sectional malaria prevalence surveys • Cross sectional evaluation of Olyset Nets	Article 2 and article 3

H6: The IRS campaigns achieved poor coverage, as defined by the proportion of structures sprayed. **H7:** The residual efficacy of IRS went below optimal level (80% mosquito mortality 24h post-exposure in WHO cone bioassays) before the end of the high transmission season.	**<u>Objective 5:</u> To identify limitations in the implementation of and protection conferred by IRS against malaria vectors during the project** 5.1. To estimate IRS acceptability 5.2. To estimate the coverage of IRS campaigns 5.3. To estimate the duration of the optimal residual efficacy of IRS campaign and compare it with the duration of the high malaria transmission	• Population census a • Demographic and health annual surveys • Cross sectional malaria prevalence surveys • Longitudinal IRS residual efficacy monitoring • Data collected during the IRS campaigns	Article 1
RQ5: How could IRS implementation and ITNs distributions improve in the future?	**<u>Objective 6:</u> To identify possible improvements in IRS to guide future campaigns.** **<u>Objective 7:</u> To identify possible improvements in ITN distributions to guide future campaigns.**	• MDA surveys • Population census a • Demographic and health annual surveys • Cross sectional malaria prevalence surveys • Longitudinal IRS residual efficacy monitoring	Article 1 Article 2

RQ6: Shall IRS and ITNs be deployed together in the future, or shall Magude proceed with one intervention alone?	**<u>Objective 8:</u> To assess, from an entomological perspective, whether the deployment of IRS and ITN together added value compared to the deployment of one intervention alone.**	• Entomological surveillance • Insecticide resistance monitoring • Human sleeping patterns • Longitudinal IRS residual efficacy monitoring	Article 4
RQ7: What would have been the added value of implementing additional vector control interventions and which intervention could be suitable for Magude?	**<u>Objective 9:</u> Identify suitable additional new interventions to deploy in Magude.**	Based on findings from all articles, current evidence for new interventions and addition data on the district.	
RQ8: Were the factors that prevented the interruption of malaria transmission during the Magude project similar to those that prevented interruption during the Garki project?	**<u>Objective 10:</u> To compare factors that impeded the interruption of malaria transmission during the Magude project with those that prevented it during the Garki project.**	Based on findings from all articles of the Magude project and from results from the Garki project.	

6. Materials, methods and results

Article 1 (accepted for publication). **Fernández Montoya L,** Máquina M, Marti-Soler H, Sherrard-Smith E, Alafo C, Opiyo M, et al. The realized efficacy of indoor residual spraying campaigns falls quickly below the recommended WHO threshold when coverage, pace of spraying and residual efficacy on different wall types are considered

Objectives addressed:

- Objective 2: To evaluate whether local malaria vectors were amenable to the implemented vector control tools during the project
- Objective 5: To identify limitations in the implementation of and protection conferred by IRS against malaria vectors during the project
- Objective 6: To identify possible improvements in IRS to guide future campaigns

The realized efficacy of indoor residual spraying campaigns falls quickly below the recommended WHO threshold when coverage, pace of spraying and residual efficacy on different wall types are considered

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Abstract

Indoor residual spraying (IRS) has been and remains an important malaria control intervention in southern Mozambique, South Africa and Eswatini. A better understanding of the effectiveness of IRS campaigns is critical to guide future elimination efforts. We analyze the three IRS campaigns conducted during a malaria elimination demonstration project in southern Mozambique, the "*Magude project*", and propose a new method to calculate the efficacy of IRS campaigns adjusting for IRS coverage, pace of house spraying and IRS residual efficacy on different wall types. *Anopheles funestus sensu lato* (*s.l.*) and *An. gambiae s.l.* were susceptible to pirimiphos-methyl and DDT. *Anopheles funestus s.l.* was resistant to pyrethroids, with 24h post-exposure mortality being lower for *An. funestus sensu stricto* (*s.s.*) than for *An. parensis* (collected indoors). The percentage of structures sprayed was above 90% and percentage of people covered above 86% in all three IRS campaigns. The percentage of households sprayed was above 83% in 2015 and 2016, but not assessed in 2017. Mosquito mortality 24h post-exposure stayed above 80% for 196 days after the 2016 IRS campaign and 222 days after the 2017 campaign and was 1.5 months longer on mud walls than on cement walls. This was extended by up to two months when 120h post-exposure mortality was considered. The district-level realized IRS efficacy was 113 days after the 2016 campaign. While the coverage of IRS campaigns in Magude were high, IRS protection did not remain optimal for the entire high malaria transmissions season. The use of a longer-lasting IRS product could have further supported the interruption of malaria transmission in the district. To better estimate the protection afforded by IRS campaigns, National Malaria Control Programs and partners are encouraged to adjust the calculation of IRS efficacy for IRS coverage, pace of house spraying during the

campaign and IRS efficacy on different wall types combined with wall type distribution in the sprayed area.

Introduction

Indoor residual spraying (IRS) has been and remains a cornerstone intervention in malaria elimination efforts in southern Mozambique [1, 2]. Historically, it has been the core intervention in several initiatives that aimed to eliminate malaria in the southern part of the country, South Africa and Eswatini [3]. Between 1960 and 1969, malaria elimination was attempted using IRS with DDT [4]. During the Lubombo Spatial Development Initiative (LSDI, 2000-2011), IRS with bendiocarb was used in combination with treatment with artemisinin-based combination therapy (ACT) [3]. Since 2015, the Mozambique, South Africa, Eswatini (MOSASWA) initiative has been implementing IRS in Maputo Province, first using Actellic® 300CS (Syngenta Crop Protection AG, Switzerland) and DDT, and later SumiShield™ 50WG (Sumitomo Chemical Company Ltd., Japan) and Fludora® Fusion (Bayer CropScience, Germany) [1]. From 2015 to 2018, the Magude project, designed to evaluate the feasibility of malaria elimination in Mozambique with available tools at the time, implemented annual rounds of IRS with DDT and Actellic® 300CS, on top of programmatically distributed insecticide treated nets (ITNs) and combined with mass drug administration, and standard diagnosis and treatment [5].

Although great reductions in malaria incidence or prevalence were systematically observed during all these initiatives, the fact that none of them managed to interrupt local malaria transmission [2, 6] calls for a thorough analysis to understand the limitations of the used interventions. IRS was the backbone for transmission

reduction in all initiatives. Mozambique plans to continue using IRS to accelerate towards elimination in the south, and to reduce transmission in the highest burden areas and manage insecticide resistance throughout the country [7]. South African and Eswatini also continue to use IRS to progress towards malaria elimination [8]. IRS in general, continues to be a key vector control tool globally, not only for the control of malaria, but also for the control of several other vector-borne disease such as dengue, leishmaniasis and chagas disease [9]. A better understanding of the effectiveness of IRS campaigns will be critical to guide future malaria elimination efforts in Mozambique and southern Africa and efforts to control other vector-borne diseases.

IRS reduces malaria transmission by killing susceptible mosquitoes that rest indoors on sprayed walls, or by reducing indoor vector-host contact through its excito-repelling properties that prevent mosquito entry into houses or reduce the time they spend inside. Its effectiveness therefore depends on the resting and feeding behaviors of local malaria vectors [10], vector susceptibility to the IRS active ingredients [11], the IRS coverage that is achieved [12], the quality of spraying [13] and the residual efficacy of the IRS product over time [14]. IRS is considered to be most effective in areas where the local vectors rest indoors and are susceptible to the active ingredient of the IRS product. Its effectiveness increases with higher spray coverage as well as using active ingredients with a longer residual efficacy.

IRS campaigns are commonly evaluated by quantifying their operational coverage and -to a lesser extent - the product's residual efficacy, but these indicators do not provide a complete picture of the potential effectiveness of an IRS campaign. Coverage is commonly reported as the percentage of houses or structures sprayed out of all those identified during IRS campaigns. Since some houses/structures may not be identified or are not accessible, this indicator can overestimate the actual coverage.

Residual efficacy is often measured as the number of months during which mosquito mortality 24h post-exposure to a sprayed wall remains above 80% [15]. However, there is evidence that IRS leads to significant reduction in malaria prevalence (compared to no IRS) even after the mosquito mortality are below 80% [16, 17]. This may be linked to the delayed mosquito mortality induced by some IRS products (e.g. mortality 48 or 72h after exposure to the insecticide) [18, 19], or to other effects that sublethal exposure to IRS products may have on mosquitoes [20, 21]. In addition, efficacy measured through WHO cone bioassays, even when considering delayed mortalities, only reflects the duration of IRS efficacy on an individual sprayed wall. Since not all houses are sprayed during a campaign, a proportion of indoor resting mosquitos will be able to rest on unsprayed surfaces and hence will not be killed or affected. Furthermore, a product's residual efficacy varies between different surface types [22-27] and as such, not all houses will have the same capacity to kill mosquitoes. Finally, IRS campaigns can take several months to be completed. Therefore, by the time the last houses are sprayed, the residual efficacy in the first sprayed houses would have partially waned off, affecting the overall ability of IRS to kill mosquitoes and hence the overall community protection of IRS [28]. These factors have not been systematically considered in the evaluation of IRS campaigns to-date but are likely to result in a lower realized IRS efficacy compared to estimates based on more frequently reported indicators.

In the present study, we examine the IRS campaigns conducted during the Magude project to understand their potential effectiveness and to identify gaps in the protection of IRS that may have jeopardized the interruption of malaria transmission in the district. We report the susceptibility of local vectors to deltamethrin, DDT, pirimiphos-methyl and bendiocarb, the operational and effective coverage of the IRS campaigns and the residual efficacy of Actellic® 300CS on cement and mud walls. We

propose a new method to estimate the residual efficacy of IRS in a more realistic manner, which combines IRS coverage, the pace of spraying, the residual efficacy on different wall surfaces and the distribution of these wall types in the district into a new metric: the realized district-level IRS residual efficacy. We subsequently link this residual efficacy with seasonality of malaria transmission in the district to understand whether IRS effectively covered the high malaria transmission season during the Magude project.

Materials and Methods

Study area

Magude district is a rural district in southern Mozambique that borders with South Africa (Kruger National Park) on the west (Fig 1). It covers approximately 6,961 km^2 and, in 2015, had 48,448 residents and 4,133 non-residents divided over 10,965 households [29]. Detailed socio-demographic information on the district is provided elsewhere [6, 29]. Previous epidemiological analyses have shown that the high malaria transmission season in the district traditionally extends from November to April [29]. The main malaria vectors in southern Mozambique are *An. arabiensis* and *An. funestus s.s.* [3, 30-34]. In Magude, *An. arabiensis* was responsible for approximately 74% of all mosquito bites during the Magude project [35]. The district's long IRS history is outlined in Table 1.

Fig. 1. Map of the study areas. Villages/neighborhoods in Magude and Manhiça districts where adult mosquitoes were collected for insecticide resistance monitoring and/or WHO cone bioassays to evaluate the residual efficacy of Actellic® 300CS. The subnational administrative boundaries were obtained from the Humanitarian Data

Exchange (https://data.humdata.org/dataset/cod-ab-moz) under a CC-BY-IGO license (https://data.humdata.org/faqs/licenses).

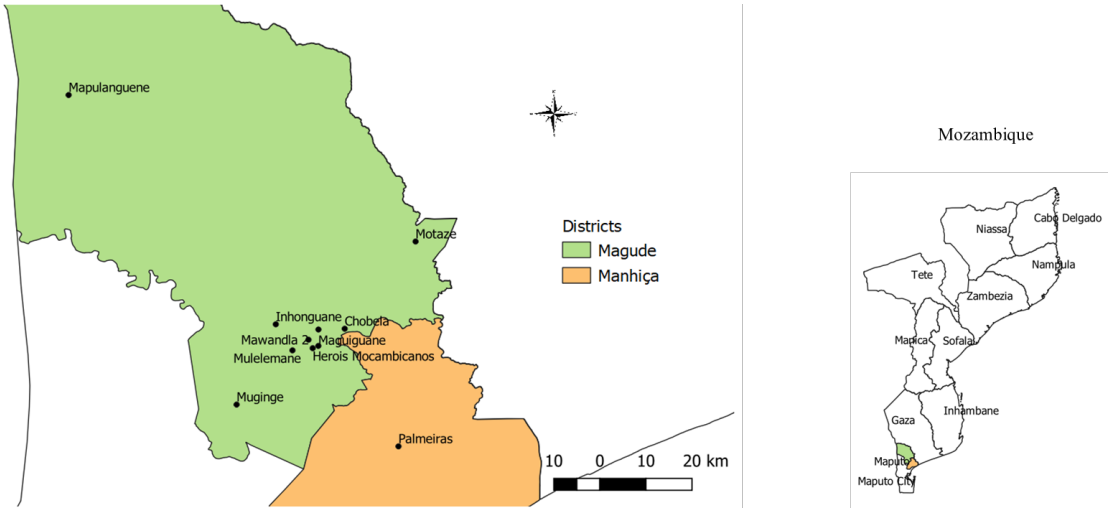


Table 1. History of indoor residual spraying (IRS) campaigns in Magude district.

Year (start IRS)*	Active ingredient	Coverage	Reference
2017	Pirimiphos-methyl	District level	[68]
2016	Pirimiphos-methyl	District level	[68]
2015	dichlorodiphenyltrichloroethane	District level	[68]
2014	Deltamethrin and DDT	Focal (Motaze)	[5] Personal communication NMCP
2013	Bendiocarb and Deltamethrin	District level	Personal communication NMCP
2012	No IRS conducted		
2011	DDT and Bendiocarb	District level	
2008-2010	Bendiocarb, Lambda-cyhalothrin and DDT	District level	
2007	Bendiocarb, K-otrine and DDT	District level	
2005, 2006	DDT and Bendiocarb	District level	

* IRS typically starts before the onset of the rainy season (August-October)

Implementation of indoor residual spraying (IRS) in 2015, 2016 and 2017

District-wide IRS was implemented by Goodbye Malaria (GBM) in 2015 (3rd of August to 7th of November), 2016 (22nd of August to 30th November) and 2017 (21st of August to 16th December). In 2015, IRS was conducted with dichlorodiphenyltri-chloroethane (DDT) on thatched or mud-walled houses (47% of houses sprayed) and pirimiphos-methyl (Actellic® 300CS, Syngenta Crop Protection AG, Basel, Switzerland) on concrete-walled houses (53% of houses sprayed). In 2016 and 2017, only Actellic® 300CS was used in the IRS campaigns.

Insecticide resistance testing

Insecticide susceptibility of *An. gambiae s.l.* and *An. funestus s.l.* to DDT (4%), bendiocarb (0.1%), deltamethrin (0.05%), and pirimiphos-methyl (0.25%) was assessed by means of standard WHO tube bioassays [36]. Wild blood-fed anopheline mosquitoes were collected indoors (from 6-10 am) using a mouth aspirator and a torch from April to September and in December of 2015, from February to August of 2016, from August to November of 2017 and from April to July of 2018. Mosquitoes were collected from the following villages/neighborhoods: Bairro 2000, Muginge, Motaze, Chobela, Maguiguane, Mulelemane, Mawandla 2, Mapulanguene, Herois Moçambicanos and Nhonguene (Fig1). Collected *An. funestus s.l.* (1,042 adult females in 2015) and *An. gambiae s.l.* (1,024; 3,753; 508 and 412 adults females respectively in 2015, 2016, 2017 and 2018) mosquitoes were transferred to a climate-controlled insectary located in Manhiça district at the facilities of the Centro de Investigação em Saúde de Manhiça (CISM) (28±2°C, 75±5% RH, 12:12h day:night light cycle). Females from the same village were pooled into the same cage, given *ad libitum* access to a 10% dextrose

solution (D-(+)-Glucose ≥99.5% (GC), Merck, Germany) and were allowed to oviposit. Larvae were reared in purified water (Elix® Advantage 3 Water Purification System and Millipore® Milli-DI) and fed with Tetramin® Baby fish food (**Tetra Holding GmbH,** Germany). WHO susceptibility tests were conducted with the 2-5 days old female adults that emerged. After exposure, mosquitoes were kept in the holding tubes for 24 hours with *ad libitum* access to a 10% dextrose solution (D-(+)-Glucose ≥99.5% (GC), Merck, Germany). Mortality was assessed 24-hrs post-exposure and mosquitoes stored individually on silica gel afterward (SiO2-Silica Gel Beads, Merck, Germany).

All tested mosquitoes were morphologically identified to either belonging to the *An. gambiae* complex or the *An. funestus* group using the dichotomous key of Coetzee [37]. A random subset of mosquitoes of each species group (approx. 22% of total sample size) were identified to species level by Polymerase Chain Reaction (PCR) as described by Scott *et al*. [38], and Koekemoer *et al*. [39], respectively.

IRS operational and effective coverage, spray periods and reasons for not spraying

Data on the operational coverage of IRS (i.e. the number of houses and structures sprayed and found and the number of people living in sprayed households) was obtained from reports produced by GBM that implemented the campaigns. Data on IRS effective coverage (i.e. the number of households that reported receiving IRS) and on the reasons for households not being sprayed were assessed through structured questionnaires administered to Magude residents during the district-wide mass drug administration (MDA) campaigns in January 2016 after the first IRS campaign and in February 2017 after the second IRS campaign. The date when households where sprayed was collected from

the households of the randomly selected participants of each malaria prevalence cross-sectional survey conducted during the Magude project (May of 2017 and 2018). During these surveys, field workers copied the spray date from the sticker placed by the spray operators on one door of each sprayed household, when this was available. Details on MDAs and the demographic and health platform, their implementation periods and data collection forms, are provided elsewhere [6, 29].

Monitoring the residual efficacy of Actellic® 300CS

The residual efficacy of Actellic® 300CS after the 2016 and 2017 campaign was evaluated through regular WHO standard cone bioassays conducted on the sprayed walls of a subset of nine cement and nine mud/clay houses, the two most common types of houses in the district [29]. Houses were selected from daily lists of sprayed houses. *The number of houses was based on* logistical feasibility. Neighboring unsprayed houses of the same wall types served as controls. During the 2016-2017 season, monitoring started 24h after IRS application and continued for a total of 12 months (August 2016 to September 2017). During the 2017-2018 season, monitoring started approx. one month after IRS application and continued for 10 months (November 2017 to August 2018). WHO cone bioassays were conducted in the same houses over the residual efficacy monitoring period. Residual efficacy was not evaluated after the 2015 campaign.

WHO standard cone bioassays were conducted using either mosquitoes from an insecticide susceptible *An. arabiensis* colony (KGB) or wild caught *An. funestus s.l*. or *An. gambiae s.l.*. KGB originates from Kanyemba, Zimbabwe and were colonized in 1975 and kept under standard insectary conditions as described by Hunt et al. [40] before a colony was started at CISM in 2015 in its climate-controlled insectary. Susceptibility of the

colony to pirimiphos-methyl (the active ingredient in Actellic® 300CS) was confirmed using the WHO susceptibility bioassays [36] in July 2016 and June and July 2018 (S1 Table). When the number of mosquitoes from the susceptible KGB colony were insufficient to conduct WHO cone bioassays, either wild *An. funestus s.l.* collected in Palmeira, Manhiça district, or wild *An. gambiae s.l.* collected from Muginge and Simbe, Magude district, were used to conduct the WHO bone bioassays. Figure 2 shows the months when each of these types of mosquitoes were used. *An. funestus s.l.* and *An. gambiae s.l.* mosquitoes were collected indoors in the morning between 6-10 am. Specimens were obtained using a mouth aspirator and a torch. Female mosquitoes were selected and used in the WHO cone bioassays the following day. After the test, they were identified to species morphologically using a stereomicroscope and the dichotomous key of Coetzee [37]. The susceptibility of the *An. funestus s.l.* mosquito population from Palmeira and *An. gambiae s.l.* mosquito population from Magude to Actellic® 300CS was confirmed through several WHO susceptibility bioassays conducted during the study period (S2 Table). These tests were conducted with unfed 2-5 day old female offspring of the wild caught mosquitoes. Mosquito collection and rearing was done as described in the resistance monitoring section above.

In each house, WHO cone bioassays were conducted during the morning hours (6-10 am). WHO cones were positioned at four different heights (approx. 0.4m, 0.8m, 1.2m and 1.6m) arranged diagonally across a single wall. Ten 2-5 day-old unfed female mosquitoes susceptible to the insecticide sprayed were introduced in each cone and kept inside for 30 minutes [12, 15]. After this period, mosquito knock-down was

recorded and the mosquitoes were transferred to paper cups and transported to a climate-controlled insectary with *ad libitum* access to a 10% dextrose solution (D-(+)-Glucose ≥99.5% (GC), Merck, Germany). Mortality among exposed and control mosquitoes was recorded 24h post-exposure and also 48h, 72h, 96h and 120h post-exposure to assess delayed mosquito mortality. After the 2016 IRS campaign, mortality 48h and 72h after exposure was only recorded from month 8 post-spraying (when 24h mortality fell below 80%) and 96h and 120h mortality from month 11 post-spraying. The dates, house type, mosquito species used, and results of individual bioassays are provided in S3 File.

Data analysis

All analyses were conducted with R statistical software version 4.1.0. [41].

Vector resistance to insecticides

Mosquito mortality was assessed 24h after mosquito exposure to insecticide-treated or control papers and was calculated as the percentage of mosquitoes that died out of the total number of mosquitoes exposed. When control mortality was higher than 20%, the bioassay was discarded. When it was between 5% and 20%, the mortality of the exposed mosquitoes was corrected using Abbott's formula [36]. Resistance status was defined according to WHO guidelines as: susceptibility (mortality 98-100%); suspected resistance (mortality 90-97%), and confirmed resistance (mortality below 90%) [36].

Operational and effective IRS coverage and reasons for not spraying

Operational house and structure-level coverage was calculated per IRS round based on the number of houses or structures sprayed out of those found during the campaign (as

reported by the Goodbye Malaria). Population-level operational coverage was calculated as the number of people that were protected (as reported by the Goodbye Malaria) divided by the total number of residents in the district (as recorded through the census of the population and the demographic and health surveys [29]). Household-effective level coverage was calculated as the number of households that claimed receiving IRS of all the households for which spraying status was recorded during MDAs. Reasons for a household not being sprayed (as reported during the MDAs) are reported as frequencies.

Residual efficacy of Actellic® 300CS as measured through cone bioassays

Differences in mortality across cone heights were analyzed using Poisson regression models fitted using maximum likelihood (R package mixlm [42]), where Abbott's adjusted mosquito mortality 24h post exposure was estimated by the cone height (lower, middle, upper) and wall surface (mud, cement), with the number of houses in which cone bioassays were conducted as the offset. This method was used because it allows to compare mortalities at different cone heights over time. Since there were no significant differences across cone heights (assessed at the 95% confidence level), data from individual cones was grouped for each house and test. The calculation of the district-level realized residual efficacy explained below requires daily estimates of mosquito mortality in WHO cone bioassays on different wall types. To estimate such daily mosquito mortalities with robust credible intervals, a logistic binomial Bayesian model was fitted to the mosquito mortalities observed in the WHO cone bioassays at each observation time post-exposure (24h, 48h, 72h, 96h and 120h), for each wall type and for mosquitoes exposed to treated and control walls separately. This method

estimates daily mosquito mortality from the observed bioassay results at discrete points in time post-spraying. It simulates a sequence of random samples of mosquito mortalities from WHO cone bioassay that converge to the observed distribution of mosquito mortalities in the discrete WHO cone bioassays. In other words, it is a way to conduct a robust interpolation of observed bioassay results to obtain daily mosquito mortality values. A Hamiltonian Monte Carlo sampling methods was used and programmed using the R package RStan, the R interface to Stan programming language [43]. Four chains were initialized to assess the convergence of 1,000 iterations, the first 500 of each were discarded as burn in. The posterior distributions of parameters (4,000 iterations) and 90% Bayesian credible intervals were estimated, posterior checks were performed using R package shinystan (version 2.50.0) [44, 45] and visually confirmed to fit the data. Bioassays where control mortality 24h post-exposure was >20% were discarded from the analysis. Estimated daily mosquito mortality 24h post-exposure was corrected with Abbott's formula when control mortality was >=5% and <=20% [15, 46]; estimated 48h, 72h, 96h and 120h mortalities post exposure were corrected when their respective control mosquito mortality was >=5% but not discarded if mortality exceeded 20% to avoid losing data. We report the resulting mosquito mortalities 24h, 48h, 72h, 96h and 120h post exposure over time with their 95% confidence intervals and the number of days during which such mortalities remained above the WHO thresholds of 80% with their confidence intervals [15].

District-level realized IRS efficacy

Residual efficacy, as measured through WHO cone bioassays, represents the maximum mosquito killing efficacy of a sprayed wall over time. However, it does not represent the

mosquito killing capacity of an IRS campaign in a sprayed area over time. This is due to the fact that: 1) efficacy is different for different types of walls and each area has a specific distribution of wall types; 2) by the time the last houses are sprayed during a campaign, the first sprayed houses may have started losing their efficacy, and 3) not all houses are sprayed. In order to obtain an estimate of the district-level realized IRS efficacy (i.e. the actual capacity of an IRS campaign to kill indoor resting mosquitoes at any given point in time since the beginning of the campaign), daily estimates of 24h-post-exposure mosquito mortality from the cone bioassays were adjusted by the distribution of wall types in the district, the pace of household spraying during the campaign (i.e. percentage of households actually sprayed at any given day after the campaign started, out of all households visited in the district at the end of the campaign) and the achieved effective IRS coverage. To do so, a weighted average of the estimated daily mosquito mortalities across wall types was calculated by giving cement houses a weight of 53% and mud/clay plastered houses a weight of 47%. These values are based on the proportion of houses of each type that were sprayed during the 2015 IRS campaign [6]. Since household wall types were not recorded during the malaria prevalence cross-sectional surveys or the 2016 and 2017 IRS campaigns, the weighted average mortality was assumed to represent the average residual efficacy of a household in the district. For each sprayed household, its daily IRS residual efficacy was calculated for 365 days from the time of spraying. Subsequently, for every day since the start of the campaign, the residual efficacy of each household with a spray date was summed and divided by the total number of houses sprayed for which the spray date was known. This represents the maximum daily residual efficacy that would have been achieved since the beginning of the campaign if all households would have been sprayed

in the district. However, since a percentage of the households were not sprayed, the daily maximum residual efficacy was scaled to the percentage of households that were actually sprayed during the campaign.. The result is the percentage of indoor resting mosquitoes that the IRS campaign could have killed in the district every day from the beginning of the campaign and is referred to here as the 'realized district-level IRS residual efficacy'. The decay of this efficacy over time after the 2016 campaign is reported. WHO recommends reporting the number of weeks/months during which mosquito mortality 24h-post exposure stays above 80% for the evaluation of the residual action of insecticide impregnated surfaces [15]. Hence, this measure has been frequently reported across scientific literature. Although it is known that IRS continues to reduce malaria burden (compared to no IRS) beyond the point when mortality in mosquitoes exposed to spray walls falls below 80% [16], we report the number of days during which mosquito mortality remained equal to or greater than 80% (here called "optimal realized district-level IRS residual efficacy) to facilitate the comparison with results provided in other publications. Data was analyzed using R statistical software version 4.1.0. [41].

Ethical considerations

Ethical approval for the monitoring of residual efficacy of IRS was obtained from Manhiça Health Research Centre Institutional Bioethics Committee for Health (CIBS-CISM/68/2015). Approval for monitoring insecticide resistance was obtained from the Manhiça Health Research Scientific Committee (CCI/135/Nov 2015). The household owner (>18 years old) where (i) mosquitoes were collected indoors for resistance monitoring or where (ii) the WHO cone assays were performed monthly, were informed

about the purpose of the study in the local language (Xichangana or Portuguese) and gave their oral informed consent. They were free to withdraw from the study at any moment. All other studies from which data were drawn in the present study were approved by CISM's institutional ethics committee, Hospital Clinic of Barcelona's Ethics Committee, and the Mozambican Ministry of Health National Bioethics Committee. The study protocol to implement and evaluate the impact of MDAs was also approved by the pharmaceutical department of the Ministry of Health of Mozambique and registered as Clinical Trial NCT02914145. More details on the ethical consideration of the population census, household surveys, cross-sectional surveys and MDAs are provided elsewhere [6].

Results

Susceptibility status of *An. funestus s.l* and *An. gambiae s.l* and species composition

Anopheles funestus s.l. was only collected in sufficient numbers for susceptibility testing in 2015. *An. funestus s.l.* was susceptible to DDT, bendiocarb and pirimiphos-methyl, but resistant to deltamethrin (Table 2). Of the 22% of the *An. funestus s.l.* mosquitoes that were identified to species molecularly, the majority were either *An. funestus s.s.* (55.8%) or *An. parensis* (41.6%). Out of the 173 exposed and 93 control *An. funestus s.l.* mosquitoes used for resistance testing against deltamethrin, we identified to species 106 of the exposed and 65 of the control mosquitoes. Among the exposed mosquitoes, 40 were *Anopheles funestus s.s.* and 66 *An. parensis.* Among the control mosquitoes, 38 were *An. funestus s.s.* and 28 *An. parensis*. Among the exposed *Anopheles funestus s.s.*,

17 died post-exposure (42.5%, n=40), and among the exposed *An. parensis*, 58 died post-exposure (87.9%, n=66). Differences between *An. funestus s.s.* and *An. parensis* mortalities were statistically significant χ^2 = 22.641, df = 1, p<0.0001). After implementation of district-wide IRS with DDT and Actellic in 2015, *An. funestus s.l.* mosquitoes were no longer collected in sufficient numbers to evaluate whether resistance to DDT or pirimiphos-methyl emerged in this species after IRS.

An. gambiae s.l. was susceptible to DDT, bendiocarb, pirimiphos-methyl and deltamethrin throughout the Magude project (Table 2). *An. gambiae s.l.* resistance to pirimiphos-methyl was suspected in Muginge in 2018, but its susceptibility to this insecticide was confirmed in the same village a year later (Table 2). Most (93%) of the identified *An. gambiae s.l.* mosquitoes were *An. arabiensis.*

Table 2. Insecticide susceptibility of F1 generation *An. funestus s.l.* and *An. gambiae s.l.* from Magude district, 2015-2018. Italics are used to indicate suspected resistance (mortality 90-97%); bold numbers indicate confirmed resistance (mortality below 90%).

		Bendiocarb 0.1%		**DDT 4%**		**Deltamethrin 0.05%**		**Pirimiphos-methyl 0.25%**	
		Percent mortality (n)		**Percent mortality (n)**		**Percent mortality (n)**		**Percent mortality (n)**	
		Treated	**Control**	**Treated**	**Control**	**Treated**	**Control**	**Treated**	**Control**
An. gambiae s.l.									
2015	Bairro-2000	100 (197)	3.2 (94)	99.1 (134)	9.5 (63)	98.7 (98)	14.6 (48)	100 (112)	8.9 (45)
	Muginge			100 (86)	4.5 (44)			100 (97)	4.1 (49)
2016	Bairro-2000	*94.3* (122)	0 (74)	100 (166)	0 (94)	100 (88)	0 (62)	100 (140)	1.4 (71)
	Chobela	100 (116)	1.5 (67)	100 (224)	5.9 (119)	98.1 (214)	0.8 (124)	100 (113)	2.6 (70)
	Herois Mocambicanos	100 (102)	0 (41)						
	Maguiguane			100 (44)	13.6 (22)	100 (127)	0 (72)	100 (167)	7.5 (93)
	Mapulanguene			100 (97)	0 (48)				
	Mawandla 2			100 (100)	0 (49)			100 (49)	0 (24)
	Motaze			100(48)	0 (24)	100 (47)	0 (24)		
	Muginge			100 (100)	0 (49)	100 (98)	0 (50)		
	Mulelemane	100 (91)	4.2 (48)	100 (49)	0 (25)			100 (23)	0 (15)
	Nhongane			100 (96)	0 (48)			100 (96)	0 (44)
2017	Maguiguane	100 (21)	0 (10)	100 (45)	0 (24)	100 (112)	1.9 (53)	100 (67)	3.2 (31)
	Motaze	100 (20)	0 (10)			100 (56)	0 (28)	100 (14)	3.2 (31)
	Simbe	100 (78)	0 (30)			100 (78)	0 (30)	100 (70)	6.6. (30)
2018	Muginge			*97.4 (86)*	11.7 (51)	100 (100)	16 (50)	*95.4* (101)	12 (50)

2019	Muginge			100 (100)	0 (50)			100 (97)	2 (50)
An. funestus s.l.									
2015	Bairro-2000	100 (72)	8.3 (60)	100 (37)	0 (38)	**67.6** (155)	8.3 (96)	100 (231)	8.3 (144)
	Muginge							100 (71)	0 (20)

Percentage indicates percent mortality 24h following 1h exposure to the insecticide; number between parentheses indicates the number of mosquitoes tested.

IRS operational and effective coverage, campaign duration and reasons for households not being spraying

In general, the IRS campaigns took place between the months of August and December and were completed within 3 to 4 months (S3 Figure). A summary of the campaigns and their outcomes is given in Table 3. The house and structure operational coverage (i.e. percentage of houses and structures sprayed out of those found during each campaign) was >90% for all campaigns. Population-level operational coverage (i.e. percentage of people living in sprayed household) was >86% in all campaigns. The household level effective coverage, as measured shortly after each IRS campaign, were 83% in January 2016 and 90% in February 2017 with little spatial heterogeneity [6]. This indicator was not assessed after the 2017 campaign.

The most commonly reported reason for a household not being sprayed was the fact that nobody was at home at the time the spray team visited the compound (51.5-64.1%), followed by the spray team not visiting the household (27.1-28.1%) and the household rejecting IRS (6.4-10.3%). In some cases (10.9-14.1%), the interviewee did not know why the household was not sprayed.

Table 3. Coverage and duration of the IRS campaigns implemented during the Magude project.

	2015 campaign	2016 campaign	2017 campaign
Period	3rd August - 7th November	22nd August- 30th November	21st August- 16th December
Campaign duration	3 months + 4 days	3 months + 8 days	3 months + 25 days
Household level-effective coverage[1]	83% (MDA2, Jan 2016)	89.7% (MDA4, Feb 2017)	ND
- By administrative subdivision (MDA)			

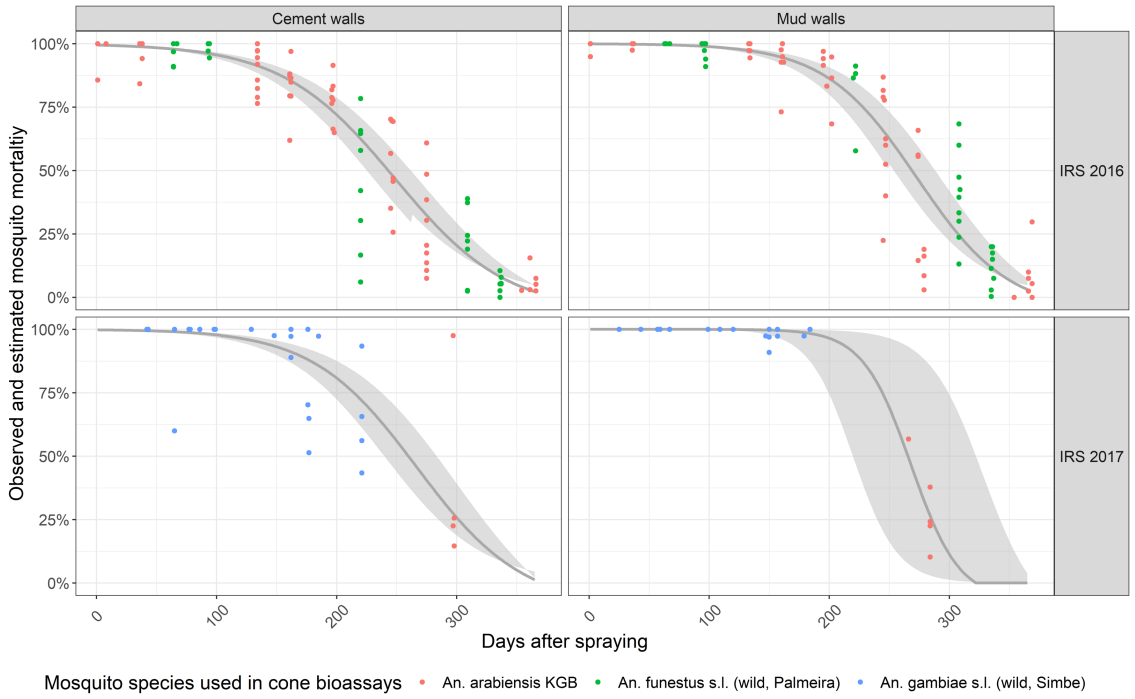
Magude Sede	81.3%	89.9%	ND
Mahele	91.4%	90.9%	ND
Mapulanguene	83.7%	86.2%	ND
Motaze	83.4%	90.0%	ND
Panjane	82.2%	85%	ND
Population level operational coverage[2]	92.6%	86.1%	88.6%
House-level operational coverage[3]	92.6%	94.5%	98.4%
Structure-level operational coverage[4]	91.6%	92.6%	96.5%

1 Proportion of households sprayed of all households in Magude district. Results previously reported in [6];
2 Number of people that were protected (as reported by the Goodbye Malaria) divided by the total number of residents in the district.
3 Number of houses sprayed out of those found during the campaign (Results reported by Goodbye Malaria Initiative)
4 Number of structures sprayed out of those found during the campaign (Results reported by Goodbye Malaria Initiative)
ND: not determined

Residual efficacy of Actellic® 300CS, 24h mortality

The residual efficacy of the 2016 IRS campaign (i.e. mosquito mortality >80% 24h after exposure) was estimated to be approximately 6 months (179 days, 95% CI: 163-196) on cement walls and 7 months (217 days, 95% CI: 199-236) on mud/clay plastered walls (Fig 2). The residual efficacy of the 2017 IRS campaign was estimated to be over 6.5 months (202 days, 95% CI: 182-224) on cement walls and close to 8 months (238 days, 95% CI: 194-292) on mud walls (Fig 2).

Fig 2. Residual efficacy of Actellic® 300CS in Magude on two different wall types after the 2016 and 2017 IRS campaigns in Magude district. Observed (point data, after Abbott's correction) and estimated (lines) mosquito mortality 24h post-exposure to insecticide-treated mud/clay-plastered and cement walls. Point colors represent the species of mosquitoes used in cone bioassays at each point in time (*An. arabiensis* KGB colony mosquitoes, wild-caught *An. funestus s.l.* or wild-caught *An. gambiae s.l.*).



Residual efficacy of Actellic® 300CS, delayed mortality

Delayed residual efficacy could not be estimated for the 2016 IRS campaign as delayed mortalities were only assessed from month 8 post-spraying onwards. But observed Abbott's corrected mosquito mortalities measured 72h post-exposure remained above 80% for 247 days on cement and 274 days on mud walls, 96h post-exposure mortality were already below 80% when it was measured for the first time (day 335) and 120h post-exposure mortality was above 80% for 235 days post-campaign in mud houses, but below 80% in cement houses at that same time point.

After the 2017 IRS campaign, estimated Abbott corrected mosquito mortality after exposure to treated mud walls was above 80% for an additional two weeks when 48h and 72h post-exposure mortality were considered, for approx. another 3 weeks when 96h post-exposure mortality was considered, and approximately another 1.5 months when 120h post exposure

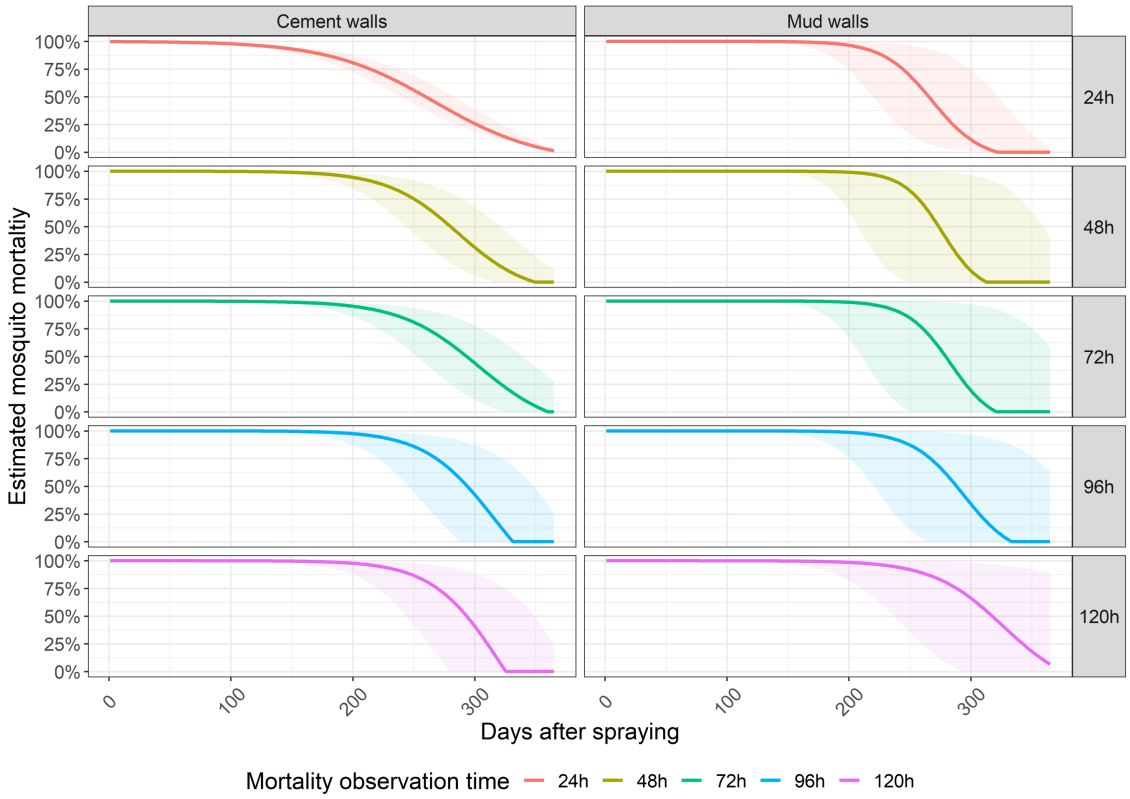
mortality was considered, compared to 24h mortality. In cement houses, 48h post-exposure mortality remained above 80% for an additional month and one week, 72h mortality for an additional month and two weeks, and 96h and 120h mortality for an additional two months, compared to 24h mortality data (Table 4).

Mortality among control mosquitoes in WHO cone bioassays ranged from 0% to 11% 48h post-exposure, 2-14% 72h post-exposure, 2-27% 96h post-exposure and 3-29% 120h post-exposure.

Table 4. Duration of optimal IRS residual efficacy (i.e. mosquito mortality >80%) in mud and cement walls, as estimated through WHO cone bioassays and expressed in days.

	2016 campaign	**2017 campaign**				
End point	**24h**	**24h**	**48h**	**72h**	**96h**	**120h**
Mud walls	217 (199,236)	238 (194, 292)	253 (194, 335)	255 (189,344)	261 (195,346)	280 (203, >365)
Cement walls	179 (163,196)	202 (182,224)	242 (207, 280)	251 (213,295)	261 (213,314)	262 (213,317)

Fig 3. Estimated Abbott's corrected mosquito mortalities 24h-120h after exposure to mud or cement walls sprayed with Actellic® 300CS during the 2017 campaign in Magude district.

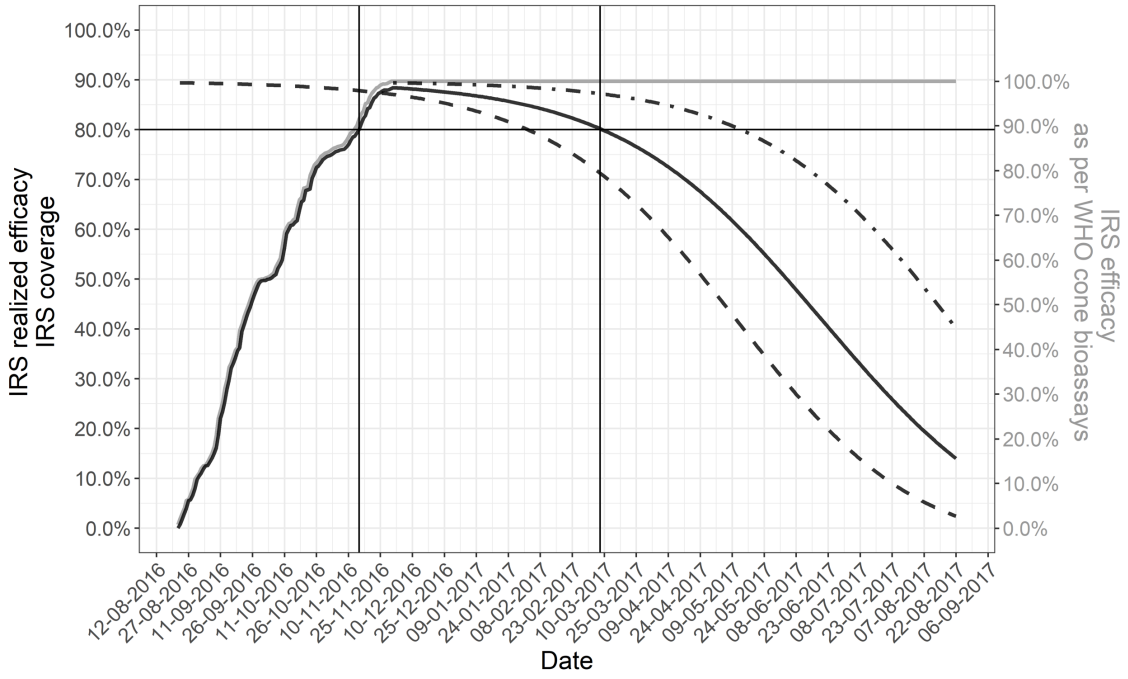


District-level realized IRS efficacy

Figure 4 shows the district-level realized efficacy overtime as a result of the 2016 IRS campaign in Magude district (i.e. the percentage of indoor resting mosquitoes that the IRS campaign could have killed on any given day after the campaign started).

Fig 4. District-level IRS realized efficacy of the 2016 IRS campaign in Magude district. Grey solid line: IRS effective coverage (household level). Black solid line: Realized IRS residual efficacy in the district considering IRS coverage, the pace of spraying, residual efficacy in mud and cement walls and the distribution of these wall types in the district. To illustrate the effect of adjusting residual

efficacy by pace of spraying, the dashed and dotted dashed lines represent how residual efficacy would have evolved if it started to decay at the beginning or the end of the campaign respectively. Vertical lines mark the date when the campaign started to kill more than 80% of the mosquitoes resting indoors and when it started to kill less than 80% again.

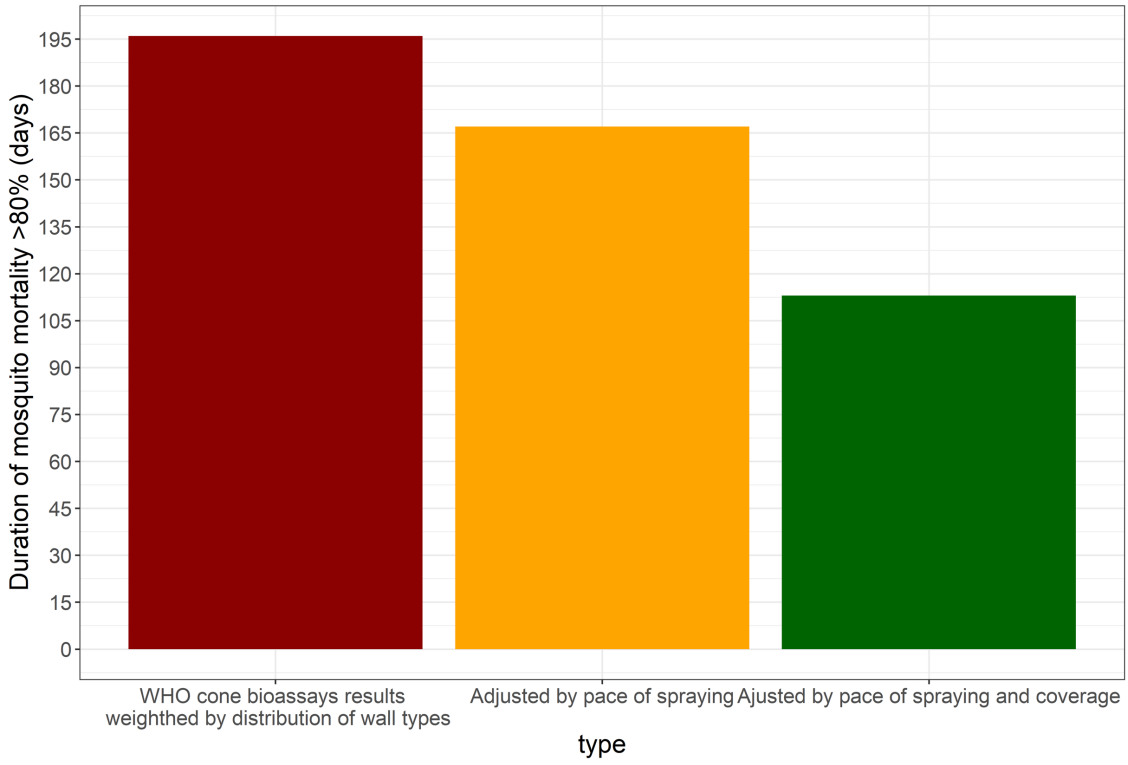


Considering the distribution of wall types in the district, the average duration of optimal residual efficacy of a sprayed wall in the district (measured as mosquito mortality 24-post exposure in cone bioassay remaining above 80%) was 6 months and 13 days (196 days, 95% CI: 179-213) after the 2016 IRS campaign, and 7 months and 8 days (222 days, 95% CI: 190-255) after the 2017 campaign (Fig 5). After correcting this for the pace of house spraying (shown in S4 Figure), if all households would have been sprayed, the district-level duration of optimal residual efficacy would have been 5 months and 15 days (167 days, 95% CI: 150-184) after the 2016 IRS campaign, which was achieved between 27th October 2016 and 12th April 2017 (Fig 5). After the 2017 IRS

campaign it would have been 6 months and 9 days (191 days, 95% CI: 158-226) and achieved between 29th October 2017 and 8th May 2018 (Fig 5).

Further adjustments for the IRS household-level effective coverage (i.e. percentage of household reported to be sprayed during the MDA campaigns of all district households), the optimal district-level realized efficacy after the 2016 campaign was shortened to 3 months and 20 days (113 days, 95% CI: 97-147) and achieved between 16th November 2016 and the 9th of March 2017 (Fig5). Household level effective coverage was not measured after the 2017 campaign and hence the district-level realized efficacy after this campaign could not be estimated.

Fig 5. Reduction in the estimated duration of the 2016 IRS campaign residual efficacy after adjusting for wall type distribution, pace of household spraying and IRS coverage.



Discussion

The present study examined the IRS campaigns conducted during the Magude project to understand their effectiveness and gaps in protection that could help to explain why local malaria transmission was not interrupted in Magude and guide future malaria elimination efforts.

Local *An. funestus s.l.* and *An. gambiae s.l.* were susceptible to pirimiphos-methyl and DDT, the insecticides used for IRS in the 2015, 2016 and 2017 campaigns. *An. funestus s.l.* exhibited resistance to deltamethrin. Pyrethroid-resistance in *An. funestus s.l.* is wide-spread in southern Mozambique [31-33, 47]. However, in Magude district, *An. funestus s.l.* mortality after exposure to deltamethrin in 2015 (>67%) was substantially higher than mortalities observed in

the neighboring districts of Chokwe (0% mortality in 2009 [33]) and Manhiça (33% mortality in 2009 [32]; 3-10% in 2014 [31]). These differences could be due to mosquito rearing or testing conditions, to the use of mosquitoes that were not truly representative of the local mosquito population or to true differences in the frequency of resistant mosquitoes between districts. A common limitation in all studies assessing discriminatory dose bioassay mortality using F_1 offspring of wild caught female mosquitoes is how representative the tests are of the whole population. That is, if all offspring emerge from eggs laid by a few females, the survival at bioassay testing may simply reflect the specific phenotypes of those females rather than the distribution of phenotypes in the vector population. In our study, large amounts of blood-fed female mosquitoes were collected from several houses to increase the genetic diversity in the sample, but the percentage of adult females that laid eggs was not monitored. The previous studies in Manhiça and Chokwe have also not reported this critical information [31-33]. Ambient conditions are also known to affect resistance test results and could also explained the observed difference in resistance profiles [48]. However, differences could also reflect true differences in the vector population caused by natural barriers that prevent or limit gene-flow between mosquito populations, differences in selection pressure from historical agricultural practices or vector control interventions, variations in resistance status across species of the same complex [49] combined with inter-district differences in species distribution. It is worth noting that Manhiça, Chokwe and Magude districts all have large agricultural plantations where different insecticides may have been used over time to protect sugar cane or rice crops. The composition of the tested vector sample differed between Manhiça and Magude. In Magude, *An. funestus s.s.* accounted for 55.8% of the *An. funestus s.l.* mosquitoes, while in the Manhiça study it accounted for 95%.

Although our assessment of the difference in the frequency of pyrethroid resistance among *An. funestus s.s.* and *An. parensis* is inconclusive due to the low number of *An. funestus s.s.* mosquitoes in the sample, our results would suggest a higher frequency of resistant individuals among *An. funestus s.s.* than among *An. parensis.* If these differences were confirmed, they could justify the inter-district differences in resistance observed. All in all, the inter-district differences in resistance to deltamethrin highlight the difficulty to extrapolate insecticide resistance results across areas. They should be further investigated molecularly and with large sample sizes collected in spatiotemporally diverse positions over a year to confirm that they are due to true differences in local vectors and not to artifacts during testing procedures. As new interventions targeting resistance mosquitoes emerge, such differences may be important to guide their deployment.

This is the third study that reports *An. parensis* resting on indoors walls [50, 51]. This is an important finding since *An. parensis* was recently incriminated as a malaria vector and implicated in residual malaria transmission in South Africa [52]. Although believed to be mainly zoophilic, *An. parensis* has been observed to bite humans outdoors [53]. During the Magude project, *An. parensis* accounted for 5.8% of residual vector bites [35] and was found feeding outdoors, indoors before people went to bed, and indoors while people were in bed, showing its potential to transmit malaria in different environments. Finding *An. parensis* resting indoors in the morning during the manual mosquito collections indicates that IRS could target a part of this vector population.

In contrast to *An. funestus s.l.*, *An. arabiensis* from Magude district was susceptible to pyrethroids. This could indicate that this species manages to avoid or reduce exposure to

insecticides through behavioral resistance. The ability of *An. arabiensis* to enter houses to blood feed but subsequently avoid contact with LLINs or IRS has been documented elsewhere [54, 55]. Given the number of large-scale agricultural plantations in and around Magude district, pyrethroids may have been used to protect crops in the area. If they were, the differences in the preferred breeding sites of *An. arabiensis, An. funestus s.s.* and *An. parensis* may have altered their exposure to pyrethroids, which could have contributed to the observed differences in their susceptibility to this class of insecticide [52].

The high proportion of houses and structures sprayed out of those found during the three IRS campaigns (>91%) suggests that IRS was well accepted by the population of Magude. This is further supported by the fact that rejection of IRS was only reported by 6-10% of people asked for the reason why their household were not sprayed. The population and household level coverage (>86% and >83%, respectively) indicate that the effective IRS coverage was high and above the WHO recommended coverage of 80% [56] during the Magude project. Although those two indicators were not measured after the 2017 campaign, effective coverage was likely to be equally high in this campaign as: i) the number of structures found during the campaign was similar, and; ii) the percentage of structures sprayed was higher compared to the two previous campaigns.

Interesting to note is the fact that the percentage of structures and houses sprayed of those found as reported by the IRS campaign was lower than the percentage of district households sprayed in all three IRS campaigns. As shown by the reasons for the households not being sprayed, this is likely due to the spray teams missing some households. This highlights the

fact that the actual IRS coverage may be lower than the coverage reported after IRS campaigns, which could impact the efficacy of malaria control and elimination efforts.

Mosquito mortality 24h post-exposure in WHO cone bioassays remained above the WHO efficacy threshold of 80% [15] for over 7 months in mud houses, and around 6 months in cement houses. Although both KGB colony mosquitoes and wild collected mosquitoes were used in the cone bioassays, the pattern of residual efficacy decay seems to follow a similar and typical pattern [17]. Great differences in the duration of Actellic® 300CS's residual efficacy have been observed across countries and wall types, with its efficacy ranging anywhere from 3 to 11 months [22-27, 57-59], and this tends to be true for other IRS products [60]. It has been argued that such differences could be related to the quality of spraying [26], differences in wall properties [61] (e.g., wall smoothness or coating used in different settings) or due to environmental conditions (e.g., temperature and humidity) [62] as well as potential differences in wall modifications post-spraying [28] that were not measured in the present study. Regardless of the reason, the variability in study results highlight the importance of measuring the residual efficacy of IRS products locally, to inform the selection of IRS products and identify the optimal time for IRS campaign implementation.

Killing malaria vectors before they can actually transmit malaria to humans (in the days between the moment they get infected to the moment they become infectious after the sporogonic cycle, or in the days between blood meals of already infectious mosquitoes) is expected to reduce malaria transmission [12]. In Magude, mosquito mortality 5 days post exposure to Actellic® 300CS extended optimal efficacy by between one and two months, depending on wall type, which is similar to previous observations in India [18]. This highlights the

importance of assessing delayed mosquito mortalities to understand the real effect of IRS in reducing the ability of the vector to transmit malaria. However, the high mortality among control replicates observed at 48h, 72h, 96 and 120h time points indicates that a broader discussion is needed to identify the best methods to estimate IRS induced delayed mosquito mortalities.

Traditionally, the potential impact of an IRS campaign has been assessed by reporting operational IRS coverage and a product's residual efficacy (as measured by WHO cone bioassay) separately [12, 15]. But this provides incomplete information on the true potential mosquito killing effect of an IRS campaign. First, it does not take into account that houses are sprayed gradually and hence, residual efficacy does not decay in all houses at equal pace from the start of the campaign. Secondly, differences in residual efficacy across wall types implies that some houses have a higher mosquito killing capacity than others. As a result, some houses will be more effective at killing indoor resting mosquitoes than others at any given point in time during and after each campaign. Thirdly, unsprayed houses will provide surfaces for mosquitoes to rest on without being killed, reducing the overall capacity of the IRS campaign to kill indoor resting mosquitoes in the targeted area. By combining these factors, a more realistic metric of IRS efficacy, the "district-level realized IRS efficacy", is presented here. Surprisingly, this new metric shows that the optimal realized district-level efficacy of the 2016 IRS campaign (with Actellic® 300CS) was 3 months and 20 days, almost 3 months shorter than the optimal residual efficacy measured through standard WHO cone bioassays alone. Based on WHO cone bioassay data alone, one would assume that the 2016 IRS campaign effectively covered the entire high malaria transmission season, but the realized efficacy was achieved mid-November (shortly after the start of the rainy season and a month and half before the high malaria incidence season) and lost

early March (almost two months before the traditional high malaria transmission season ended). Although delayed mortality may extend the duration of the realized efficacy for an additional month or so, the rapid decrease in efficacy towards April may leave communities less protected at the end of the high malaria transmission season, a time when the effect of MDA had already faded away. This could explain the annual increases in malaria incidence observed during April, May and June throughout the Magude project [6]. Rains are still frequent and intense during those months [29], which could have created adequate conditions for vector populations to proliferate and drive transmission in the absence of effective IRS.

To identify strategies that could have closed this gap, it is crucial to understand the behavior of local vectors, as the effectiveness of vector control interventions depend upon them. The main malaria vector species during the Magude was *An. arabiensis* which accounted for 74% of all human exposure to vector bites [35]. *An. arabiensis* is known for the plasticity of its behaviors. It can feed on animals or humans, depending on host availability and feed indoors or outdoors, at dusk, dawn or during the night depending on the location of its hosts [54, 63]. It has been observed to rest indoors when its hosts are primarily indoors [64], but to rest both indoors and outdoors when its hosts are outdoors [65]. As said before, *An. arabiensis* is known for its ability to avoid contact with vector control interventions [54, 55] and has been found to exhibit outdoor resting tendencies following the application of IRS or deployment of ITNs [66, 67]. Given the variation of *An. arabiensis* across areas upon host availability and local situation, an evaluation of its local behaviors is necessary to understand the effect that different vector control intervention could have on the local population of this species. In our study, most of the mosquitoes that were collected indoors during the manual collections for resistance monitoring

purposes, both before and after the implementation of IRS campaigns, were *An. arabiensis*. Although we did not measure how frequently this vector rests indoors (compared to outdoors) in Magude, the fact that *An. arabiensis* were found resting indoors combined with the high IRS coverage suggests that IRS controlled *An. arabiensis* partially but not fully. In addition, IRS is likely to have controlled *An. funestus s.s.*, known to be a major vector in the region, as this vector species was no longer found in indoor collection for resistance monitoring after the implementation of the first IRS campaign. These facts suggest that IRS was effective, at least to some extent, at controlling the main local vectors during the Magude project and leads us to conclude that the use of a longer-lasting IRS product will have contributed to further reduce malaria transmission.

Our proposed new methodology to estimate the IRS residual efficacy in a more realistic manner has some limitations: it requires 1) knowing the distribution of different house types in the district, which is information that is often not available unless a census of the population has been recently conducted, 2) understanding the different residual efficacies on those different wall types, which is often not assessed in programmatic settings or only in a few geographic locations, and 3) knowing the pace of spraying (i.e. the proportion of structures sprayed on any given day out of all structures sprayed during the campaign), information that is often not reported after IRS campaigns but may be collected as part of the campaign monitoring process. One could omit information on differences in residual efficacies between wall types, and use a simplified version of our proposed realized IRS residual efficacy, but we argue that IRS coverage and the pace of spraying will be important indicators to better understand the efficacy of IRS campaigns.

Beyond shedding light into the reasons why malaria transmission was not interrupted during the Magude project, this study highlights the need to rethink the data and indicators used to evaluate the potential effectiveness of IRS campaigns. The large differences in residual efficacy estimates obtained through WHO cone bioassays compared to those obtained by considering IRS coverage, the pace of spraying, the residual efficacy on different wall types and the distribution of such wall types in the district, shows the need to improve current methods to estimate IRS efficacy. Finally, the impact that the pace of household spraying had in determining the time when IRS campaigns reached optimal efficacy, highlights the need to evaluate different IRS implementation strategies to design the most effective IRS campaigns.

Conclusion

The IRS campaigns implemented during the Magude project achieved high coverage and acceptability. However, its realized residual efficacy considering IRS coverage, the pace of spraying, residual efficacy on different wall types and distribution of such wall types in the district, fell short to provide optimal protection during the entire high malaria transmission season, which could be one of the reasons why local malaria elimination was not achieved. The use of a longer-lasting IRS product could have contributed to further reducing malaria transmission by increasing the protection provided during the final months of the high transmission season. An accurate estimation of IRS residual efficacy and an evaluation of vector behaviors and insecticide resistance is critical to select IRS products and to inform the overall design of vector control strategies. Countries should consider more realistic indicators, such as

the realized IRS efficacy proposed here, to obtain more accurate estimates on the efficacy of their IRS campaigns.

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Supporting information files

S1 Table. Susceptibility of CISMs *An. arabiensis* KGB colony to pirimiphos-methyl

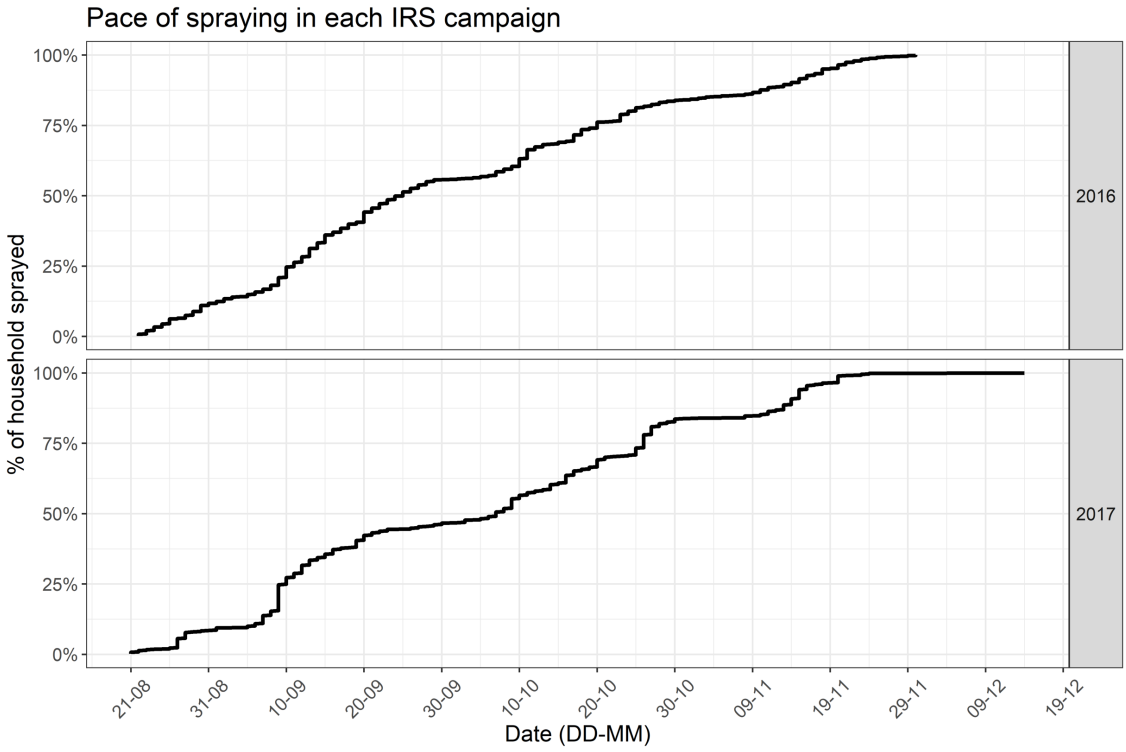
Test date	Mortality in controls	Abbott's adjusted Mortality in exposed (%)
27.07.16	0 (20)	100(36)
07.06.18	0 (51)	98.7(76)
18.07.18	7.3(41)	98.3 (65)

S2 Table. Susceptibility of F1 generation of wild-caught *An. funestus s.l.* and *An. gambiae s.l.* to pirimiphos-methyl.

Species	Locality	Test date	Mortality in controls (%)	Mortality in exposed (%)
An. funestus s.l.	Palmeira, Manhiça district	19.05.2016	0 (22)	100 (44)
		21.07.2016	0 (30)	100 (56)
		29.08.2017	4.2 (24)	100 (45)
An. gambiae s.l.	Muginge/Simbe, Magude district	14.12.2017	6.7 (30)	100 (70)

S3 File. Raw uncorrected mortality data for mosquitoes from all WHO cone bioassays conducted in Magude district from 2016 to 2018 (data is not provided inside of this thesis, it will be available when the paper is published)

S4 Figure. Pace of household spraying during the 2016 and 2017 IRS campaigns.



S5 File. Dates on which households received IRS during the 2016 and 2017 campaigns in Magude, as recorded for those households of randomly selected participants in the malaria prevalence cross-sectional surveys in May of 2017 and 2018. (data is not provided inside of this thesis, it will be available when the paper is published)

Article 2 (Under review) **Fernández Montoya L**, Alafo C, Martí-Soler H, Máquina M, Malheia A, Sacoor C, et al. An evaluation of LLIN ownership, access, and use during the Magude project in southern Mozambique.

Objectives addressed:

- Objective 4: To identify limitations in the implementation of and protection conferred by LLIN against malaria vectors during the project
- Objective 7: To identify possible improvements in ITN distributions to guide future campaigns.

An evaluation of LLIN ownership, access, and use during the Magude project in southern Mozambique

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Abstract

The Magude Project assessed the feasibly of eliminating malaria in a low transmission setting in southern Mozambique using a package of interventions. This study measured the ownership, access and use of long-lasting insecticide treated nets (LLINs) and inequalities in these indicators across household wealth, size and population subgroups, to understand the protection that LLINs provided during the project. Data were obtained from various household surveys. At least 31% of the nets distributed during the 2014 and 2017 campaigns were lost during the first year post-distribution. Most nets (77.1%) in the district were Olyset Nets. LLIN access never exceeded 76.3% and use varied seasonally between 40% and 76.4%. LLIN access limited LLIN use during the project, especially during the high transmission season. LLIN ownership, access and use were lower in harder-to-reach localities, in poorer and larger households. Children and women below 30 had poorer access to LLINs than the overall population. Net use was lowest among school-aged children and young adults, especially among young males, and highest in children under 5, pregnant women, in older adults and in households that received indoor residual spraying (IRS). This study revealed that LLIN mass-distribution campaigns alone are not sufficient to achieve the high level of net protection needed during elimination programs and that reviewing the LLIN allocation scheme, top-up distributions and/or community engagement campaigns is needed, also to reduce inequalities in populations' access to LLINs.

Introduction

Mozambique is one of the six countries that accounts for approximately half of all malaria deaths worldwide [1]. Although most of the country-wide efforts concentrate on reducing the malaria burden, malaria eliminating initiatives have been implemented in the southern part of the country in the context of broader attempts to eliminate malaria in southern Africa [2, 3]. In Mozambique both control and elimination efforts have relied heavily on vector control. Until 2000, malaria prevention relied mainly on indoor residual spraying. Since 2000, it relied on the distribution of insecticide-treated nets (ITNs), later replaced by long-lasting insecticidal nets (LLINs), while the use of indoor residual spraying (IRS) has been limited to a few districts in Central Mozambique and to the southern province of Maputo to accelerate malaria elimination in this region, South Africa and Eswatini [2, 3]. LLINs have been used as a core vector control intervention since 2005, first distributed to target groups and then through mass distribution campaigns every 3 years since 2014, with the aim to provide at least one net for every two persons in a household [4].

LLIN efficacy relies on their ability to kill mosquitoes when they come into contact with the net, and their ability to prevent vectors from biting humans (as they provide a physical barrier and/or repel host-seeking mosquitoes). LLIN effective protection in the field depends on net use, which depends on a population's access to LLINs [5]. Once in use, net efficacy depends on its physical integrity (absence of holes), ability to preserve the bio-availability of insecticides on the net's surface [6, 7] and on the behavior of local vector populations [8], as LLINs cannot prevent bites from vectors that bite outdoors, or indoors before people use a net. Evidence from the field shows that LLIN ownership, access, physical integrity and residual bio-efficacy can all decrease

rapidly over time after net distribution [9-11], that LLIN use can vary markedly across seasons [12] and that vector compositions and behaviors can change as a results of the implementation of vector control interventions [13, 14].

LLINs were one of the two vector control interventions implemented during the Magude project. The project aimed to interrupt malaria transmission in Magude district, southern Mozambique, through a combination of interventions targeting the vector (LLINs and annual district-wide indoor residual spraying or IRS) and the parasite reservoir (mass drug administration, MDA, and standard diagnosis and treatment) simultaneously [15], but local malaria transmission was not halted [15, 16].

This study aimed to evaluate the extent of protection that was provided by LLINs during the Magude project, to better understand why malaria transmission could not be interrupted locally. Results from this study also inform the design of additional strategies to cover the gaps in LLIN protection in future malaria elimination efforts in the region. Using data collected through the district demographic and health platform, malaria prevalence cross-sectional surveys, and mass drug administration campaigns, we evaluate LLIN ownership, access, and use during the Magude project, as well as differences in these indicators across district localities, household wealth and size, age group and gender.

Materials and methods

Study site

The study was conducted in Magude district, southern Mozambique. A baseline census in 2015 registered 48,448 residents (and 4,133 non-residents) and 10,965 households. Additional

detailed demographic, health and malaria incidence information can be found elsewhere [17]. Magude, like most of Mozambique, has year-round malaria transmission with seasonal variations presenting higher incidence between November and April. Two LLIN mass distribution campaigns were conducted by the National Malaria Control Programme (NMCP), one before (May 2014) and one during the Magude project (December 2017). In May 2014, 35,432 LLINs (Olyset, Sumitomo Chemical Ltd, Japan; Permanet 2.0, Vestergaard Frandsen, Switzerland) were distributed in the district [17], and in December 2017, 44,400 LLINs (Dawa Plus 2.0, Tana Netting, United Arab Emirates). Additionally, LLINs were continuously distributed through the Expanded Program of Immunization (EPI) and antenatal care services (ANC), although the total number of nets and net brand(s) distributed through these channels are unknown. Community engagement activities were conducted throughout the Magude project to increase the acceptability of MDA and IRS and the use of LLINs.

Data sources

The present analysis draws from data collected through multiply studies and surveys conducted throughout the Magude project. A summary of each study with its sampling strategy is provided below. More extensive description of the malaria prevalence cross sectional surveys, mass drug administration surveys, the census of the population and demographic and health surveys are provided elsewhere [15-17]. Table 1 shows the LLIN indicators that were monitored, as well as the sample size of each study.

- A **census of the population** (January-June 2015) was conducted before the Magude project began. The questionnaire included questions at the household, individual and

net levels, including the number of members and nets that each household had, the net brands, the number of people that slept under each net and whether people used the net the night before the survey. In the individuals' surveys, only answers of residents were recorded (i.e. visitors were excluded) [17].

- **Health and demographic surveys** (2 surveys; August-September 2016 and September-December 2018) were conducted to update a subset of data that were collected during the census of the population, and included the entire Magude population. The questionnaire included questions on number of household members and nets in each household and whether people used the net the night before the survey, but did not record the net brand nor the number of people that slept under each net [17].
- **Malaria prevalence cross-sectional surveys** (4 surveys, every May from 2015 to 2018) were conducted to measure malaria prevalence at the end of the high transmission season. An age-stratified simple random sample of participants, with oversampling of children under 15 years of age, was drawn from the census of the population annually. Each participant was asked about the type of net they slept under, the channel of net acquisition and whether they slept under the net the night before the interview.
- **Mass drug administration surveys** (4 surveys at each MDA round: November 2015, January 2016, December 2016 and February 2017) were administered to each person found during each MDA round, regardless of whether they received the drugs or not. The percentage of the population that responded to these surveys was 89.6% in November 2015, 77.7% in January 2016, and 80.7% in both December 2016 and

February 2017. The questionnaire included questions on whether people used the net the night before the survey, and the reason for not using it.

Questionnaire data (interviews) were collected using tablets and Open Data Kit (ODK [18]) forms and sent daily to a Server Data Base at CISM (Manhiça Health Research Center, Manhiça, Mozambique)

Table 1. Data sources, their sample sizes and LLIN indicators estimates derived from each source

	2015			2016				2017		2018	
Time	**Jan-June**	**May**	**Nov**	**Jan**	**May**	**Jun-Aug 16**	**Dec**	**Feb**	**May**	**May**	**Sep-Dec**
Type of study	**Population census**	**Prevalence Survey**	**MDA 1**	**MDA 2**	**Prevalence Survey**	**HDS 1**	**MDA 3**	**MDA 4**	**Prevalence Survey**	**Prevalence Survey**	**HDS 2**
Sample size	10800 households, 48448 people 24302 nets	1035 people	43431 people interviewed	37666 people interviewed	1657 people (age stratified sample)	10648 households, 49274 people	39759 people interviewed	39748 people interviewed	3865 people (age stratified sample)	3354 people (age stratified sample)	10149 households, 51436 people
LLIN channels of acquisition											
LLIN brands											
LLIN attrition since last campaign											
LLIN reasons for loss											
LLIN ownership											
LLIN access											
LLIN use											
LLIN sharing: no of people that slept under each net											
LLIN reasons for not use											

Prevalence Survey: Malaria prevalence cross sectional surveys
MDA: Mass drug administration campaigns
HDS: Health and demographic surveys

Data analysis

LLIN attrition since the 2014 mass distribution campaign

LLIN attrition was calculated retrospectively, using data from the 2015 population census and from the health and demographic surveys in 2016 and 2017. The number of nets present in a household was compared with the number of nets distributed during the mass distribution campaign to estimate the minimum percentage of nets lost.

LLIN ownership, access and use and inequalities across household and population subgroups

Bednet ownership, access and use indicators were estimated following the recommendations of Roll Back Malaria and the World Health Organization [19, 20].

- Ownership (calculated from census data and health and demographic surveys):
    - proportion of households with at least one LLIN out of all households in the district
    - proportion of households with at least one LLIN for every two persons out of all households in the district (referred here to as “optimal access”)
- Access (calculated from census data and health and demographic surveys):
    - proportion of individuals with access to a net in their household out of all individual in the district.
    - Proportion of individuals sleeping in households that had one net for every two people out of all individual in the district.

- Use (calculated from census data and health and demographic surveys, MDA surveys and malaria cross-sectional prevalence surveys)
    - proportion of individuals that slept under a net the night before being interviewed.

To understand inequalities in LLIN ownership and access across localities and types of households, the above indicators were calculated for each of the five administrative posts, each level of household wealth and each household size (i.e. the number of members in a household). Household wealth was calculated using the Multidimensional Poverty Index (PI), an adaptation of the poverty index originally developed by the Oxford Poverty and Human Development Initiative [21], using data from the Magude census of the population and by classifying the household into three groups according to their deprivation scores: 0-2; 3-4; 5-6. The higher the number of deprivations, the poorer the household. A complete explanation of the calculation of the Multidimensional Poverty Index (PI) is provided elsewhere [22].

Differences in LLIN ownership and access across households of different deprivation levels were calculated using data from the 2015 census, as this was the only survey with sufficient data to estimate the Multidimensional Poverty Index (PI). Differences across household size and administrative post were calculated based on pooled data from the 2015 census and the health and demographic surveys in 2016 and 2018. To explore differences in net access across sex and age groups, we calculated the percentage of people living in households with at least one net for every two people, disaggregated by sex and by age group (i.e. under 5 years of age, between 5

and 14 years of age and 15 years of age) for each survey, and statistical analyses were performed using the Chi-square test of inequality.

To estimate LLIN use from the malaria prevalence cross-sectional surveys, weights were assigned to participants' answers based on their age strata as the random sample was age-stratified. Resulting point estimates are provided together with their 95% confidence intervals (CIs), using the svycipro function of the R package survey. To estimate LLIN use from MDA surveys, point estimates together with the 'best-case' and 'worst-case' scenario are provided, as those surveys did not reach the entire population (between 76% and 80% of the population was surveyed). For the best-case scenario, we assume all individuals who were missed slept under the net the night before the survey. For the worst-case scenario, we assumed none of those individuals slept under a net.

Additional analyses were conducted using the census and the health and demographic surveys to understand i) the behavioral gap in LLIN use (i.e., the percentage of people that used the net of those living in a household with at least one LLIN, disaggregated by sex and the age groups; ii) if and how the population shared the net (i.e. calculation of the percentage of nets that were shared by one, two or three or more people) in households with different numbers of people per net, and iii) the effect of IRS on LLIN use (i.e. the percentage of people sleeping under a net in sprayed vs. unsprayed household).

Reasons for not using an LLIN to sleep

In surveys conducted during the MDA campaigns (high transmission seasons), participants were asked for the reason for not using a net to sleep the night before through a closed-ended question. During the MDA surveys the following options were provided: I don't have a net; I dislike the net; the net was not hung; it is too hot; other reason (please specify). The percentage of individuals reporting each answer is reported together with 95% Wald CIs. Estimates represent between 76% and 80% of the Magude population, depending on the survey round.

Type of nets used by residents and channels of net acquisition

We report the frequency of each net brand found in the district during the 2015 population census. Malaria prevalence cross-sectional survey data (2015, 2016, 2017, 2018) were used to estimate the percentage of participants that slept under an LLIN or a net impregnated in the last 12 months and the frequency of different channels of acquisition (distributed by a health facility, national malaria program, Centro de Saude de Manhiça, bought or unknown). Weights were assigned to participants' answers based on their age strata (see above) and analyses were performed using the svycipro function of the R package survey.

Ethical consideration

All studies were approved by CISM's institutional ethics committee, Hospital Clinic of Barcelona's Ethics Committee, and the Mozambican Ministry of Health National Bioethics Committee. The study protocol to implement and evaluate the impact of MDAs was also approved by the pharmaceutical department of the MoH of Mozambique and registered as

Clinical Trial NCT02914145. More details on the ethical considerations of the population census, household surveys, cross-sectional surveys and MDAs are provided elsewhere [16].

Results

LLIN attrition since mass distribution campaigns

In Jan-Jun 2015, less than one year after the 2014 mass distribution campaign, the total number of nets in the district (including those not distributed during the mass campaign of 2014) was 25,011 [17]. This number was 22,502 in June-August 2016 and 30,274 in September-December 2018. This implies that at least 31.4% and 36.5% of the nets that were distributed during the 2014 mass distribution campaign were lost during the first and second year after distribution, respectively, and that 31.8% of the nets distributed during the 2017 mass campaign were lost within a year of distribution

LLIN ownership and access

The proportion of households that owned at least one net decreased from 81.5% in 2015 (Jan-June) to 78.8% in 2016 (Aug-Sept) but increased to 91.1% in 2018 (Sep-Dec) after the mass distribution campaign of December 2017. The proportion of households that owned at least one net for every two persons decreased from 61.9% in 2015 to 54.4% in 2016 and increased again to 59.2% in 2018. The proportion of individuals that had access to an LLIN within their household was 73.7% in 2015, decreased to 68.2% in 2016 and increased to 76.3% in 2018. A summary of net ownership and access values obtained during the population census and subsequent district-wide health and demographic surveys is provided in Table 2.

Table 2. LLIN ownership, access, use, use provided access and use among sprayed and unsprayed household throughout the Magude project. Indicators estimated from MDA surveys are presented with their best-case and worst-case estimates in parenthesis. Confidence intervals for indicators estimated from the malaria prevalence cross-sectional surveys are calculated with survey methods taking into consideration individual weights based on their age-strata and are presented in square brackets. No confidence intervals are provided for indicators calculated from the census or demographic and health surveys, as these covered the entire population in the district.

			2015			2016				2017		2018	
			Population census	Prevalence Survey	MDA 1	MDA 2	Prevalence Survey	HDS 1	MDA 3	MDA 4	Prevalence Survey	Prevalence Survey	HDS 2
			Jan-June	May	Nov	Jan	May	Jun-Aug 16	Dec	Feb	May	May	Sep-Dec
Ownership	% of households with at least one LLIN		81.5					78.8					91.1
	% of households with at least one LLIN for every two people		61.9					54.4					59.2
Access	% of individuals with access to an LLIN in their household		73.7					68.2					76.3
	% of individuals who lived in households with at least one net per every two people		54.5					45.6					50.0
	% of children <5 years-old who lived in households with at least one net per every two people		48.9					39.9					45.2
	% of pregnant women who lived in households with at least one net per every two people							49.6					50.8
Individual level use	% individuals who slept under an LLIN the previous night	Overall	25.4	40.9 [36.7-45.0]	67.9 (61.0, 75.2)	76.3 (59.4, 81.7)	64.4 [61.6-67-0]	40.00	67.8 (54.7, 74)	70.3 (56.7, 76)	72.2 [70.0-74.0]	70.4 [67.7-73]	57.1
		In HH with at least one net for every two people	35.0					55.1					66.1

		In HH with less than one net for every two people	14.0					27.6					49.2
	% of < 5 year-olds who slept under an LLIN the previous night	Overall	27.2	39.5 [35.4-44.0]	73.6 (67.9, 75.6)	78.8 (61.8, 83.4)	68.6 [63.9-73.0]	43.8	71.9 (65.3, 74.5)	74.4 (68.1, 76.5)	80.8 [79.0-82.0]	76.7 [74.4-79]	64.6
		In HH with at least one net for every two people	36.7					59.5					74.1
		In HH with less than one net for every two people	18.0					34.0					58.2
	% of 5-14 year-olds who slept under an LLIN the previous night	Overall	21.7	40.3 [33.8-47]	69.8 (52.3, 77.4)	75.6 (56.7, 81.6)	60.5 [54.7-66.0]	36.5	65.2 (52.6, 71.9)	68.2 (54.2, 74.8)	70.3 [67.5-73.0]	70.7 [68.3-73.0]	58.3
		In HH with at least one net for every two people	31.0					52.2					67.5
		In HH with less than one net for every two people	11.1					25.0					50.5
	% of >15 year-old who slept under an LLIN the previous night	Overall	26.9	41.7 [35.0-49.0]	70.8 (56.8, 76.6)	76.0 (58.7, 81.5)	65.4 [61.5-69]	40.9	68.0 (52.8, 75.2)	70.1 (54.8, 76.6)	70.6 [67.0-74.0]	68.0 [63.2-72.0]	54.6
		In HH with at least one net for every two people	36.7					55.5					63.7
		In HH with less than one net for every two people	14.2					26.9					45.9
	% of men who slept under an LLIN the previous night	Overall	24.0	34.8 [29.0-41.0]	69.9 (55.2, 76.2)	75.4 (52.8, 82.8)	63.5 [63.5-68]	38.5	66.1 (50.2, 74.3)	68.4 (52.3, 75.9)	72.4 [69.1-75.0]	69.4 [65.4-73.0]	53.0
		In HH with at least one net for every two people	33.3					53.8					62.4
		In HH with less than one net for every two people	13.2					26.5					46.1
	% of women who slept under an LLIN the previous night	Overall	26.6	45.5 [39.6-51.0]	71.9 (65.7, 74.3)	77.1 (64.7, 80.8)	65.0 [61.4-68.0]	41.2	69.0 (58.3, 73.9)	71.6 (60.1, 76.2)	72.3 [69.5-75.0]	71.0 [67.4-74.0]	60.2
		In HH with at least one net for every two people	36.4					56.0					69.1
		In HH with less than one net for every two people	14.6					28.4					51.9
		Overall		42.9 [9-85]	73.1[a]	77.9[b]	77.8 [50.5-92]	48.6	72.0[c]	77.9[c]	90.0 [64.4-98.0]	92.3 [53.1-99.0]	62.8

	% of pregnant women who slept under a net the previous night	In HH with at least one net for every two people						59.7					70.9
		In HH with less than one net for every two people						38.8					55.6
Relation between LLIN use and IRS	% individuals who slept under an LLIN the previous night	In sprayed HH	27.0	43.1 [37.8-49]	73.0[a]	78.7[b]	67.5 [64.4-70.0]	41.7	69.4[c]	71.5[c]	71.6 [68.9-74.0]	73.2 [69.8-76.0]	
		In unsprayed HH	23.2	37.1 [30.4-44]	60.7[a]	66.4[b]	48.5 [40.8-56.0]	37.7	56.0[c]	59.5[c]	67.6 [62.0-73.0]	60.8 [54.5-67.0]	

HH= household, HDS= Health and demographic survey, a: based on answers from 89.6% of the district residents, b: based on answers from 77.7% of the district residents, c: based on answers from 80.7% of the population

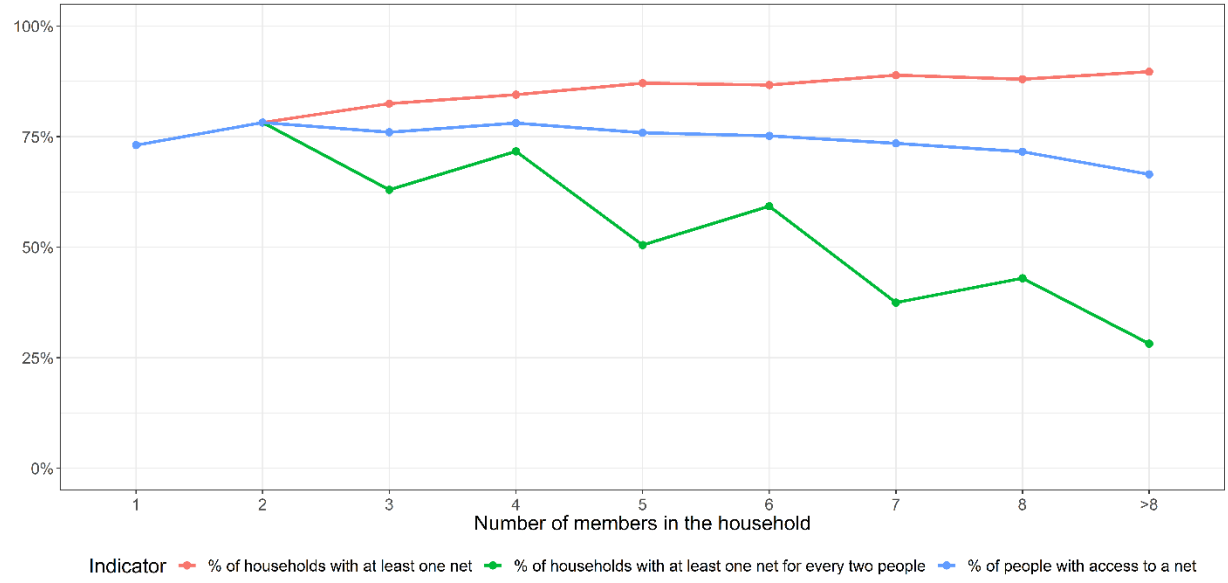
There were significant differences in ownership and LLIN access across household sizes (χ^2, df=8, p<0.0001 for all tests). The percentage of households with at least one LLIN increased slightly with increasing household size (i.e. number of members in the household), but the percentage of households with at least one net for every two people (optimal ownership) and the percentage of people with access to a net decreased with increasing household size. The percentage of households with at least one net was 73.1% for households with one person and 89.7% for households with more than 8 members. The percentage of households with at least one net for every two people was 73.1% for households with one member and 28.2% for households with more than 8 members. The percentage of people with access to a net in households with one member was 73.1% but in those of more than 8 members it was 66.5% (Fig. 1).

Small but significant differences in LLIN ownership and access were observed across household deprivation levels (χ^2, df=2, p<0.0001 in all tests). Wealthiest households presented better LLIN ownership and access levels. In 2015, optimal ownership was 3.9% higher and household member access to a net was 5.4% higher in the wealthiest households. These differences increased in 2016, when optimal ownership was 11.5% higher and member access to a net was 11.2% higher in the wealthiest households, compared to the poorest ones (S1 Table).

There were also significant differences in LLIN ownership and access across administration subdivisions of the district (χ^2, df=4, p<0.0001 in all test). In 2015 and 2016, the lowest percentage of households that owned at least one LLIN was observed in Panjane (77.9% and 74.3%, respectively) with the highest values observed in Motaze (90.8% and 84.3%,

respectively). Likewise, the lowest percentage of households with at least one net for every two people was observed in Panjane (51.5% and 43.2%, respectively) and the highest values in Motaze (72.0% and 58.5%, respectively). Residents' access to sleeping under an LLIN was lowest in Mapulanguene (71.3% and 61.6%, respectively) and highest again in Motaze (82.9% and 73.1%, respectively) (S1 table).

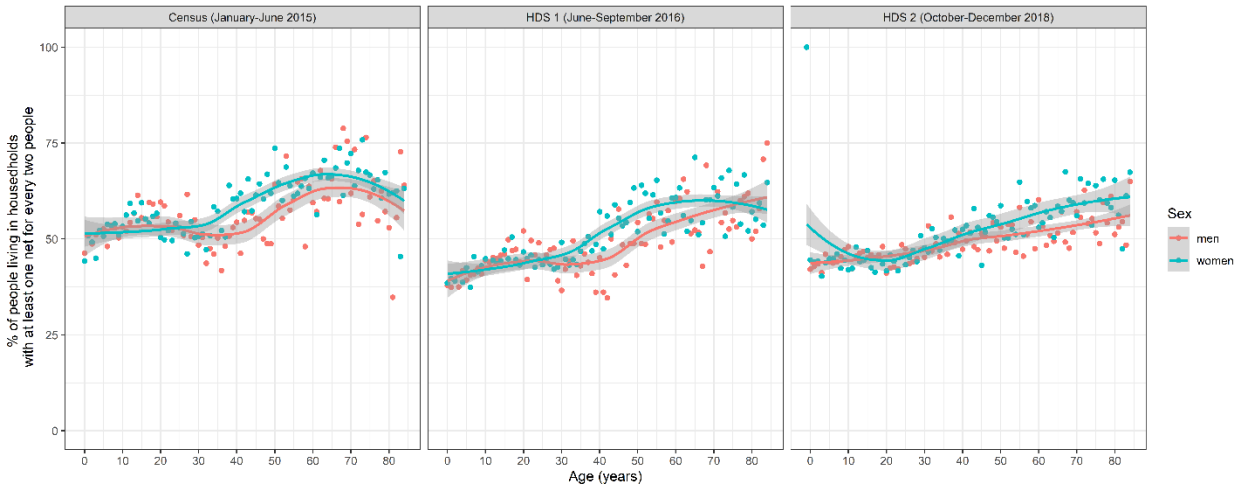
Fig 1. Percentage of households in Magude district during the project with at least one LLIN, of households with a least one LLIN for every two people, and of household members with access to a net, segregated by the size of the households.



The percentage of people that lived in a household with at least on net for every two people varied with age and sex. It was lowest in the youngest age groups, increased among school-aged children and young adults, decreased again in adults between 20 and 40 years of age, and

subsequently increased in older ages. In those over 30 years of age, it was higher among women than men, but in those below 30 years of age it was similar across sexes (Fig. 2).

Fig. 2. Percentage of people living in households with at least one LLIN for every two people, seperated by gender and age. Grey shaded areas represent the locally estimated scatterplot smoothing of the curves.



LLIN use

Out of the 24,302 nets for which information was collected during the 2015 population census (97% of all nets in the district), 47.9% had been used the night before, 51.2% had not been used the night before and 0.8% of the respondents did not know if the net was used the night before.

Between January and June 2015 (before the project started) 25.4% of residents reported sleeping under a net. During the project, individual LLIN use varied seasonally, with a maximum LLIN use of 76.4% (76.0-76.8) in January 2016 (MDA data, high transmission season) and a

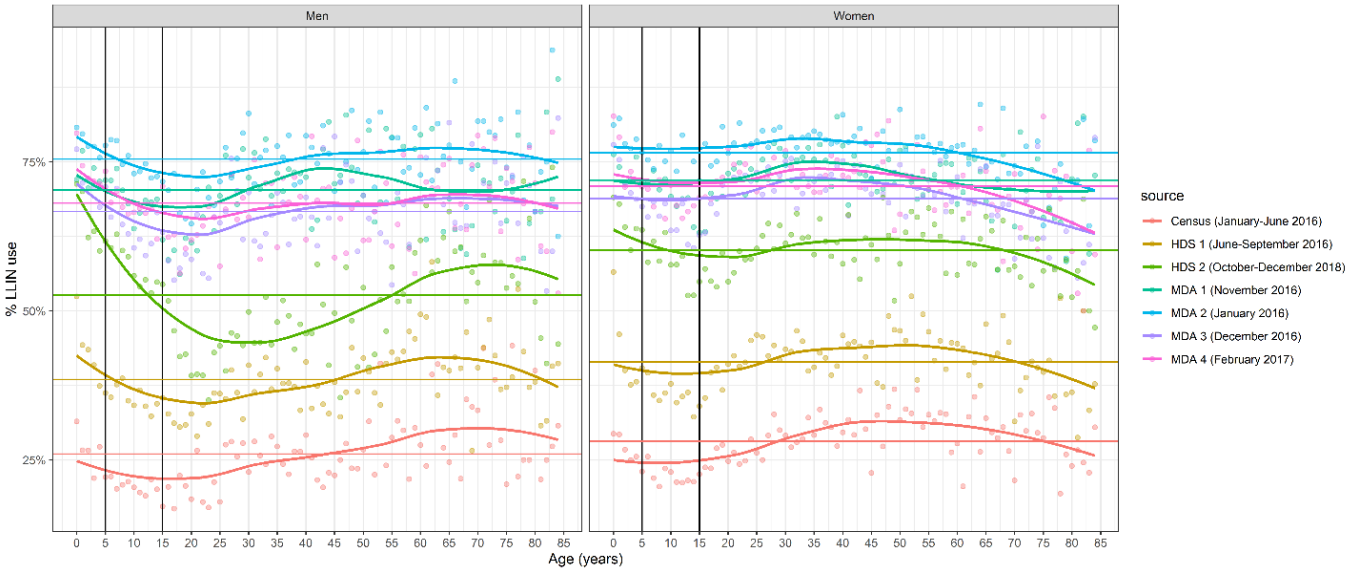
minimum of 40% in June-August 2016 (DHS data, low transmission season) (Table 2). Individuals living in households with at least one net for every two persons were more likely to sleep under a net than those sleeping in households with less than one net for every two persons (χ^2, $p<0.001$, for all years) (Table 2)

LLIN use varied between administrative posts (S2 Table). Use was generally higher in Magude Sede (the district's capital) and Motaze (the second most urbanized locality) than in the more rural areas of Magude (χ^2, $p<0.001$). Use was higher in the richest households than in the poorest ones, with differences up to 14% (χ^2, $p<0.001$) and higher in smaller households than in larger ones before the project started, with difference up to 14.5% (χ^2, $p<0.001$), but similar across household sizes during project itself.

LLIN use varied considerably with age and gender (Fig. 3). Differences between men and women were more accentuated during the low transmission months. Among men, LLIN use was generally highest in children under 5 years of age, was especially low in men between the ages of 18 and 30, increased steadily until the age of 65 and 75 to decline again in older men (Fig. 3). The same pattern was observed in both transmission seasons, although differences across age groups were more accentuated in the low transmission season. Among women, LLIN use was generally higher in women between the age of 30 and 55 than in women of other ages. During the high transmission season, net use was lowest in women of older ages and during the low transmission season in female adolescents until the age of 20 (Fig. 3). As observed from the census of the population of 2015 and health and demographic survey of 2016 and 2018 (Table

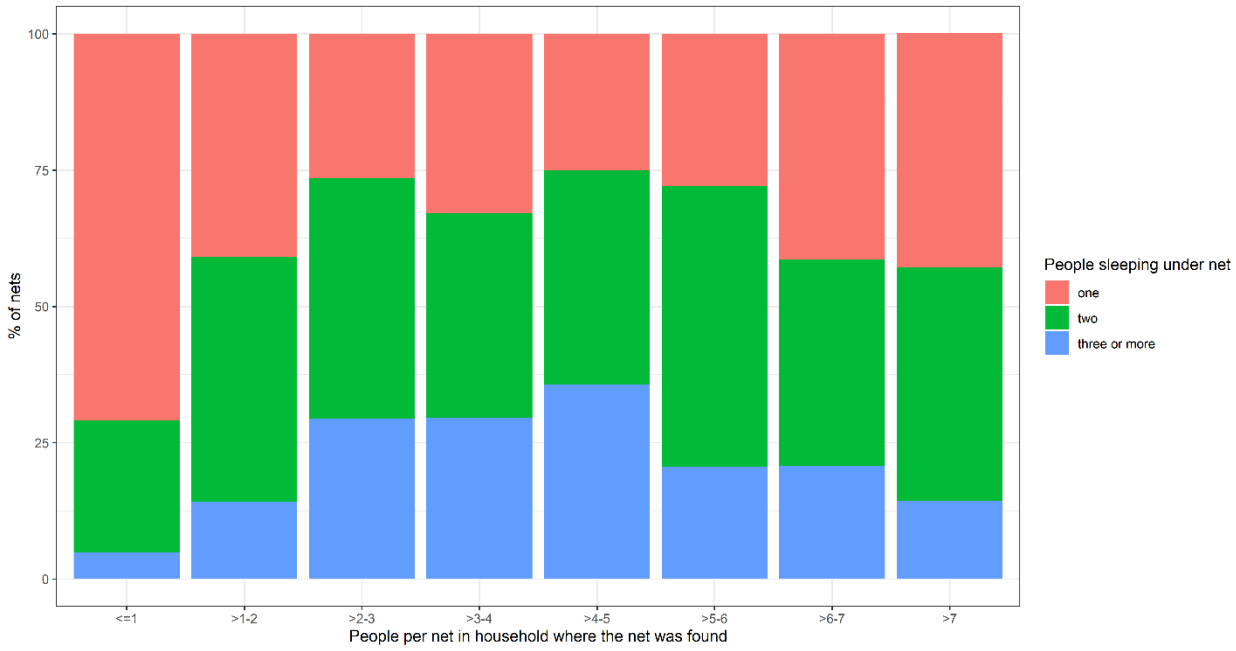
2), pregnant women were more likely to sleep under a net that non-pregnant women (χ^2, p=0.001), although those differences were not large.

Fig. 3. **LLIN use across age and gender in Magude district during the project.** Each line represents data from a single survey or study conducted during the Magude project. Each point is the average bednet use found for the corresponding age across individuals of that age in the corresponding study. MDA estimates represent between 77.7% and 89.6% of the population.



At the time of the population census conducted at the beginning of the project (January-June 2015), the number of people per net was 1.7 (SD: 0.8) with a median of 2 (IQR 1-2), and 47.5% of nets found in all households (n=24,302) were used by one person, 38.5% by two persons and 14.1% by three or more persons. The percentage of nets used by three or more persons increased with the number of people per net in the household up to 5 people per net, and decreased thereafter (Fig. 4).

Fig. 4. Number of people sleeping under a net according to the number of people per net in a household where the net was found.



Reasons for not using an LLIN to sleep

The main reasons for not using an LLIN (data from MDA surveys conducted during the high transmission seasons) was not having a net (56.3% [55.4-57.2], 61.8% [60.8-62.8], 67.1% [66.3-68.0] and 77.6% [76.9-78.4] in November 2015, January 2016, December 2016 and February 2017, respectively), followed by the bednet not being hung (22.6%[21.8-23.2], 23.3%[22.4-24.2], 18.8%[18.2-19.5] and 14.7% [14.1-15.3], respectively), disliking of the bednet (10.9% [10.3-11.5], 8.3% [7.8-8.9], 8.2[7.7-8.7] and 5.0% [4.6-5.4], respectively) or that it was too hot (7.8% [7.3-8.3], 5.5% [5.1-6.0], 4.4% [4.1-4.9] and 1.4% [1.3-1.7], respectively). The proportion of individuals who claimed not using the net due to not having one increased over time across the four MDA rounds.

Type of nets used by residents

In May 2015, 2016 and 2017, 79.0% (65.8-88.0), 91.0% (88.8-93.0) and 91.2% (89.6-93.0) of respondents, respectively, used a net that was impregnated during the last 12 months or a long-lasting insecticidal net the night before the interview (data from the malaria prevalence cross sectional surveys). In May of 2015, 2016, 2017 and 2018, 95.5% (84.7-99), 81.4% (78.4-84), 85.1% (83.2-87) and 96.5% (95.1-98) of respondents, respectively, reported to have received the net they used from a health facility or the NMCP and 0.4% (0.5-3), 3.4.% (2.3-5), 2.9% (2.1-4) and 0.7% (0.3-2), respectively, reported to have bought the net. In the population census during 2015, the majority of the 24,302 nets present in households for which information could be collected (97% of all nets in the district), were Olyset® Nets (77.1%), followed by Permanet® 2.0 (21.1%), Netprotect® (0.5%), Interceptor® (0.5%), Duranet® (0.1%) and DawaPlus® (0.1%), and of 0.9% the brand was unknown.

Discussion

The present study evaluated LLIN ownership, access and use during the Magude project and inequalities in these indicators across district subdivisions, household sizes, household wealth, and an individual's sex and age. Such information is critical to improve our understanding of the protection that LLINs confer during malaria control and elimination programmes, including the Magude project, and to identify ways to improve LLIN access and use.

Most nets that were found in Magude district during the project were obtained from the NCMP or a health facility (>88%), suggesting they were received during the LLIN mass distribution campaign, or through the ante-natal care services (ANC) or expanded programs of immunization (EPI). Less than 3.4% of the nets had been bought. From the start of the project up to December 2017 (when the next LLIN mass distribution campaign took place) most residents slept under an Olyset® Net, as this brand accounted for 77.1% of the nets identified in the district.

The percentage of households that owned at least one net ranged from 78.8% to 91.1%, suggesting that there were gaps in household coverage shortly after the mass distribution campaigns. Household's optimal LLIN ownership and individual access ranged from 54.4% to 59.2% and from 68.2% to76.3%, respectively, during the project. The percentage of households with optimal LLIN ownership and the percentage of people with access decreased from 61.9% and 73.7% at the start of the project to 54.4% and 68.2% during the second year of the project, respectively. Despite the distribution of over 25% more nets in the 2017 campaign compared to 2014 campaign, optimal LLIN ownership and individual access in 2018 increased but remained at levels similar to those measured at the beginning of the project, 59.2% and 76.3%, respectively. Given that the rate of net loss after both campaigns was similar (approx. 31% during the first year), this finding suggests that the distribution of larger quantities of nets in 2017 did not improve LLIN ownership or access. This could be due to an inadequate distribution of nets. Indeed, we observed inequalities in LLIN optimal ownership and access during the project, with larger households and those located in more remote areas being more frequently underserved than wealthier households or households located in easier-to-reach areas. This suggests that the

households that are missed during the campaigns may have been those that are located in harder-to-reach areas and that LLIN allocation strategies during the campaign did not adequately cover the needs of larger households. Combined with the fact that larger households showed lower LLIN survival over time elsewhere in Mozambique [23], the protective efficacy of LLINs in larger households may even have been lower in Magude district. Inequalities were also observed by household wealth, with wealthier households owning more nets, which has been observed in other countries [24, 25]. These inequalities exacerbated over time after the mass distribution campaign. Although we did not investigate the reasons, this may be due to poorer households using the nets for other purposes than sleeping (e.g. fishing or the protection of fruits and seedlings [26]) or due to a more rapid deterioration and subsequently disposal of nets because of e.g. poorer storage conditions or net care practices [27]. This should be assessed in greater detail, as a more rapid net loss in poorer households leads to a greater gap in protection by LLINs.

LLIN attrition rates in Magude were higher than those reported in other provinces in Mozambique (Tete, Nampula and Inhambane) after the mass distribution campaign in 2017 [23]. Although we did not assess the reasons for net loss, the most frequently reported reason in the other provinces included throwing the net away, that the net was destroyed or that it was used for other purposes [23]. The rapid loss of LLINs, combined with deficient LLIN access, compromised the protection that LLINs could have provided throughout the Magude project, and suggests that a more frequent distribution of LLINs and/or increasing community awareness to ensure the survival of nets over time would be warranted. Although currently not recommended by the World Health Organization (WHO) for programmatic settings, top-up campaigns could

have been implemented in Magude district to compensate for LLIN attrition and the inequalities in net ownership as the necessary data for decision-making were recorded in the health and demographic platform.

Surprisingly, even though LLINs were reportedly being distributed during antenatal care services throughout the Magude project, access was lower among children than among adults and lower in women below 30 than in older ages. This could be due to the combination of low use of antenatal care services (between 25% and 31% of pregnant women never used antenatal care services [16]) or immunization services, as well as to LLIN stock-outs in health facilities, and should be further investigated.

The majority of Magude residents who slept under a net before the project started either used a net that was impregnated over the last 12 months or a LLIN (79% in 2015 and >91% in other years), which suggests that LLINs could have provided both personal protection (i.e. reducing vector-host contact) and contributed towards reducing population densities of local pyrethroid-susceptible vectors in the district. LLIN use increased throughout the project from a baseline level of 25.4% in January-June 2015 to 64.4% and 76.3% in January and May 2016, respectively, and to 70.3% and 72.2% in February and May 2018, respectively. Since the percentage of households with optimal LLIN ownership and the percentage of people with access to a net did not increase during the project, the observed increase in LLIN use is likely due to community engagement campaigns that were implemented during the Magude project. This suggests that reaching the target of 2 people per net in a household through mass distribution

campaigns would not have been enough alone to achieve high levels of LLIN use in Magude. Although the average number of people per net at the beginning of the project (~1 year after distribution) was 1.7, with a median value of 2, more than 25% of the nets located in households with more than two people per net were used by a single individual. This shows the need to revise the allocation strategies during mass distribution campaigns, or to promote net sharing by two individuals during community engagement campaigns.

LLIN use varied seasonally, reaching a maximum of 76.3% in the high transmission months but being as low as 40% during the low transmission season. During the high transmission seasons, LLIN use seems to have been limited by LLIN access, as the most frequently reported reason for not using a net to sleep was not having one, and the percentage of participants reporting this reason increased over time as LLIN access decreased. The fact that LLIN use exceeded LLIN access at times during this season shows the population's willingness to use a net during this season. This suggests that increasing LLIN access in Magude would have increased LLIN use, at least during the high transmission season, which could have further reduced malaria transmission. During the low transmission season, LLIN use was highly limited by human behavior, in addition to poor access. The percentage of people sleeping under a net among those living in households that had at least one net for every two people in this season was only 55.1% (June-August 2016). Seasonal variations in LLIN use have been observed in several other countries and have been commonly linked with vector abundance and/or heat [12, 28-32]. Raising awareness of the risk of contracting malaria during this season is critical to increase LLIN

use. This is especially important in Magude, as a significant proportion of transmission occurred during this season during the project [16].

As seen in other African settings [33-37], LLIN use was lowest in school-age children and young adults, especially among young males, highest in children under 5 and in older adults, and in general higher among women than among men. Since young adults (5-15 years old) have been observed to act as important reservoirs of malaria parasites in neighboring countries [38, 39], and the infection rates in Magude residents of >5 years of age were similar or -at times- higher than in those <5 yo [16], the low levels of net use in this age group may have contributed toward sustaining malaria transmission during the Magude project. The variation of LLIN use with age also suggests that the common disaggregation of LLIN use in the three age groups as recommended by WHO (under 5, 5-15 and >15 years of age) [20] may not accurately reflect the age-related differences in net use and highlights the importance of implementing community engagement activities targeting specific age groups, especially young males.

LLIN use was slightly higher among people living in sprayed households (i.e. covered by IRS) than among those living in unsprayed households. Although the reasons were not evaluated in the present study and the number of unsprayed households was very low, this suggests that deploying IRS in combination with LLINs may have had a positive impact on LLIN use. Such synergistic associations has been previously observed in Magude [16] and elsewhere [40, 41], which highlights the potential added value of deploying the two interventions together.

At the start of the project, 14% of nets were used by three or more people. Net sharing among more than two individuals likely continued during the project as LLIN use exceeded LLIN access at specific points in time. The downside of sharing a net with more than 2 persons is that this can reduce the protection provided by LLINs as people's limbs can be against or stick out from underneath the net due to limited space availability, which allows mosquitoes to feed on the user(s). A study conducted in Kenya showed that malaria prevalence in children who slept with two or more additional people under a net was similar to that in children that did not use a net to sleep [42]. In Guinee Bissau, a similar trend was seen with hospital visits by children [43]. As such, net sharing preferences should be taken into consideration during mass distribution campaigns, either by distributing larger nets to households where more than two people share a net (e.g. due to limited sleeping space) and/or raise community awareness on best LLIN use practices through community engagement campaigns.

This study draws from different surveys to report LLIN ownership, access and use at different time points. This provides valuable insights into how access decreases after the LLIN mass distribution campaigns, how inequalities in LLIN ownerships and access evolve over time and how seasonality affects LLIN use. This approach also has several limitations, as the surveys were not specifically designed to measure LLIN indicators. The first limitation is that the surveys during the MDA campaigns represented between 77.7 and 89.6% of the Magude population, and as such our point estimates do not represent the entire population of the district. Nonetheless, by estimating the best-case and worst-case scenario intervals, a representative range of confidence in the values is provided. Second, attrition rates were evaluated retrospectively, and

may have been underestimated as some nets were likely received through ANC and EPI services rather than through the LLIN mass distribution campaign. Finally, the reasons for net loss, and for not using a net during the low transmission season were not quantified, which is critical information to guide future behavioral change campaigns aimed at improving the impact of LLINs.

Conclusion

LLIN ownership, access and use were heteroegenous and sub-optimal during the Magude project. People living in hard-to-reach areas, poorer and larger households, and young males were associated with poorer LLIN access and lower LLIN use during the project. The combination of LLINs and IRS had a positive effect on LLIN use. Mass-distribution campaigns alone were not enough to achieve the high level of LLIN protection needed during the malaria elimination program. To ensure high and equal levels of LLIN protection, future mass LLIN campaigns in Mozambique and elsewhere, especially in elimination settings, should a) revise LLIN allocation scheme to ensure equal LLIN ownership and access, b) consider LLIN top-up campaigns to fill the gaps in LLIN access resulting from LLIN allocation schemes and attrition post-campaign, and c) raise community awareness to ensure high LLIN use, especially during the low transmission season, among school-aged children and young males in harder-to-reach areas and in the poorest households. Further research is needed to investigate the reasons for: 1) current net allocation strategies leading to inequalities in bednet ownership and access, 2) the poor LLIN use observed during the low transmission season, 3) the low use observed in young males, 4) the faster net loss observed in Magude compare to other districts in Mozambique, and 5) LLIN access being

lower in children under 5 and pregnant women despite continuous LLIN distribution through ANC and EPI.

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Data availability

Data are available upon request from CISM's institutional ethics committee (sozinho.acacio@manhica.net), Hospital Clínic of Barcelona's Ethics Committee (CEIC@clinic.cat), and the Mozambican Ministry of Health National Bioethics Committee (jflschwalbach@gmail.com) for researchers who meet the criteria for access to confidential data.

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Supporting information

S1 Table. LLIN ownerships and access by locality and wealth index in Magude district

	% of HH with at least 1 net		% of HH with at least 1 net for every 2 people		% of people with access to an LLIN within their household	
By locality	2015	2016	2015	2016	2015	2016
- Magude Sede	80.1	79.2	61.1	55.6	72.4	68.8
- Motaze	90.8	84.3	72.0	58.5	82.9	73.1
- Panjane	77.9	74.3	51.5	43.2	68.7	60.3
- Mahele	86.1	72.6	61.5	48.3	75.8	62.8

- Mapulanguene	78.4	70.7	58.4	45.1	71.3	61.6
By wealth index (MPI)*	2015	2016	2015	2016	2015	2016
0	83.0	85.7	63.9	59.9	75.0	74.2
1	82.9	80.0	61.4	53.1	74.3	67.7
2	77.4	74.2	60.0	52.6	69.6	63.0

** The MPI was reclassified into three categories according the number of deprivations: Category 1 (0-2); Category 2 (3-4); Category 3 (5-6). The higher the number of deprivations, the poorer the household.*

S2 Table. LLIN use by administrative post in Magude district. Data sources are the same as for Table 1 in the main manuscript.

By locality	Magude	Motaze	Panjane	Mahele	Mapulanguene
2015 (Jan-Jun)	27.2	27.3	14.3	16.0	11.5
2015 (November)	70.7	79.1	62.3	73.0	58.3
2016 (January)	75.7	85.0	70.3	78.7	69.7
2016 (Jun-Aug)	42.6	41.4	30.2	30.9	20.4
2016 (December)	68.3	73.3	60.7	57.0	71.1
2017 (February)	70.3	75.8	64.1	67.6	60.9

Article 3 (Accepted for publication) **Fernández Montoya L**, Alafo C, Martí-Soler H, Máquina M, Comiche K, Cuamba I, et al. Overlaying human and mosquito behavioral data to estimate residual exposure to host-seeking mosquitoes and the protection of bednets in a malaria elimination setting where indoor residual spraying and nets were deployed together.

Objectives addressed:

- Objective 3: To evaluate where and when residual human-vector contact occurred during the project
- Objective 4: To identify limitations in the implementation of and protection conferred by LLIN against malaria vectors during the project

Overlaying human and mosquito behavioral data to estimate residual exposure to host-seeking mosquitoes and the protection of bednets in a malaria elimination setting where indoor residual spraying and nets were deployed together

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Abstract

Characterizing persistent malaria transmission that occurs after the combined deployment of indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) is critical to guide malaria control and elimination efforts. This requires a detailed understanding of both human and vector behaviors at the same temporal and spatial scale. Cross-sectional human behavior evaluations and mosquito collections were performed in parallel in Magude district, Mozambique. Net use and the exact time when participant moved into each of five environments (outdoor, indoor before bed, indoor in bed, indoor after getting up, and outdoor after getting up) were recorded for individuals from three different age groups and both sexes during a dry and a rainy season. Malaria mosquitoes were collected with CDC light traps in combination with collection bottle rotators. The percentage of residual exposure to host-seeking vectors that occurred in each environment was calculated for five local malaria vectors with different biting behaviors, and the actual (at observed levels of LLIN use) and potential (i.e. if all residents had used an LLIN) personal protection conferred by LLINs was estimated. *Anopheles arabiensis* was responsible for more than 74% of residents' residual exposure to host-seeking vectors during the Magude project. The other four vector species (*An. funestus s.s.*, *An. parensis*, *An. squamosus* and *An. merus)* were responsible for less than 10% each. The personal protection conferred by LLINs prevented only 39.2% of the exposure to host-seeking vectors that survived the implementation of both IRS and LLINs, and it differed significantly across seasons, vector species and age groups. At the observed levels of bednet use, 12.5% of all residual exposure to host-seeking vectors occurred outdoor during the evening, 21.9% indoor before going to bed, almost two thirds (64%) while people were in bed, 1.4% indoors after getting up and 0.2% outdoor after leaving the house. Almost a third of the residual exposure to host-seeking vectors (32.4%) occurred during the low transmission season. The residual bites of *An. funestus s.s.* and *An. parensis* outdoors and indoor before bedtime, of *An. arabiensis* indoors when people are in bed, and of *An. squamosus* both indoors and outdoors, are likely to have sustained malaria transmission throughout the Magude project. By increasing LLIN use, an additional 24.1% of exposure to the remaining hosts-seeking vectors

could have been prevented. Since *An. arabiensis,* the most abundant vector, feeds primarily while people are in bed, increasing net use and net feeding inhibition (through e.g. community awareness activities and the selection of more effective LLINs) could significantly reduce the exposure to remaining host-seeking mosquitoes. Nonetheless, supplementary interventions aiming to reduce human-vector contact outdoors and/or indoors before people go to bed (e.g. through larval source management, window and eave screening, eave tubes, and spatial repellents) will be needed to reduce residual exposure to the outdoor and early biting *An. funestus s.s.* and *An. parensis.*

Introduction

Mozambique is one of the four countries with the highest malaria burden in the world [1]. Reducing and eventually eliminating malaria in its most southern province (Maputo province) has been considered critical to make progress towards malaria elimination in South Africa and Eswatini as well. Although Maputo province has been targeted by regional initiatives aiming at accelerating malaria elimination, such as LSDI (Lubombo Spatial Development Initiative) [2] and MOSASWA (Mozambique, South Africa and Eswatini) [3], neither of these initiatives -nor previous attempts to eliminate malaria in sub-Saharan Africa- have succeeded in interrupting transmission. There is an urgent need to improve our understanding of the limitations of current control interventions in order to optimize them and/or implement novel or supplementary interventions [2, 4], if we are to achieve malaria elimination in sub-Saharan Africa.

Malaria control has historically relied heavily on controlling malaria vectors through indoor residual spraying (IRS). Although IRS led to great reductions in the malaria burden in Africa during the Global Malaria Eradication Programme (GMEP) in the 1950s and 1960s, it was not sufficient to interrupt malaria transmission in Africa. It was concluded that IRS failed due to rapidly evolving insecticide resistance and the fact that some mosquito species were not resting indoors [5-8]. Since 2000, and due to renewed efforts to eliminate malaria, insecticide treated nets (ITNs), which were later replaced by long-lasting insecticidal nets (LLINs), have become the most widely used vector control intervention. ITNs, and to a lesser extent IRS, have contributed

most to the observed reductions in malaria cases in Africa between 2000 and 2015 [9]. Challenges for LLINs include resistance to pyrethroids [10], the main insecticide class used in nets, and mosquitoes biting people when they are not under the net (either outdoors or indoors) [11].

IRS and LLINs target different mosquito behaviors. IRS reduces the survival of mosquitoes that rest on treated wall surfaces and, hence, vector population densities. LLINs protect people by killing mosquitoes, repelling them when they approach the net and by acting as a physical barrier, preventing vector-host contact. As pyrethroid resistance is widespread in Africa, the combination of IRS with a non-pyrethroid insecticide and LLINs (which are currently pyrethroid-based) could have an additional impact on malaria transmission, compared to implementing a single intervention [12], and can help to mitigate for the effects of insecticide resistance [13]. Such combinations could therefore play a critical role in accelerating malaria elimination in low transmission settings. However the scientific evidence of the added value of combining IRS with LLINs is limited and not always in agreement [14, 15], which lead the WHO to call for additional evidence in malaria transmission foci, including low transmission settings [13].

Besides evaluating the added epidemiological value of combining the two interventions, we need to understand their gap(s) in protection, which was evaluated during the Magude project [16]. The project assessed the feasibly of eliminating malaria in a low transmission setting in southern Mozambique using a package of interventions targeting the malaria parasites and vectors simultaneously. Vector control consisted of the implementation of annual district-wide IRS in addition to LLINs that are mass-distributed every three years. Although the project achieved significant reductions in malaria incidence and prevalence, malaria transmission was not interrupted [17]. Hence, this project provides a unique opportunity to understand the gaps in protection (i.e. persistent interactions between humans and mosquitoes) that remain in a low malaria transmission setting after the combined deployment of the two core vector control interventions. To-date, such evaluations have focused on comparing the impact of the individual versus combined interventions on standard entomological indicators through mathematical models [18], through empirical data from experimental hut trials that mimic semi-field conditions [19] or through field studies [12, 20-22]. But to accurately characterize residual malaria transmission, both human and vector behavioral data are needed to identify the place and time

where and when humans and malaria vector species interact [13]. Although methods to quantify human exposure to mosquito bites were already developed in 2006 [23], very few studies have since collected empirical data to evaluate these human-vector interactions [24, 25], and even fewer studies have collected human and mosquito behavioral data at the same time and in the same place [26-31]. In addition, no study has evaluated human-vector interactions in a low transmission setting where LLINs are combined with area-wide IRS.

Here, using both human and vector behavioral data that were collected in parallel in Magude between 2015 and 2017, we 1) estimate the proportion of residual exposure to five host-seeking vector species (i.e. mosquito species that survived the combined deployment of LLIN and IRS and were found carrying sporozoites) experienced by residents of Magude in each of five different environment: outdoors before going indoors, indoors before going to bed, indoors while in bed, indoors after getting up and outdoors after leaving the house; 2) assess the actual personal protection that LLINs conferred to Magude residents against the five different local malaria vector species; 3) estimate the maximum personal protection that LLINs could have conferred if all residents would have used a net to sleep; and 4) characterize the residual exposure to host-seeking mosquitoes that would have remained in each environment even if all residents would have used a net to sleep every night. To our knowledge, this is the first study to characterize the residual exposure to bites of different vector species (five) with distinct host-seeking patterns in an area with combined deployment of LLINs and IRS, and to report the protective efficacy of LLINs against those different vector species during both the low and high malaria transmission season.

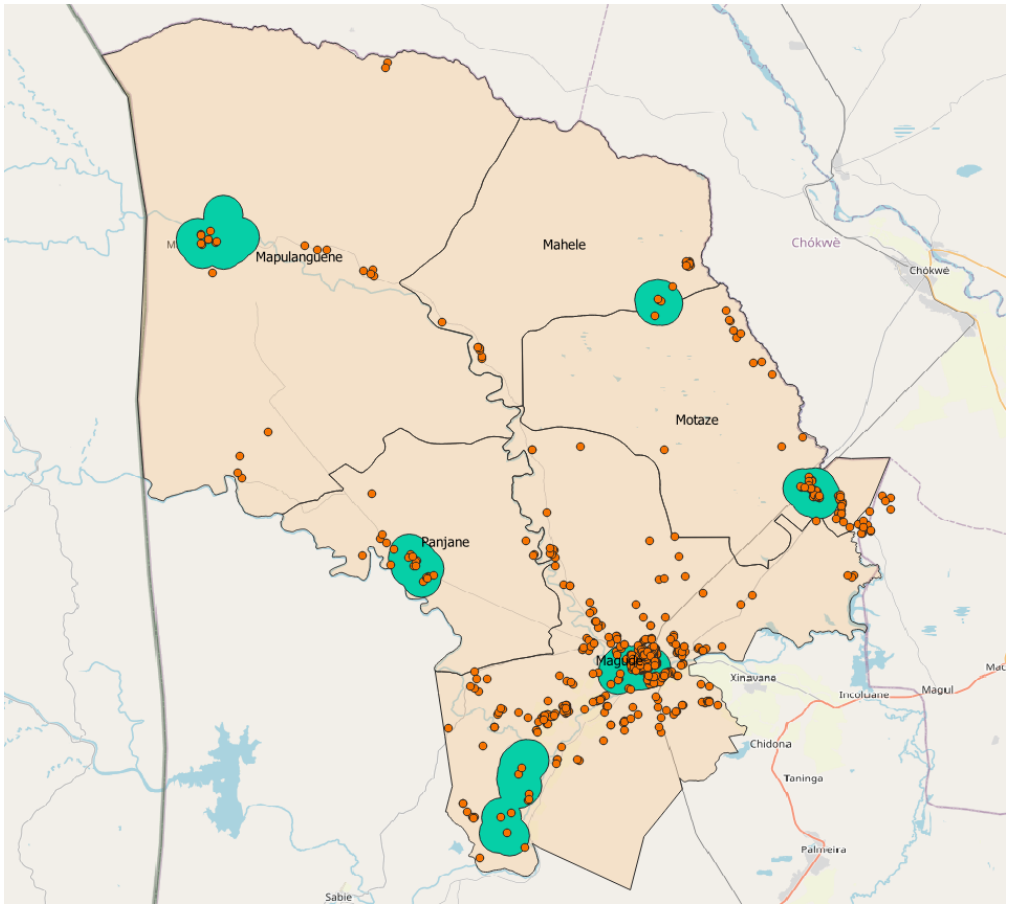
Materials and Methods

Study site and status of vector control interventions

The study took place in Magude (Fig. 1), a rural district located in Maputo Province, southern Mozambique. There were a recorded 48,448 residents in the district in 2015, and malaria prevalence by rapid diagnostic test ranged from 9.1% in May 2015 to 1.4% in May 2018. A

comprehensive description of the district demographic, socio-economic and health characteristics is provided elsewhere [32].

Fig 1. Map of Magude district. Red dots represent the households that were enrolled in the human behavioral study; the green areas are areas where entomological surveillance was conducted. The subnational administrative boundaries have been taken from the Humanitarian Data Exchange (https://data.humdata.org/dataset/cod-ab-moz) under a CC-BY-IGO license (https://data.humdata.org/faqs/licenses).



The National Malaria Control Program distributed 35,432 LLINs during their mass distribution campaign in May 2014 [32]. In 2015, the percentage of households with at least one ITN for every two people was 53.2% [32]. The district received two rounds of IRS before and during this study, the first round (before our study) between August 2015 and October 2015 using dichlorodiphenyltrichloroethane (DDT) and pirimiphos-methyl (Actellic 300CS, Syngenta Crop Protection AG, Basel, Switzerland) and another round (during this study) between September

2016 and November 2016 using Actellic only. Apart from vector control activities, four rounds of mass drug administration (MDA) were implemented between 2015 and 2017. Based on data collected during the MDA campaigns immediately after the IRS rounds, 83% and 89.7% of the households were sprayed during 2015 and 2016, respectively [16, 17]. More details on all interventions and their impact on malaria prevalence in the district are described elsewhere [17].

Definition of transmission seasons

Based on a previous analysis showing that the incidence of malaria peaks two months after the peak rainfall [32], we considered the high transmission season to start in November (one month after the rainy season starts but one month before malaria peaks to account for immature mosquito development times) and the low transmission season in May (one month after the end of the rainy season, to account for mosquito longevity).

Human behavior cross sectional evaluation

Human behavior was evaluated during both a low (17th August to 2nd November 2016) and a high transmission season (22nd February to 19th April 2017), based on the assumption that human behavior may differ between seasons due to e.g. climate conditions, perceived malaria risk and socio-economic activities. An age-stratified random sample of participants of three age groups (5 to 11 years, 12 to 17 years, and 18 years or older) was drawn from the district population using the population census database and respecting the proportion of people from each age group in each administrative division. The sample size allowed to estimate the percentage of exposure to host-seeking mosquitoes prevented by LLINs in each of the three age groups at 95% confidence with a 10% margin of error and it was calculated assuming a point estimate of 50% due to the lack of previous similar measurements in the country.

Human behavior was evaluated by means of close-ended structured interviews conducted by a trained field worker (S6). In addition, participants were given a digital watch (DigiTime DT23, Xonix Field Ranger or Xonix-BW007) and asked to record the actual time they (i) entered the house in the evening/night (time after which the participant did not go out of the house anymore), (ii) went to bed in the evening/night, (iii) got up in the morning and (iv) left the

house in the morning, using a time-tracking card (S1 Fig). In both seasons, participants were visited during three consecutive days. On the first day, the field worker explained the study to the participants, obtained written informed consent from them or from their caretakers (for those in the 5-11 year-old group), provided the participants with a time-tracking card and a digital watch and instructed the participants how to complete the card using the watch. On the second and third day after this initial visit, the field worker conducted the structured interview and digitized the information from the participant's time-tracking card. The first interview was considered a test round meant to ensure that participants had understood the use of the watch, the time-tracking card and the interview questions. During the structured interviews, participants were asked if they (i) used an LLIN to sleep the night before, (ii) used any other measures to prevent mosquito bites, (iii) left their bed during the night and (iv) worked at night. For the youngest age group (5-11 years old), their adult caretaker was asked to fill out the time-tracking card and respond to the survey questions on behalf of the child.

Entomological surveillance

Vector surveillance started in May 2015, and data up to August 2017 have been included in the analysis to match the duration of the first phase of the Magude project. Mosquitoes were collected monthly in six sentinel sites in Magude district (Fig 1). In each sentinel site, mosquitoes were collected in fifteen representative houses during two consecutive nights every month. Collections took place indoors in 10 households and outdoors within the compound of 5 other households using miniature CDC light-trap (Model 512, John W Hock, Florida, USA). These traps were combined with Collection Bottle Rotators (Model 1512, John W Hock, Florida, USA) in 6 households (3 indoors; 3 outdoors, every night) to collect mosquitoes from time of trap placement to 6pm, and subsequently at 2h intervals until 6am, after which mosquitoes were collected in the final bottle until the team visited the house again. Every month, houses were randomly assigned a trap type (i.e. CDC-light trap with or without a rotator) and a collection environment (indoors or outdoors). Indoors, the CDC light-trap was placed at the foot-end of a bed with the trap opening approx. 1.5m above the ground. One or two adult volunteers (>15 years old) from the selected household were asked to sleep in the bed under an LLIN. Participants

not owning a net were provided with a WHO-approved LLIN. Outdoors, CDC light traps were baited with a BG-Lure cartridge (Biogents AG, Germany) and CO_2 that was generated through a mixture of 10g commercially available yeast (Instant Yeast, Smart Chef, Best Brands S.A., Tunesia), 100g white refined household sugar and 1L of regular tap water to mimic indoor conditions (i.e. a human sleeping next to the trap). The outdoor traps were placed in close proximity to the house with the trap opening approx. 1.5m above the ground, and were protected from the weather, theft, animals and/or children by available objects in the environment (mostly trees, or tall vegetation). Due to suspicion of arboviral diseases transmission in Mozambique, which has since been confirmed [33, 34], no comparison against Human Landing Catches (HLC), the current gold standard methodology to assess human biting rates, were performed. As such, 'exposure to hosts-seeking mosquitoes' is reported throughout this study, rather than 'vector bites'. Every morning after a collection night, the team visited the house to collect the mosquitoes and used a digital structured questionnaire to gather information on the collection conditions for data quality purposes (see data analysis section below).

Anopheline mosquitoes were identified morphologically to species using a stereomicroscope and the keys of Gillies and Coetzee [35]. Individuals belonging to the *Anopheles gambiae* s.l and *An. funestus* s.l complex were identified to species by multiplex polymerase chain reaction using the wing and leg [36, 37]. *Plasmodium falciparum* sporozoites in mosquitoes were detected by means of enzyme-linked immunosorbent assay using the head and thorax of the mosquitoes [38].

Data collection and analysis

Data from both studies were collected with tablets (Huawei, Model S7-701u) using Open Data Kit (https://opendatakit.org/). The analysis focused on evaluating the residual exposure of Magude residents to malaria vector species that survived the combined deployment of IRS and LLIN, the personal protection that LLINs conferred against exposure to host-seeking mosquitoes that survived or did not come in contact with IRS and LLIN products, and the personal protection they would have provided if all residents would have used a net. The exposure to residual host-seeking

mosquitoes was quantified for five different environments where humans and mosquito vectors typically interact during the evening, night and early morning: i) outdoors, before people go indoors, ii) indoors, before people go to bed, iii) indoors, while people are in bed, iv) indoors, after people have gotten up, and v) outdoors, after people got up and left the house. Estimates are given for the two distinct malaria seasons: the low and high transmission season. We first analyzed the progression of our study participants through those environments, and the differences between seasons, age groups and gender. We then analyzed the host-seeking behavior of the local vector species during the low and high transmission seasons and subsequently overlapped both human and vector behaviors to obtain estimates of human exposure to host-seeking vectors in each environment and in both seasons. Finally, we estimated the proportion of exposure to the different host-seeking vector species that LLINs prevented through personal protection, and the proportion of exposure LLINs could have prevented if all residents would have used a net, again through personal protection. We compared LLIN personal protection across seasons and age groups.

Human behavior

Only participants that reported sleeping indoors the night before the interview (99% of all participants) and who provided complete and chronologically consistent information for the time goings indoors, to bed, time of getting up and leaving the house were included in the analysis. The median time of the day at which participants went indoors, to bed, got up and left the house and the median amount of time they spent indoors (before going to bed, in bed, and after getting up) is reported together with the 90^{th} and 10^{th} quantiles to provide a measure of dispersion, since values were not normally distributed. Differences across seasons, age groups and gender were evaluated using the non-parametric tests (Mann-Whitney-Wilcoxon, Kruskal-Wallis rank sum test or Dunn's Test for pairwise multiple comparison). The percentage of people who used an LLIN the night before the interview, used other mosquito protection measures and/or left the bed during the night was estimated and their 95% CI calculated using the normal approximation method. These percentages were compared across seasons, age groups and gender using Chi-square tests.

Vector species composition, densities, sporozoite rates, and time of biting

Vector collections that met the exclusion criteria (S3) were disregarded in the analysis. Species composition was estimated based on results of molecular species identification. Sporozoites in mosquitoes were detected by means of enzyme-linked immunosorbent assay [38]. Species composition and the number of host-seeking mosquitoes per person per time interval were calculated for the high and low transmission seasons separately. The number of host-seeking mosquitoes per person was calculated for each collection time interval by dividing the number of host-seeking mosquitoes collected at each time interval by the number of people sleeping in the room with the trap (or by one for outdoor collections) and by the minutes within the time interval. The rates obtained for each species and for each time period (e.g. 18:00-20:00) were averaged over a season to obtain season-representative values. The peak biting time of each species was considered to be the time interval with the highest rate of host-seeking mosquitoes per person.

Exposure to host-seeking vectors adjusted for human behavior

The indicators used in the present analysis are an expansion of those proposed by Monroe *et al*. [24] and Killeen *et al*. [23]. All equations are provided in S2 Table. For each participant, we estimated the number of host-seeking mosquitoes that each participant is exposed to in each one minute intervals ($\mathrm{B}_{I,t}$, where *t* is expressed in minutes) through a modification of the method proposed by Killeen *et al*. [23]. We added the host-seeking mosquitoes per minute along the period of time that each participant spent in each environment to obtain the total residual exposure to host-seeking mosquitoes in each environment: outdoors before going indoors ($\mathrm{B}_{O,bb}$), indoors before going to bed ($\mathrm{B}_{I,bb}$), indoors and in bed ($\mathrm{B}_{I,b}$), indoors after getting up ($\mathrm{B}_{I,ab}$) and outdoors after getting up ($\mathrm{B}_{O,ab}$). For the purpose of calculating outdoor residual exposure to host-seeking mosquitos, we assumed that participants where outdoors between (i) 4pm (when mosquito collections started) and the time they reported going indoors, and (ii) between the time they reported leaving the house and 8am (when mosquito collections stopped). We assumed that participants were still exposed to host-seeking vectors while sleeping

under a net, and that net users had an 81.1% reduction in exposure compared to people not using a net. This value is based on the percentage of mosquitoes that were observed to blood feed (18.9%) when participants in an experimental hut trial in Tanzania were sleeping under used Olyset® Nets (Sumitomo Chemical Company Ltd , Japan) [39]. We choose this value since i) Olyset Nets accounted for 77.1% of all nets in Magude district, ii) no local measurement on feeding inhibition were available, iii) it represented feeding inhibition of a mixture of wild *An. gambiae* and *An. funestus* mosquitoes (similar to our vector composition), and iv) the Olyset Nets in the trial had been in domestic use for 4 years and the Olyset Nets in Magude district were distributed approx. 2.3 to 3 years prior to this study. Observed feeding inhabitations with new but deliberately holed Olyset Nets were similar, with reported values of 82%, 83.8% and 84.2%, with the exception of a single study that reported 96.3% [39-42]. The limitations of the feeding inhibition parameter value are further explored in the discussion.

We estimated the proportion of residual exposure to host-seeking vectors in each environment both at the observed levels of bed net use and in the hypothetical situation that all residents used a net. To estimate the proportions, we summed the residual exposure to host-seeking mosquitoes experienced by all participants in a given environment and divided this value by the total across all environments. Proportions are reported with their 95% confidence intervals.

The proportions of residual exposure to host-seeking vectors occurring in the low transmission season at observed levels of bednet use ($\pi_{r,low}$) and assuming all residents used a net to sleep ($\pi_{p,low}$) were calculated by dividing the number of host-seeking vectors that all study participants were exposed to during the study night in the low transmission season by the number of host-seeking vectors they were exposed to during the low and high transmission seasons combined. This proportion was reported together with the 95% confidence interval using the normal approximation method.

Actual and maximum personal protective efficacy of LLIN against host seeking vectors

The actual personal protection conferred by LLINs in Magude district ($P^*_{S,C}$) was calculated as the percentage of exposure to host-seeking vectors (that survived or did not come in contact with

IRS and LLIN products) that LLINs prevented at the observed levels of bednet use: $P^*_{S,C} = 100\text{x}(1 - \frac{\text{B}_r}{\text{B}_{ru}})$, where B_r is the total number of host-seeking vectors that study participants were exposed to during one night at the observed levels of bednet use and B_{ru} the total number of host-seeking vectors that they would have been exposed to if none of them would have used nets to sleep. The 95% confidence intervals of $P^*_{S,C}$ were calculated using the normal approximation method.

The maximum personal protection that an LLIN could confer to each participant (P^*_S), i.e. the maximum percentage of exposure to host-seeking mosquitoes preventable through personal protection of a net, was estimated for each individual participant, rather than for the entire study population as other studies have proposed [23, 24], to provide a more accurate measure of variability in the estimate. For each participant, we calculated $P^*_S = 1 - \frac{B_p}{B_u}$, where B_p is the total number of host-seeking vectors that the participant would have been exposed to if they used the bednet to sleep, and B_u if they did not use a bednet to sleep. Because we observed that the distribution of the individual P^*_S was not normal (see S4) we reported median values plus their 10th and 90th percentile for different seasons, age groups and species. Note that LLINs can provide community protection, whereby even community members who do not sleep under a net gain some protection due to reduction in the number of infected mosquitoes that are killed by LLINs that are used by other members. This community-level effect is however ignored in our analyses.

Ethical Clearance

Ethical approval was obtained from the Manhiça Health Research Center's Institutional Bioethics Committee for Health (CIBS-CISM/072/2015 for our human behavior study; CIBS-CISM/043/2015 for our entomological surveillance) and local administrative authorities (52/SDSMASS/024.1). Before commencing any of the two studies, field workers informed participants of the objectives, risks and benefits of the studies, and how their data are protected and used, as well as of their right to withdraw from the study any time. For the human behavioral study, a written informed consent was provided and read out loud to all study participants. Only those that signed were

enrolled in the study. Parents or official guardians signed the informed consent and responded to the survey on behalf of their children aged 5 to 11 years. Children between the age of 12 to 17 years provided consent themselves. For the entomological surveillance study, verbal informed consent was obtained from an adult member of the household to place the mosquito traps indoors or outdoors.

Results

Study participants

During the low transmission season, a total of 576 individuals were visited and 350 individuals were recruited of which 331 completed both interviews (168 women and 163 men). During the high transmission season survey, 536 individuals were visited, of which 331 individuals were recruited and completed both interviews (184 women and 147 men). The number of participants that slept indoors the night before the interview and that provided chronological values on their time-tracking card was 283 during the low and 289 during the high transmission season. The main reasons for unsuccessful visits included participants not being present at the time of the survey (53.5% of unsuccessful visits during the low and 44.4% during the high transmission season) followed by migration to other places (31.7% during the dry and 42.5% during the high transmission season). Very few participants rejected participation (3.3 % during the low and 1.9% during the high transmission season). Ninety-nine percent of study participants slept indoors the night before.

Bednet use

The percentage of people that slept under a bednet the night before the interview differed significantly between seasons ($p<0.0001$). In the high transmission season, LLIN use was 66.7% (95% CI: 60.4-72.9) whereas in the low transmission season use was 39.1% (95% CI: 30.7- 47.6). Within each season, there was no significant difference in LLIN use between age groups or gender (χ^2, $p>0.05$).

Use of other measures to prevent mosquito bites

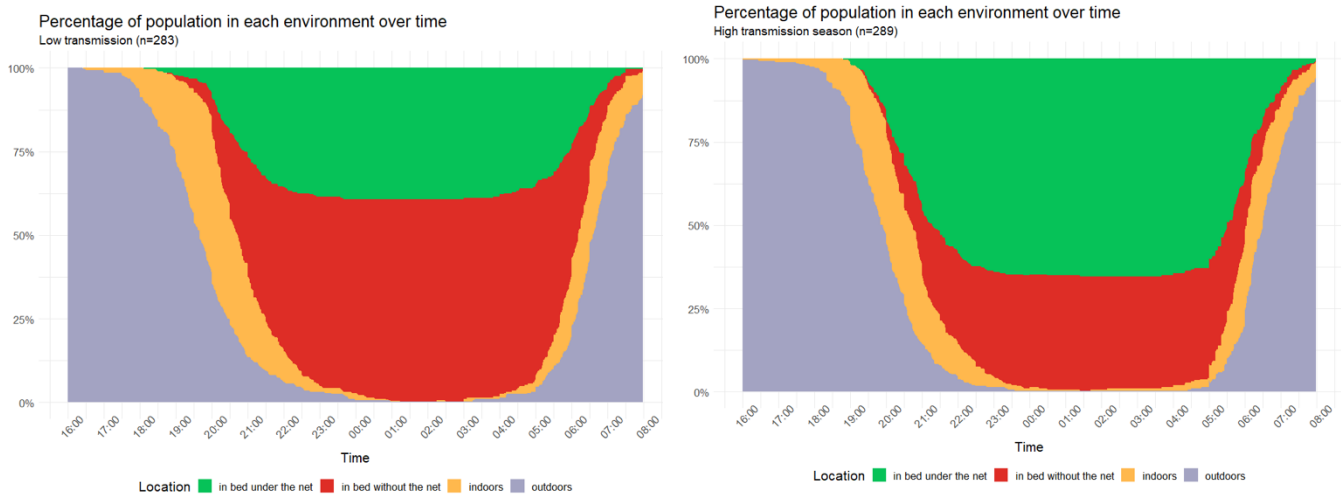
Study participants used additional measures to prevent mosquito bites beyond using an LLIN or living in a sprayed house. During the evening 52 individuals (13.9%) reported using clothing (60.8%), smoke (33.3%), charcoal (3.9%) or combining clothing and smoke (2%). During the night, 12 individuals (2.85%) reported using charcoal (33%), clothing (25%), smoke (25%) and commercial domestic insecticides (16.6%). During the morning, 6 (4.7%) individuals reported using clothing (83.3%) and smoke (16.7%). No differences were observed across age groups, sexes or betnet use (χ^2, $p>0.05$).

Human movement between environments: time going indoors, to bed, getting up and leaving the house again, and time spent indoors in bed and indoors before and after going to bed.

During the low transmission season, the time (note all times reported here are medians) at which participants went inside was 19:40 and they spent 0.8h ($p10^{th}$=0.09h, $p90^{th}$=2.7h) indoors before going to bed. They went to bed at 20:37, stayed in bed for 9.4h ($p10^{th}$=7.5h, $p90^{th}$=10.8h) and got up at 06:10, after which they spent 0.3h ($p10^{th}$=0.05h, $p90^{th}$=1.11h) indoors before leaving the house at 06:35. The total time spent indoors not in bed was 1.4h ($p10^{th}$=0.3h, $p90^{th}$=3.5h). During the high transmission season, the time at which participants went indoors was 19:55, and they spent 0.6h ($p10^{th}$=0.06h, $p90^{th}$=1.9h) indoors before going to bed. They went to bed at 20:42, stayed in bed for 9.3h ($p10^{th}$=7.4h, $p90^{th}$=10.7h) and got up at 06:03, and spent another 0.3h ($p10^{th}$=0.03h, $p90^{th}$=1.16h) indoors before leaving the house at 06:30. The total time spent indoors not in bed was 1.1h ($p10^{th}$=0.2h, $p90^{th}$=2.9h). No significant differences were observed in these times between sexes. Values for different age groups and seasons with their statistical significant differences are shown in Table 1. Overall, in the low transmissions season, people went indoors earlier, spent more time indoors before going to bed and went to bed earlier than in the high transmission seasons (Mann–Whitney U, $p<0.009$). No significant differences were observed in the time spent in bed, the time at which participants got up, the time spent indoors after getting up and the time participants left the house.

To more easily pair the human behavioral data with the mosquito behavioral data (described below) that were collected in 2h intervals, we report the percentage of the study participants in the various environments during the same 2h time periods. The percentage of participants that were indoors by 18:00 was 2.4% in the high and 8.8% in the low transmission season. At 20:00 these values were 55.7% and 64.7%, respectively, and at 22:00 96.2% and 94%, respectively. The percentage of participants that was in bed by 20:00 was 24.2% in the high and 21.2% in the low transmission season. At 22:00 these values were 85.8% and 84.8%, respectively, and by midnight 99% and 97.2%, respectively. The distribution of the study participants in each environment over time is show in Fig. 2.

Fig 2. Percentage of study participants in each environment during the evening, night and morning. The environments show are: (i) outdoors before going indoors (grey area on the left-hand side), (ii) indoors but not in bed (yellow on the left-hand side), (iii) indoors in bed using an LLIN (green) or not using an LLIN (red), (iv) indoors but not in bed after getting up (yellow on the right-hand side), (iv) outdoors after getting up (grey area on the right-hand side), during the low transmission (left panel) and high transmission season (right panel). Data including the environment of the study participants after 8am can be found in S5 Fig.



Differences in behavior time profiles across age groups and sex

In both seasons, children between 5 and 11 years of age spent more time indoors before going to bed, went to bed earlier, slept longer, got up later and left the house later than adults. In the high transmission season, they also went indoors earlier than adults. No differences were observed in the time spent indoors after getting up between children between 5 and 11 years of age and adults. In both seasons, children between 5 and 11 went indoors and to bed earlier and slept longer than children between 12 and 17. In the low transmission season they also spent more time indoors after getting up than children between 12 and 17 (Dunn, $p<0.04$). In both seasons, children between 12 and 17 spent less time indoors before going to bed and got up later than adults. In the low transmission season, they also went later indoors, slept less time and spent less time indoors after waking than adults.

Table 1. Median time of the day when participants went indoors, went to bed, got up and left the house after getting up, and the median amount of time they spent indoors before going to bed, in bed, and indoors after getting up before leaving the house. The letters (a,b,c) mark the pairs between which statistically significant differences were observed in pair-wise comparisons with Dunn Test. The * denotes that significant differences were found in all pair-wise comparisons with Dunn test (age groups) or Wilcoxon Mann Whitney (LLIN use).

	Time going indoors (HH:MM)		**Time indoors before going to bed (h)**		**Time to bed (HH:MM)**		**Time in bed (h)**		**Time getting up (HH:MM)**		**Time indoors after getting up (h)**		**Time leaving house (HH:MM)**		**Total time indoors (h)**		**Total time indoors not in bed (h)**	
	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high
Age group																		
18+ years	19:30[a]	20:02[a]	1.3 [ab]	0.9 [ab]	21:00[b]	20:56 [b]	8.7[*]	8.9 [a]	05:57[ab]	05:49 [ab]	0.4[a]	0.4	06:23[a]	06:16[a]	11[a]	10.2[a]	1.8[*]	1.4[ab]
12 - 17 years	19:59[a b]	20:01[b]	0.5 [a]	0.5 [a]	20:40[a]	20:47 [a]	9.3[*]	9.2 [b]	06:15[a]	06:04 [a]	0.2[ab]	0.3	06:38	06:24	10.5 [b]	10.4 [b]	0.9[*]	1.1[a]
5 – 11 years	19:30 [b]	19:34[ab]	0.8 [b]	0.6 [b]	20:17 [ab]	20:20 [ab]	9.9[*]	10 [ab]	06:20[b]	06:10 [b]	0.5[b]	0.3	06:46[a]	06:42[a]	11.5 [ab]	11 [ab]	1.4[*]	1[b]

Low: low transmission season
High: high transmissions season

Participants leaving the bed during the night

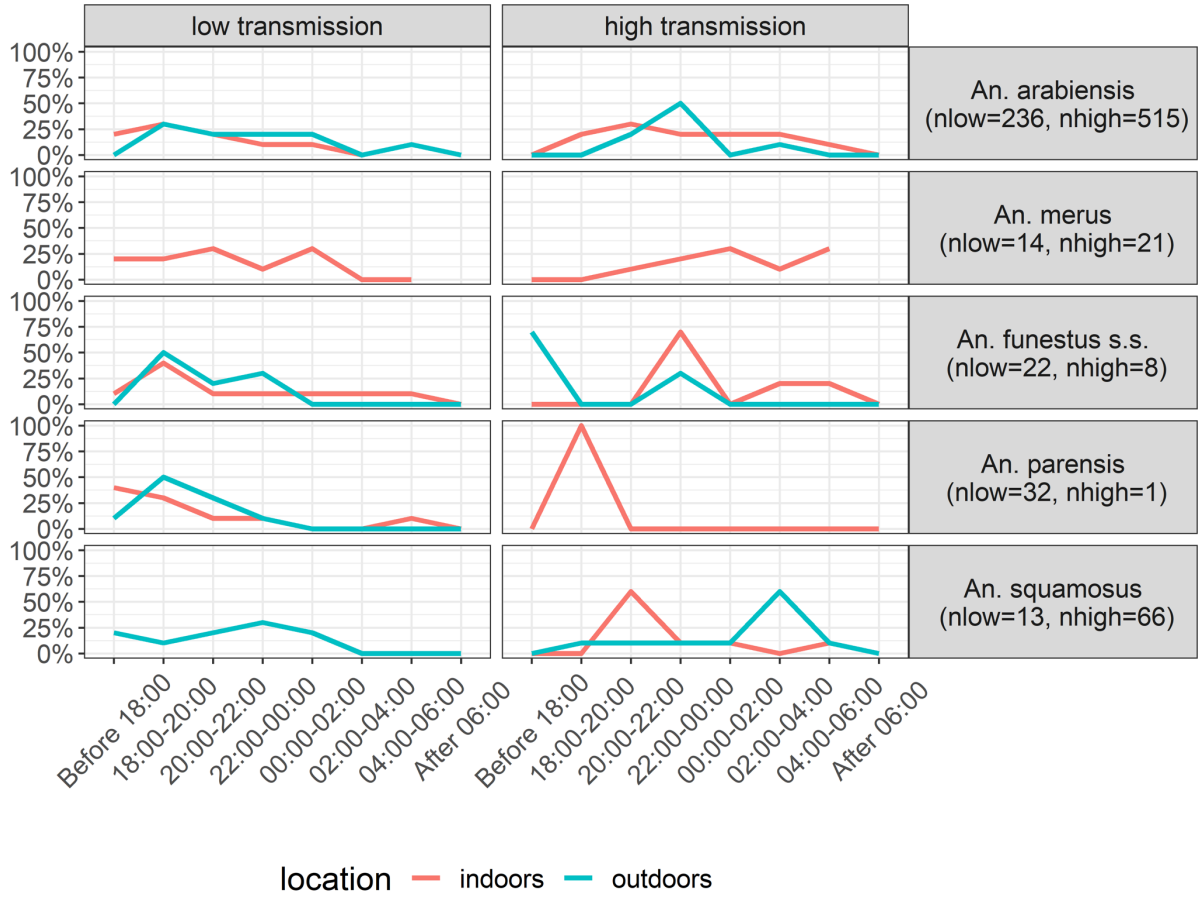
The percentage of participants that left the bed during the night was significantly higher in the low transmissions season (32%; 106 participants) than in high transmissions season (21.1%; 70 participants) (χ^2= 58.407, df = 1, p <0.0001). The main reason was to go to the toilet (71.7% of adults, 94.6% of children between 12 and 17 years old and 96.6% of children between 5 and 11 years old), followed by taking care of babies (20.7% of the adults and 2.7% of the children between 12 and 17 years old). Toilets were mostly located outdoors (96.8%, 640 responses). This may result in additional exposures to indoor (childcare) and outdoor host-seeking mosquitos (toilet visit), but as this behavior was not assessed in greater detail, the exposure occurring during these times hasn't been taken into account in the analyses below.

Vector species composition, sporozoite rates and host-seeking times

A total of 4472 *Anopheles* female mosquitoes were collected between May 2015 and August 2017 in the CDC light trap collections (both in stand-alone traps and in those combined with the collection bottle rotator) and 3593 were analyzed for the presence of sporozoites. A total of 32 (0.9%) mosquitoes were sporozoite positive during the study period. Sporozoite rates per species during the study period were as follows: *An. squamosus* 5.8% (1/17), *An. funestus s.s.* 1.04% (1/96)*, An. parensis* 1.0% (1/101) *and An. arabiensis* 0.9% (28/3021). Only *Anopheles* species found positive for *P. falciparum* malaria (i.e. incriminated as local vectors) were considered in the present analysis (we also included *An. merus*, as a positive specimen was found in September 2017). The majority of host-seeking anophelines of these five species collected (n=3848) were *An. arabiensis* (81%; n=3131) followed by *An. squamosus* (10%; n=375), *An.* parensis (3%; n=104), *An. merus* (3%; n=130) and *An. funestus s.s.* (3%; n=108). All *An. parensis* (except one individual) and more than two thirds of the *An. funestus s.s.* were collected during the low transmission season. No *An. parensis* were collected outdoors. *An. arabiensis, An. merus* and *An. squamosus* were more abundant during the high transmission season, when 70%, 61% and 88% of the mosquitoes were collected, respectively. No *Anopheles merus* were collected outdoors in any of the two seasons.

The distinct host-seeking behavior of these five vectors is shown separately for the low and high transmission seasons in Fig. 3. Overall, the peak of host-seeking activity occurred earlier in the low transmission season (between 18:00 and 20:00 indoors and outdoors) than in the high transmission season (between 20:00 and 22:00 indoors and 02:00-04:00 outdoors).

Fig 3. Host-seeking behavior of five different malaria vector species in Magude district between 4pm and 8am. The proportion of host-seeking mosquitoes collected indoors and outdoors is shown in 2 hour intervals.



During the low transmission season, 8.7% of all outdoor host-seeking mosquitoes were collected before 18:00 (when most of participants were still outdoors, see above), and 50.7% before 20:00 (when half of the participants where still outdoors). Indoors, 20% of the host-seeking mosquitos were collected between 20:00 and 22:00 (whereby approx. half of the participants where already

indoors and the other half moved indoors during this period) and 18.3% between 22:00 and 06:00 (when most of participants where in bed). During the high transmission, 4.9% of all outdoor host-seeking mosquitoes were collected before 18:00 (when most of participants were still outdoors), and 17.8% occurred before 20:00 (when half of the participants where still outdoors). Indoors, 9.5% of the host-seeking mosquitoes were collected between 20:00 and 22:00 (again with approx. half of the participants already being indoors and the other half moving indoors during this period) and 77.6% occurred between 22:00 and 06:00 (when most participants where in bed).

Residual proportional exposure to host-seeking mosquitoes in each environment at the observed levels of bednet use in Magude district

Combining human and vector behaviors at the observed levels of bed net use and looking at both seasons combined, 74.0% (95% CI: 65-6-80.9) of all host-seeking mosquitoes that Magude residents were exposed to were *An. arabiensis*, 9.9% (95% CI: 4.8-13.2) *An. squamosus*, 5.8% (95% CI: 2.7-11.5) *An. parensis*, 5.2% (95% CI: 2.3-10.8) *An. merus* and 5.1% (95% CI: 2.2-10.6) *An. funestus s.s.*. Differences between seasons are shown in Table 2. Exposure to host-seeking *An. funestus s.s.* and *An. parensis* was higher during the low transmission season than in the high transmission season.

Looking at the risk per environment, combining both seasons, the majority of residual exposure to host-seeking mosquitoes was estimated to occur when people where in bed (64%, 95% CI: 55.3-1.9), followed by indoors before going to bed (21.9%, 95% CI: 15.5-29.9), outdoors in the evening (12.5%, 95% CI: 7.7-19.5), indoors after getting up (1.4%, 95% CI: 0.2-5.6) and outdoors during the morning (0.2%, 95% CI: 0-3.8). Of the residual exposure, 32.4% (95% CI: 24.7-40.9) occurred during the low transmission season and 66.7% (95% CI: 59.0-75.2) during the high transmission season. A higher proportion of residual exposure occurred outdoors and indoors while not in bed in the low transmission season, compared to the high transmission season (Table 3).

Table 2. The contribution of different vector species to the exposure to host-seeking vectors that Magude residents experienced. 'Residual human-adjusted exposure to host-seeking vectors' shows the percentage of host-seeking mosquitoes of each vector species that residents were exposed to at the observed levels of bednet use. 'Unavertable residual human-adjusted exposure to host-seeking vectors' shows the percentage of host-seeking mosquitoes from each vector species that residents would have been exposed to if they all would have used a net when in bed.

	Seasons combined		**Low transmission season**		**High transmission season**	
Species	Actual human-adjusted exposure to host-seeking vectors (%)	Unavertable human-adjusted exposure to host-seeking vectors (%)	Actual human-adjusted exposure to host-seeking vectors (%)	Unavertable human-adjusted exposure to host-seeking vectors (%)	Actual human-adjusted exposure to host-seeking vectors (%)	Unavertable human-adjusted exposure to host-seeking vectors (%)
An. arabiensis *(n=751, nlow=236, nhigh=515)*	74.0% (65-6-80.9)	69.2% (65.6-80.9)	64.2% (48.3-77.6)	56.6% (36.8-74.6)	78.5% (68.6-86.1	75.5% (61.8-85.7)
An. squamosus *(n=79, nlow=13, nhigh=66)*	9.9% (4.8-13.2),	12.8% (6.7-22.3)	5.1% (10.2-17.4)	8.2% (16.5-26.5)	12.2% (6.6-21.0)	15% (7.2-27.8)
An. parensis *(n=33,nlow=32, nhigh=1)*	5.8% (2.7-11.5)	7.4% (3.1-15.9)	17.8% (8.5-32.7)	22.0% (9.3-42.2)	0.1% (0-5.0)	0.1% (0-8.3)
An. merus *(n=35, nlow=14, nhigh=21)*	5.2% (2.3-10.8)	4.2% (1.2-11.8)	4.1% (0.6-16.0)	3.1% (0.9-19.7)	5.8% (2.2-13.2)	4.8% (1.1-15.2)
An. funestus s.s. *(n=30, nlow=22, nhigh=8)*	5.1% (2.2-10-6).	6.4% (2.4-14.6)	8.8% (2.8-22.)	10.1% (2.5-28.8)	3.3 % (0.8-9.9)	4.5% (0.09-14.7)
All species (n=928, nlow=317, nhigh=611)	100%	100%	100%	100%	100%	100%

Table 3. Percentage of host seeking mosquitoes of each of the five vector species that Magude residents were exposed to in each of the five environments where humans and vectors have the opportunity to interact. These environments are i) outdoors before going indoors, ii) indoors before going to bed, iii) indoors while in bed, iv) indoors after getting up, and v) outdoors after leaving the house again. Percentages are given for the low and high transmission seasons separately, and for the observed levels of bednet use, or assuming a hypothetical scenario in which all residents used a net when in bed.

	Low transmission season						High transmission season				
	Outdoors evening	Indoors before going to bed	Indoors while in bed	Indoors after getting up	Outdoors morning		Outdoors evening	Indoors before going to bed	Indoors while in bed	Indoors after getting up	Outdoors morning
% of host-seeking mosquitoes at observed levels of bednet use											
An. arabiensis (n=236)	5.6% (0.7-22.8)	30.3% (15.3-50.4)	63%% (43-79.6)	1.0% (0,16.3)	0.1% (0-15)	*An. arabiensis (n=515)*	3.4% (0.8-11.4)	21.6% (13.2-33.1)	73.4 % (61.5-82.7)	1.5% (0.1-8.6)	0.1% (0-6.4)
An. merus (n=14)	0.0% (0-82.5)	25% (0-91.2)	75.0% (8.8-100)	0.0% (0-82.5)	0.% (0-82.5)	*An. merus (n=21)*	0.0% (0-51.6)	9.7% (0-60.4)	85.3% (35.4-99.6)	5.1% (0-56.4)	0.0 (0-51.6)
An. funestus s.s (n=22)	40.1% (6.2-85.2)	19.8% (0.4-75.5)	39.2% (5.6-85.2)	0.9% (0-62)	0.0% (0-61.3)	*An. funestus s.s (n=8)*	63.4% (12.7-96.7)	2.2% (0-69.8)	33.3% (1.9-87.1)	1.2% (0-69.1)	0.0% (0-68.3)
An. parensis (n=32)	49.5% (20.7-78.6)	17.7% (1.9,58.5)	32.1% (7.5-70.3)	0.6% (0-41.3)	0.0% (0-40.6)	*An. parensis (n=1)*	0.0% (0-99.5)	93.1 (0.5-100)	6.9% (0-99.5)	0.0% (0-99.5%)	0.0 (0-99.5)
An. squamosus (n=13)	100% (23.1-100)	0.0% (0-76.9)	0.0% (0-76.9)	0.0% (0-76.9)	0.0% (0-76.9)	*An. squamosus (n=66)*	29.4% (8.6-62.1)	20.9% (4.4-54.3)	46.3% (19.6-75.2)	1.2% (0-33.1)	2.2 (0-34.3)
Species combined	21.1% (10.9-36.4	25.4% (12-21)	52.7% (37.3-67.6)	0.8% (0-11.2)	0.1% (0-10.1)	Species combined	8.4% (3.9-16.5)	20.3% (12.9-30.1)	69.4% (58.8-78.3	1.6% (0.2-7.6)	0.3% (0-5.5)
% of host-seeking mosquitoes assuming all residents used the net											
An. arabiensis (n=236)	10.3% (1.3-37.4)	55.1% (29.4-78.6)	32.6% (12.8,60.1)	1.8% (0-27)	0.3% (0-24.9)	*An. arabiensis (n=515)*	6.0% (1.3,19.2)	37.9% (23.8-54.3)	53.3% (37.4,68.6	2.6% (0.2,14.5)	0.1% (0-19.7)

An. merus (nl=14)	0% (0-95.3)	54.1% (4.7-96.6)	49.5% (3.4-95.3)	0.0% (0-95.3)	0.0% (0-95.3)	*An. merus (n=21)*	0% (0-72.5)	19.5% (0-83.3)	70.2% (12.1-99.3)	10.2% (0-78.5)	0.0% (0-72.5)
An. funestus s.s (n=22)	55.8% (12-92.5)	27.6% (0.6-86.1)	15.4% (0-80.1)	1.3% (0-71.9)	0.0% (0-71.1)	*An. funestus s.s (n=8)*	78.9% (14.6-100)	2.7% (0-76.1)	17.0% (0-83.5)	1.4% (0-75.4)	0.0% (0-74.6)
An. parensis (n=32)	64.3% (22.7-92.9)	23.0% (2.5-68.3)	11.9 (0.2-59.2)	0.7% (0-40.9)	0.8% (0-48.8)	*An. parensis (n=1)*	0.0 (0-99.5)	95.7% (0.5-100)	4.3% (0-99.5)	0.0% (0-99.5)	0.0% (0-99.5)
An. squamosus (n=13)	100% (23.1-100)	0.0% (0-76.9)	0.0% (0-76.9)	0.8% (0-48.8)	0.0% (0-76.9)	*An. squamosus (n=66)*	40.2% (12-75.6)	28.5% (6.1-66.7)	26.0% (5.3-65.1)	1.7% (0-42.7)	3.0% (0-42.7)
Species combined	33.8 (17.7-54.2)	40.7% (23.2-60.7)	24.0% (10.7-44.4)	0.0% (0-76.9)	0.2% (0-15.4)	Species combined	14.1% (6.7-26.8)	34.1% (22.2-48.2)	48.4% (34.9-,62.1)	2.8% (0.3-12.4	0.5% (0-9)

Proportion of exposure to host-seeking vectors prevented by the personal protection of LLINs in Magude district

At the observed levels of bednet use and considering both seasons together, the personal protection of LLINs averted 39.2% (95% CI: 32.8-45.9) of the exposure to host-seeking mosquitoes that survived or did not come in contact with IRS and LLIN products. This percentage was lower in the low transmission seasons (20.9%, 95% CI: 11.6-34.2) than in the high transmission season (45.3%; 95% CI: 37.7-53.1). A comparison between the proportion of exposure prevented by the personal protection of LLINs and that still occurring in the different environments is shown in Fig. 4 for each season. LLINs prevented a significant higher proportion of exposure in children between the age of 5 and 11 (45.4%) than in children between the age of 11 and 17 (32.5%) or adults (38.9%). Statistically significant differences were also observed in the proportion of exposure prevented against different vector species (Kruskal-Wallis, $p<0.0001$). LLINs prevented a higher proportion of exposure to host-seeking members of the *An. gambiae* group (41.8% [95% CI: 34.4-49.5] for *An. arabiensis* and 45.4% [95% CI: 20,73.2] for *An. merus*) than from *An. squamosus* [32.0%, 95% CI: 14.2-56.3] and members of the *An. funestus* group (21.9% [95% CI: 3.8-59.7] for *An. funestus s.s.* and 13.9% [95% CI: 1.3-51.5] for *An. parensis*).

Maximum personal protection that LLINs could have conferred in Magude district

Considering both seasons combined, the maximum proportion of exposure to host-seeking mosquitoes that the personal protection of LLINs could have averted if all residents would have used a net while in bed, assuming that an increase in net use would not have led to an immediate change in vector host-seeking behaviors (see discussion) was 63.3% (p10th= 41.2, p90th=75.2; Fig. 4). This was lower during the low transmission season (50.7%, p10th=35.6, p90th=62.6) than during the high transmission season (67.5%, p10th=53.8, p90th= 76.5).

The potential personal protection that LLINs could have provided if all residents would have used a net considering both seasons was significantly different between age groups (Kruskal-Wallis, $p<0.0001$). This maximum personal protective efficacy would have been lowest for adults (57.0%; p10th=35.6, p90th=73.2) and highest for children between 5 and 11 years of

age (62.5%; p10th=47, p90th=75.8), but would not differ between the youngest and oldest child groups (61.0%; p10th=43.4, p90th=75.7).

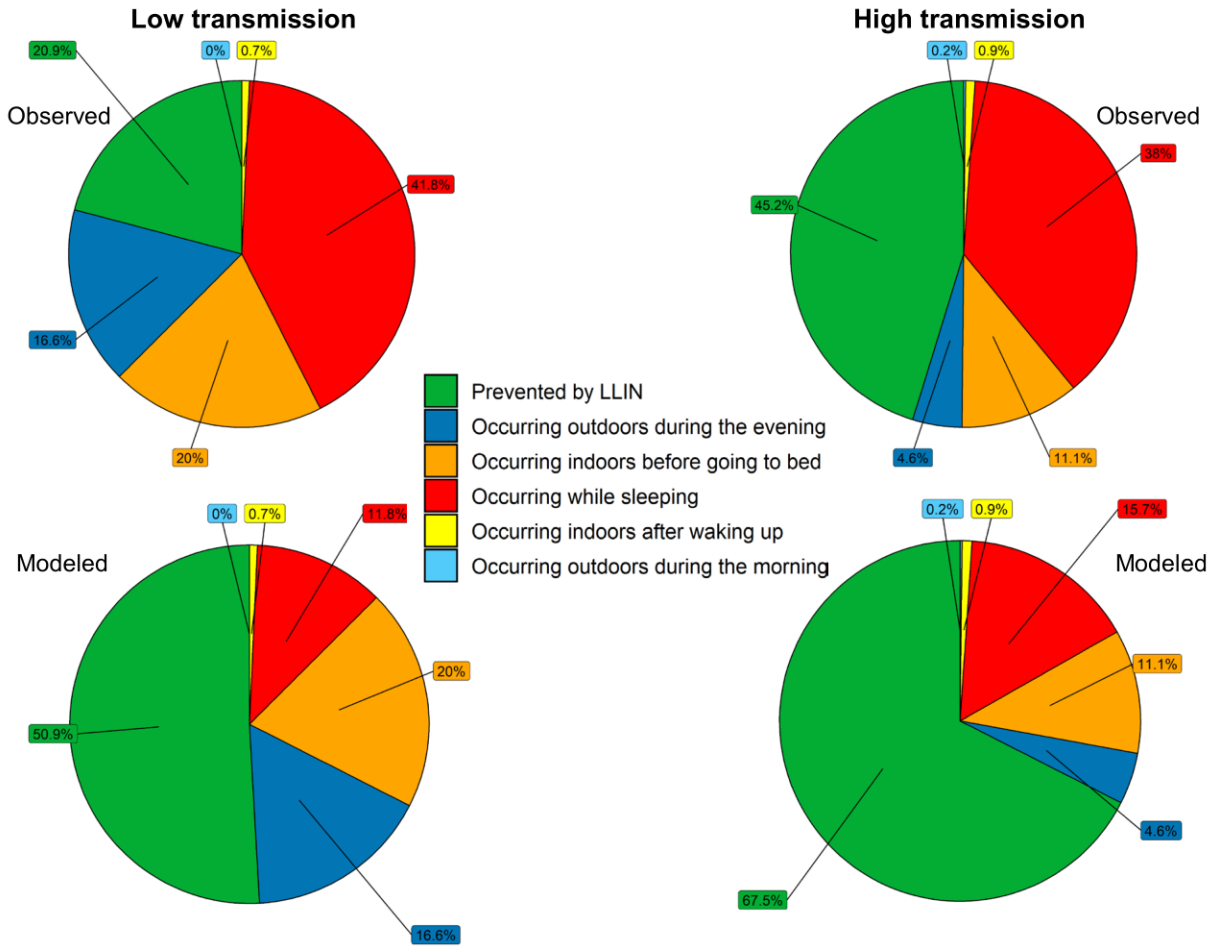
The maximum personal protection that LLINs could have conferred would also have differed significantly between the different vector species (Kruskal-Wallis, df=4, p<0.0001). LLINs would have prevented a higher proportion of exposure to host-seeking vectors of the *An. gambiae* group than to those of the *An. funestus* group, similar to the results of the previous section. Regarding the individual species, LLINs would have prevented a higher percentage of exposure to host-seeking *An. arabiensis* (67.4%; p10th=45.9, p90th=77.4), followed by *An. merus* (74.8%, p10th=55.3, p90th=80.5), *An. squamosus* (19.8%; p10th=0, p90th=66.6), *An. funestus s.s.* (42.6%; p10th=32.1, p90th=44.5) and *An. parensis* (31.7%, p10th=0, p90th=47.3).

Residual exposure to host-seeking vectors that would occur if all residents of Magude would have slept under the net every night.

Of the residual exposure to host-seeking vectors that still would have occurred if all residents would have used a net while in bed, 40.3% (95% CI: 29.8-51.6) would have happened indoors while participants are in bed due to the imperfect feeding inhibition of LLINs (see discussion), followed by 36.3% (95% CI: 26.2-47.7) indoors before going to bed, 20.7% (95% CI: 12.9-31.3) outdoors during the evening, 2.3% (95% CI: 0.4-9.1) indoors after getting up and 0.4% (0-6.2) outdoors after getting up and leaving the house. Overall, 33.4% (95% CI: 23.6-44.7) of this exposure would have occurred during the low transmission season and 66.6% (55.2-76.3) during the high transmission season. In this scenario, the contribution of members of the *An. funestus* group and of *An. squamosus* would have been higher than at the observed levels of bednet use (Table 3).

Fig 4. The proportion of exposure to host-seeking mosquitoes prevented by the personal protection of LLINs and the distribution of the unprevented exposure across the five different environments. The proportion of residual exposure to host-seeking vectors are provided at the observed (top) and modeled (bottom) net use (assuming all residents use a net while in bed). Green: exposure prevented by LLINs, dark blue: residual exposure outdoors before going indoors,

orange: residual exposure indoors before going to bed, red: residual exposure while in bed, yellow: residual exposure indoors after getting up, light blue: residual exposure outdoors after leaving the house again.



Discussion

We aimed to understand (i) the residual malaria transmission that occurred during the Magude project by characterizing residual exposure to host-seeking vectors occurring when LLINs and IRS were deployed together, (ii) the protection that LLINs conferred, and could have conferred if all residents would have used a net to sleep, against exposure to host-seeking vectors, and (iii) the residual exposure to host-seeking vectors that would have occurred even if all residents would have used a net. We hope our results help to optimize the implementation of current tools and guide the development and implementation of supplementary vector control interventions in low transmission settings in sub-Saharan Africa.

An. arabiensis was responsible for more than 74% of residual exposure to host-seeking vectors experienced by Magude residents during the Magude project. The role of *An. arabiensis* as the potential main driver of residual malaria transmission after the implementation of district-wide IRS campaigns has repeatedly been observed in southern and eastern African countries [43-45]. The other four vector species (*An. funestus s.s.*, *An. parensis*, *An. squamosus* and *An. merus*) were each responsible for less than 10% of the residual exposure to host-seeking vectors. At the observed level of bednet used, 12.5% of residual exposure occurred outdoor during the evening, 21.9% indoor before going to bed, almost two thirds (64%) while people were in bed, 1.4% indoors after getting up and 0.2% outdoor after leaving the house. Almost a third of the exposure (32%) occurred during the low transmission season. The personal protection conferred by LLINs prevented only 39.2 % of the exposure to the host-seeking vectors that survived or did not come in contact with IRS and LLIN products during the Magude project, and could have prevented a maximum of 63.3% if all residents would have used an LLIN to sleep (assuming that the increase in LLIN use does not lead to an immediate change in vector host-seeking behavior). The maximum personal protection nets could have provided differed across seasons, vector species and age groups. The personal protection of LLINs prevented a higher proportion of the exposure to host-seeking vectors of the *An. gambiae* group than to those of the *An. funestus* group, and provided better protection among children between 5 and 11 years compared to other age groups, and in the high compared to the low transmission season.

During phase I of the Magude project, residual exposure to host-seeking vectors from all vector species occurred mainly indoors (87.3%), primarily while people were in bed (64%). The latter is mainly due to the observed levels of bed net use as well as the estimated proportion of bites still occurring while people are under a net (due to the imperfect feeding inhibition of LLINs assumed in our calculations). If all Magude residents would have used a net to sleep (again assuming that the increase in LLIN use does not lead to an immediate change in vector host-seeking behavior), our estimates indicate that the personal protecting effect of LLINs alone would have prevented an additional 24.1% of exposure to host-seeking mosquitoes that survived or did not come in contact with IRS and LLIN products. .

In the hypothetical scenario that everyone would have used a net, and again assuming that an increase in LLIN use does not lead to an immediate change in vector host-seeking behavior , the highest proportion of residual exposure to host-seeking mosquitoes would still occur indoors (78.9%), and also when people are in bed (40.3%). This suggests that large gains to further reduce transmission in settings where *An. arabiensis* is the predominant residual malaria vector could be achieved by increasing the feeding inhibition of LLINs and from additional vector control interventions that reduce the indoor human-vector contact.

In contrast, our analysis of the limited number of members of the *An. funestus* group suggests that the highest proportion of residual exposure to *An. funestus s.s.* and *An. parensis* occurred outdoors during the evening, which explains the low personal protection conferred by LLINs against exposure to these species. This observed behavior could be a result of selection pressure exerted by the continuous historical implementation of insecticide-based vector control interventions, which has been observed elsewhere to shift vector behaviors to outdoor feeding [46]. Although these results will need to be confirmed by additional studies, supplementary interventions that aim to reduce the densities of outdoor biting vector populations (e.g. through larval source management or attractive targeted sugar baits) or prevent outdoor human-vector interactions (e.g. through topical repellents or impregnated clothing) will be needed to reduce the residual exposure to *An. funestus s.l.* Note that for all of the five mosquito species that we analyzed, the latter interventions are mostly needed during the evening hours (before midnight), as the proportion of residual exposure to these vectors during the early morning was very small.

LLINs provided less personal protection during the low transmission season, when almost a third of the overall exposure to host-seeking vectors recorded in Magude district occurred. This was mainly driven by the lower LLIN use, but also by the earlier vector host-seeking activity observed during this season. The seasonal variation in bednet use has been observed in several other countries [47] and highlights the need to increase LLIN use during this particular season, as malaria transmission can still persist. Additional interventions are needed to tackle the problem of early host-seeking vectors during the dry transmission season, both outdoors and indoors before people go to bed. In addition, the fact that higher number of *An. funestus s.s.* and *An. parensis* were collected during this season compared to the high transmission season, suggests

that these interventions could have a great impact in reducing the abundance of members of the *An. funestus* complex.

Since there were no differences in LLIN use between age groups, the difference in personal protection by LLINs observed between the age groups is due to differences in human behavior. The fact that young children went to bed earlier and slept longer in both seasons, and that they went indoors earlier during the high transmission season, means that the personal protection conferred to them by LLINs probably prevented a higher proportion of the exposure to host-seeking vectors in this age group than for the other age groups. This, and the fact that such behaviors can differ between regions (e.g. residents in Tengua, Milange district, Mozambique, went indoors and to bed later, slept less and got up earlier than people in Magude [48, 49]) highlight the importance of collecting local human behavioral data to accurately estimate transmission risk and the protective efficacy of LLINs, but also of other tools that aim to reduce vector-host contact.

The low number of sporozoite positive mosquitoes and the lack of data on mosquito blood meal sources prevent us from estimating the Entomological Inoculation Rate and thus from drawing firm conclusions on the relative importance of each species in sustaining residual malaria transmission during the Magude project. Nonetheless, our results do suggest that at least five species were potentially contributing to sustaining transmission during the Magude project (*An. arabiensis*, *An. funestus s.s.*, *An. parensis*, *An. squamosus* and *An. merus*) and that their contribution differs between the studied environments in which people and mosquitoes interact. Transmission by *An. funestus s.s.* and *An. parensis* is more likely to have occurred outdoors and indoors before people go to bed, while *An. arabiensis* and *An. merus* fed commonly indoors when people are in bed. Transmission by *An. squamosus* likely occurred both before people go to bed and while people are in bed. Although -based on the percentage of residual exposure attributed to each species- *An. arabiensis* may seem the most important vector of transmission, the fact that *An. funestus s.s.* can still drive transmission even if it is less abundant than *An. arabiensis* [50] suggests that *An. funestus* may still have played an important role in sustaining local malaria transmission during the Magude project.

There are, however, some limitations of the present study that may affect the accuracy of our estimations of exposure to host-seeking mosquitoes and of the personal protective efficacy of LLINs. First, due to the overall low baseline malaria prevalence and the four rounds of MDA conducted during the Magude project, very few mosquitoes were found positive for *P. falciparum*. As such, we may have excluded vectors species in our analysis. Secondly, our outdoor CDC Light traps were baited with a BG-lure (containing artificial skin compound mimics) and CO_2 to simulate a human host, but we did not validate these CDC light traps collections with CDC light traps with an actual human bait present outside. Differences in sampling efficacy may lead to changes in the proportion of host-seeking mosquitoes collected outdoors and to the over- or underestimation of the importance of this transmission environment. Thirdly, our analyses are based on participants self-reported behaviors and timings and may therefore be affected by an incapacity to properly use the digital watch provided to them, have difficulties in reading or interpreting the time recording cards, have a response-bias (e.g. claiming using the net when they did not) or a recall bias, although the latter is expected to be minimal since participants were asked in the morning about their behaviors during the previous night. Fourthly, we showed that human behaviors differ between seasons but assumed that the respective behaviors remained similar during all low and all high transmissions seasons across different years during the Magude project. Yet, there may have been unaccounted changes in human exposure to host-seeking mosquitoes due to e.g. increased awareness of malaria and/or mosquitoes during the Magude project, or exceptionally dry and wet years. Or we may have missed short-termed intra-season events that may increase exposure to host-seeking mosquitoes (e.g. those linked to agricultural activities). Fifth, the exposure experienced by the one percent of participants that did not sleep indoors or the exposure during the night when the study participants had to get up for childcare and/or to go to the toilet (between 21% and 32% of the participants) were not considered, but those behaviors could increase the overall exposure to host seeking mosquitoes. In addition, the temporal resolution of the vector behavioral data (2h) compared to the human behavioral data (1min) limit the accuracy of the estimates. However, it was sufficient to detect significant differences in LLIN protection across age groups and seasons.

The final limitation deserves special attention and a call for action. Estimates of the protective efficacy of LLINs and residual exposure to host-seeking mosquitoes are sensitive to the LLIN blood feeding inhibition chosen. As stated in the methods, we assumed an 81.1% reduction in exposure to host seeking mosquitoes when participants were under a used Olyset® Net (the main net brand observed in the district). This value is based on a study conducted in Tanzania [39], because local measurements of net feeding inhibition were not available. Data on Olyset® net feeding inhibition are available from a limited number of countries, mostly located in West Africa (Benin, Burkina Faso, Cote D'Ivoire, Nigeria) and one in east Africa (Tanzania) [51]. It is common to use the data obtained in those few countries, or to use an arbitrary value, when estimating the personal protection of LLINs in one's own country [26, 28, 52]. We selected 81.1% from all published values from Tanzania, because the experimental conditions represented the local conditions in Magude best (mosquito species composition, prior net use, see methods). This value was the lowest among all published values (except for those values for Olyset Nets used for 7 years), and therefore generates the most conservative estimates for the protective efficacy of LLINs during the Magude project. However, a wide range of feeding inhibition values has been observed across different experimental hut trials with the same net brand, and between different vector species [51]. Therefore local measurements of the LLIN feeding inhibition against local vector species are needed to i) accurately quantify the protective efficacy of nets, and ii) evaluate the residual exposure to vector bites after deployment of interventions, to better understand the gaps in the protection by LLINs.

Conclusion

The combined deployment of IRS and LLINs during the Magude project was not sufficient to prevent all malaria vector bites. The residual exposure to *An. arabiensis* indoors when people are in bed, *An. funestus s.s.* and *An. parensis* outdoors and indoors before bedtime, and of *An. squamosus* both indoors and outdoors, are likely to have sustained malaria transmission throughout the Magude project. The low transmission season should not be neglected when implementing vector control interventions during malaria elimination campaigns, as this season accounted for a third of the residents' total exposure to host-seeking mosquitoes. In areas where

the main malaria vector feeds indoors while people are in bed, like *An. arabiensis* in this study, increasing bednet use and net feeding inhibition (e.g. by improving LLIN quality and/or selecting LLIN brands after a local evaluation), can lead to significant reductions in exposure to host-seeking vectors and likely further reduce malaria transmission. However, supplementary interventions aiming to reduce human-vector contact outdoors and/or indoors before people go to bed (e.g. through larval source management, window and eave screening, eave tubes, and spatial repellents) will be needed to reduce residual biting by outdoor and earlier biting vectors such as *An. funestus s.s.* and *An parensis.*

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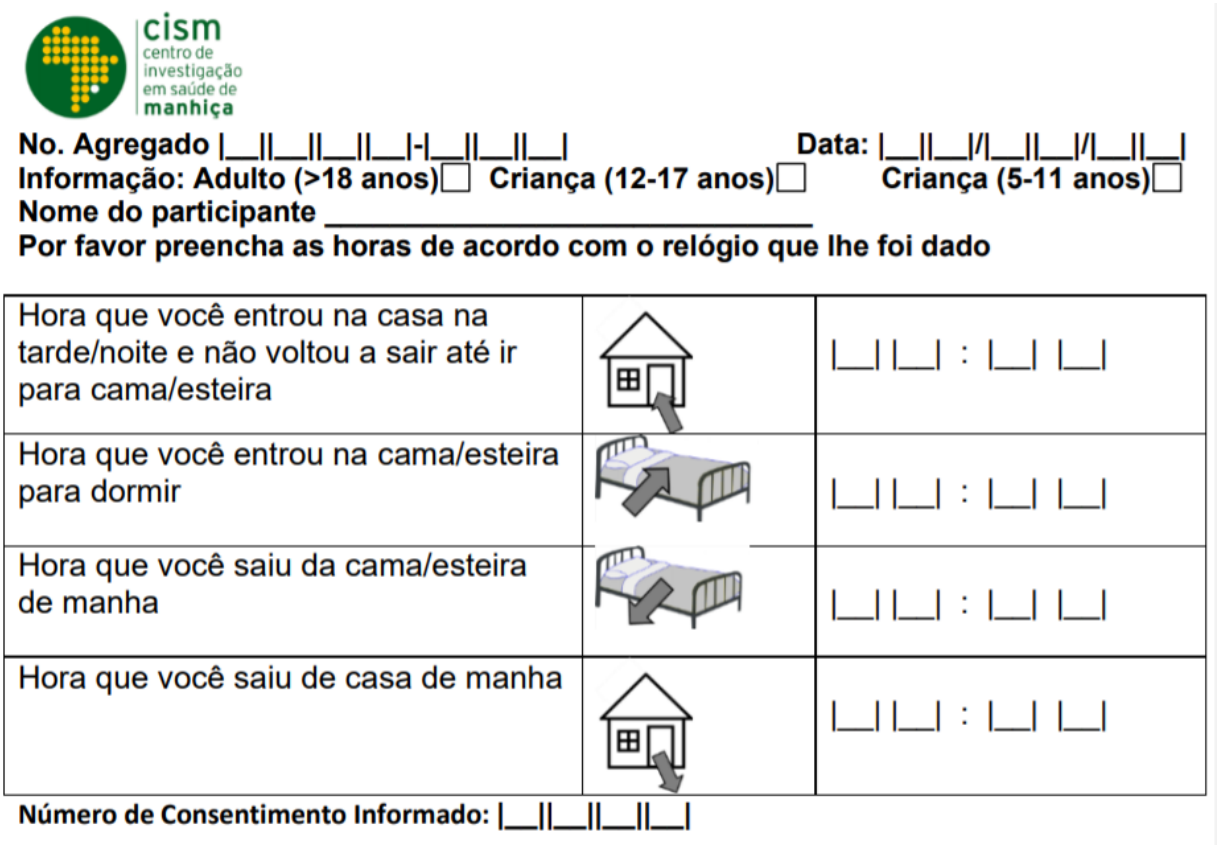
Supporting Information

S1 Fig. Time-tracking card provided to each study participant to track movement between compartments. Participant were asked to record the following: (i) time going indoors, (ii) time going to bed, (iii) time getting up and (iv) time leaving the house in the morning.



cism
centro de investigação em saúde de manhiça

No. Agregado |__||__||__||__|-|__||__||__| **Data:** |__||__|/|__||__|/|__||__|
Informação: Adulto (>18 anos)☐ **Criança (12-17 anos)**☐ **Criança (5-11 anos)**☐
Nome do participante _____
Por favor preencha as horas de acordo com o relógio que lhe foi dado

Hora que você entrou na casa na tarde/noite e não voltou a sair até ir para cama/esteira		|__| |__| : |__| |__|
Hora que você entrou na cama/esteira para dormir		|__| |__| : |__| |__|
Hora que você saiu da cama/esteira de manha		|__| |__| : |__| |__|
Hora que você saiu de casa de manha		|__| |__| : |__| |__|

Número de Consentimento Informado: |__||__||__||__|

S2 Table. Model parameters with their definitions and equations

Notation	Definition	Calculation formula
$\mathrm{B}_{I,t}$	Indoor biting rate at time t	$\frac{\sum_{t_{start}}^{t_{end}} Mosquitos_{indoors}}{People\ by\ Trap\ x\ Interval\ Minutes}$ Where the interval is the time interval in which time t is comprised and where t_{start}: start of collection time interval (e.g. 18:00) t_{end}: end of collection time interval (e.g. 20:00)
$\mathrm{B}_{O,t}$	Outdoor biting rate at time t	$\frac{\sum_{t_{start}}^{t_{end}} Mosquitos_{indoors}}{People\ by\ Trap\ x\ Interval\ Minutes}$ Where the interval is the time interval in which time t is comprised and where t_{start}: start of collection time interval (e.g. 18:00) t_{end}: end of collection time interval (e.g. 20:00)
$\mathrm{B}_{O,bb}$	Number of bites received by a person outdoor (O) in the evening before going to bed (bb) during one night	$\sum_{t=18:00}^{t\ indoors} \mathrm{B}_{O,t}$
$\mathrm{B}_{I,bb}$.	Number of bites received by a person indoors (I) before going to bed (bb) during one night	$\sum_{t\ indoors}^{t\ to\ bed} \mathrm{B}_{I,t}$
$B_{I,bu}$	Number of bites received by a person indoors (I) while in bed unprotected (bu) during one night	$\sum_{t_{to\ bed}}^{t\ get\ up} \mathrm{B}_{I,t}$
$B_{I,bp}$	Number of bites received by a person indoors (I) while in bed under the net (bp) during one night	$\rho \sum_{t_{to\ bed}}^{t\ get\ up} \mathrm{B}_{I,t}$ Where ρ if the percentage of mosquitoes that successfully bite while people are under the net
$\mathrm{B}_{I,ab}$	Number of bites received by a person indoors (I) after getting up from bed (ab) during one night	$\sum_{t_{get\ up}}^{t\ leave\ house} \mathrm{B}_{I,t}$

$\mathrm{B}_{O,ab}$	Number of bites received by a person outdoor (O) in the morning after getting up (ab=after bed)	$\sum_{t_{leave\ house}}^{t=08:00} \mathrm{B}_{O,t}$
$\mathrm{B}_{I,b}$	Number of bites received by a person indoors (I) while in bed (b) during one night	$if\ under\ net\ \ B_{I,bp}$ $if\ not\ under\ the\ net\ B_{I,bu}$
B_{I}	Residual number of bites received by a person indoors (I) during one night	$\mathrm{B}_{I,bb} + \mathrm{B}_{I,b} + .\mathrm{B}_{I,ab}$
B_{O}	Residual number of bites received by a person outdoors (O) during one night	$\mathrm{B}_{O,bb} + .\mathrm{B}_{I,ab}$
B	Residual number of bites received by a person indoors and outdoors during one night at observed levels of bednet use	$\mathrm{B}_{O} + .\mathrm{B}_{I} = \mathrm{B}_{O,bb} + \mathrm{B}_{I,b} + \mathrm{B}_{I,bu} + .\mathrm{B}_{I,ab} + \mathrm{B}_{O,ab}$ $\mathrm{B}_{I,b}$ for each participants is calculated as $B_{I,bp}$ or $B_{I,bu}$ depending on whether the participant used the net to sleep
B_{u}	Residual number of bites received by a person during on night if not sleeping under the net (u=unprotected)	$\mathrm{B}_{O,bb} + \mathrm{B}_{I,bb} + \mathrm{B}_{I,bu} + .\mathrm{B}_{I,ab} + \mathrm{B}_{O,ab}$
B_{p}	Residual number of bites received by a person during on night if sleeping under the net (p=protected)	$\mathrm{B}_{O,bb} + \mathrm{B}_{I,bb} + \mathrm{B}_{I,bp} + .\mathrm{B}_{I,ab} + \mathrm{B}_{O,ab}$
$\mathrm{B}_{\mathrm{I,nb}}$	Number of bites received by a person while indoors (I) but not in bed (nb) during one night	$\mathrm{B}_{I,bb} + \mathrm{B}_{I,ab}$
$B_{I,p}$	Number of bites received by a person indoors (I) if sleeping under the net (p=protected) during one night	$\mathrm{B}_{I,bb} + \mathrm{B}_{I,bp} + \mathrm{B}_{I,ab}$
$B_{I,u}$	Number of bites received by a person indoors (I) if not sleeping under the net (u=unprotect) during one night	$\mathrm{B}_{I,bb} + \mathrm{B}_{I,bu} + \mathrm{B}_{I,ab}$

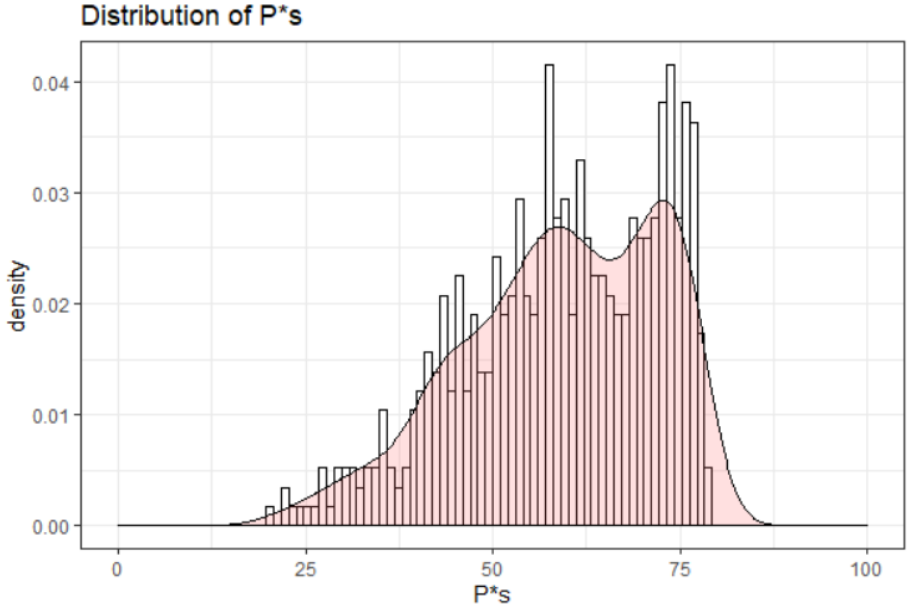
B_r	Total number of residual bites (r) that all participants together could have received given the reported levels of bed net use during one night	$\sum_{p=1}^{p=n} B$ Where p=participant and n=max number of participants in the study and $\mathrm{B}_{I,b}$ for each participants is calculated as $B_{I,bp}$ or $B_{I,bu}$ depending on whether the participant used the net to sleep
B_{rp}	Total number of residual bites (r) that all participants together could have received if they would have all used a net to sleep (p=protected)	$\sum_{p=1}^{p=n} \mathrm{B}_p$ Where p=participant and n=max number of participants in the study and where $\mathrm{B}_{I,b} = B_{I,bp}$
B_{ru}	Total number of residual bites (r) that all participants together could have received if none of them would have used a net to sleep (u=unprotected)	$\sum_{p=1}^{p=n} \mathrm{B}_u$ Where p=participant and n=max number of participants in the study and where $\mathrm{B}_{I,b} = B_{I,bu}$
$\pi_{O,bb}$	Percentage of residual bites received by a person outdoors (O) before going to bed (bb) during one night	$100x\frac{\mathrm{B}_{O,bb}}{\mathrm{B}_\mathrm{I}}$
$\pi_{I,bb}$	Percentage of residual bites received by a person indoors (I) before going to bed (bb) during one night	$100x\frac{\mathrm{B}_{I,bb}}{\mathrm{B}_\mathrm{I}}$
$\pi_{I,b}$	Percentage of residual bites received by a person indoors (I) while in bed (b) during one night	$100x\frac{\mathrm{B}_{I,b}}{\mathrm{B}_\mathrm{I}}$
$\pi_{I,ab}$	Percentage of residual bites received by a person indoors (I) after getting up during (ab=after bed) one night	$100x\frac{\mathrm{B}_{I,ab}}{\mathrm{B}_\mathrm{I}}$
$\pi_{O,ab}$	Percentage of residual bites received by a person outdoors (O) after getting up (ab= after bed) during one night	$100x\frac{\mathrm{B}_{O,ab}}{\mathrm{B}_\mathrm{I}}$

$\pi_{Op,bb}$	Percentage of residual bites received by a person indoors (I) before going to bed (bb) during one night, assuming bednet use	$100x\frac{\mathrm{B}_{O,bb}}{\mathrm{B}_{Ip}}$
$\pi_{Ip,bb}$	Percentage of residual bites received by a person indoors (I) before going to bed (bb) during one night, assuming bednet use	$100x\frac{\mathrm{B}_{I,bb}}{\mathrm{B}_{Ip}}$
$\pi_{Ip,b}$	Percentage of residual bites received by a person indoors (I) while in bed (b) during one night, assuming bednet use	$100x\frac{\mathrm{B}_{I,b}}{\mathrm{B}_{Ip}}$
$\pi_{Ip,ab}$	Percentage of residual bites received by a person indoors (I) after getting up (ab=after bed) during one night, assuming bednet use	$100x\frac{\mathrm{B}_{I,ab}}{\mathrm{B}_{Ip}}$
$\pi_{Op,ab}$	Percentage of residual bites received by a person outdoors (O) after getting up (ab=after bed) during one night, assuming bednet use	$100x\frac{\mathrm{B}_{O,ab}}{\mathrm{B}_{Ip}}$
$\pi_{r,low}$	Percentage of residual bites (r) received by participants during the low transmission season (low)	$\frac{\sum_{p=1}^{p=n\,(low\ trasmision)} B}{\sum_{p=1}^{p=n\,(high\ trasmision)} B + \sum_{p=1}^{p=n\,(low\ trasmision)} B}$
$\pi_{ur,low}$	Percentage of unavertable residual (ur) bites that participants would have received in the low transmission season if all would have used the net to sleep	$\frac{\sum_{p=1}^{p=n\,(low\ trasmision)} \mathrm{B}_p}{\sum_{p=1}^{p=n\,(high\ trasmision)} \mathrm{B}_p + \sum_{p=1}^{p=n\,(low\ trasmision)} \mathrm{B}_p}$
$P_{S,C}^{*}$	Percentage of residual bites that LLIN prevented in the population at observed levels of bednet use	$100x(1-\frac{\sum_{p=1}^{p=n} B}{\sum_{p=1}^{p=n} B_u})$

P_S^*	Percentage of bites that LLINs could prevent to a participant if he/she would have used the net to sleep	$100x(1-\frac{B_p}{B_u})$

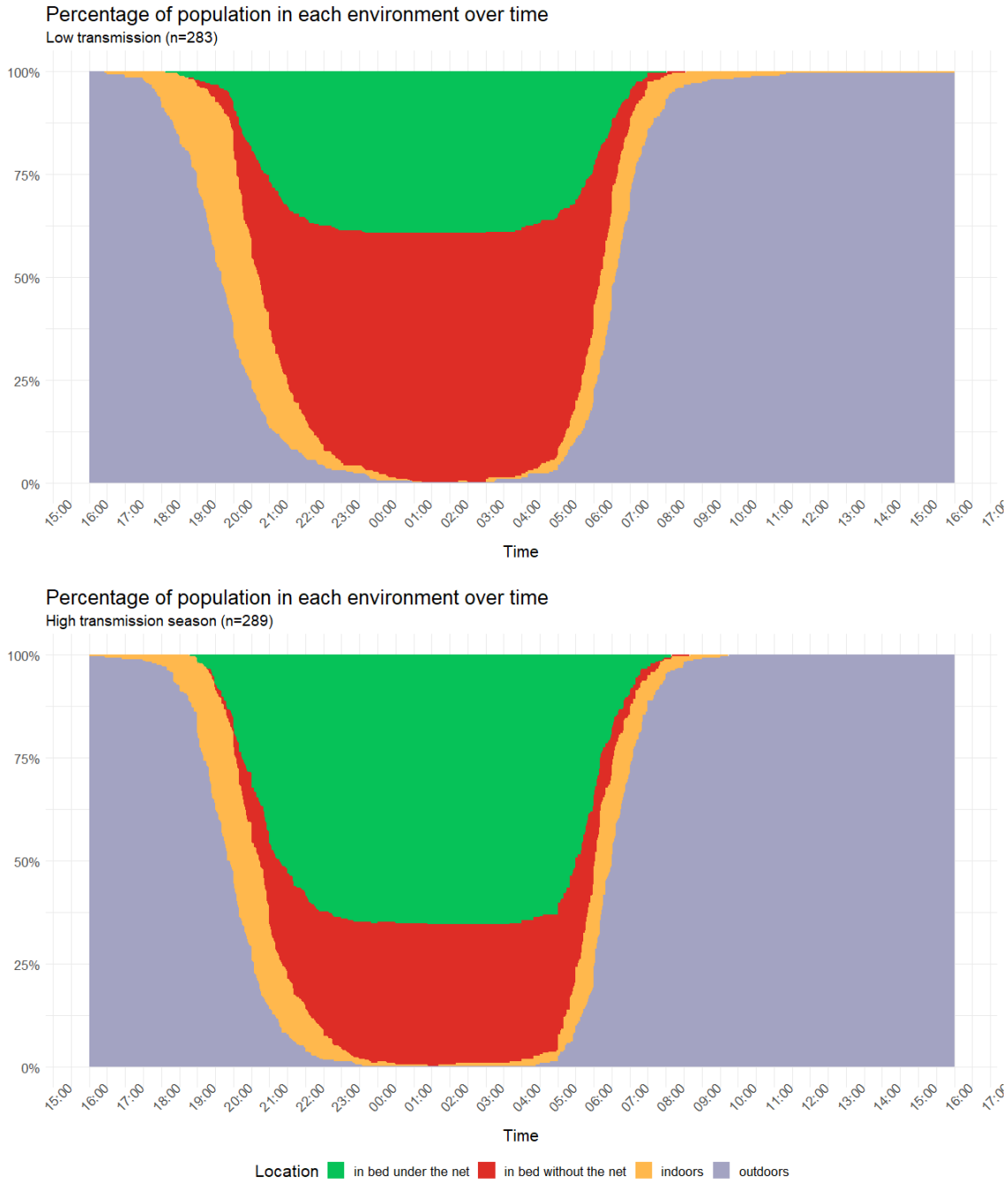
S3 Box: Exclusions criteria for mosquito surveillance data

S4 Fig. Distribution of individually calculated P_S^*. This is the maximum percentage of exposure to host-seeking mosquitoes that LLINs could have prevented for each individual (through personal protection) if they would have used the net while in bed.



S5 Fig. Location of study participants during the evening, night and morning. Percentage of participants that were outdoors (grey area), indoors but not in bed (yellow), indoors in bed using an LLIN (green) and indoors in bed but not using an LLIN (red) during the low transmission

(top panel) and high transmission season (bottom panel), including human behavioral data recorded after 8am.



S6 Structured questionnaire to assess human behaviors. (questionnaires are not included in this thesis, they will be publicly available when the articles are published)

S7 Human behavior dataset. (data is not provided inside of this thesis, it will be publicly available when the paper is
published)

S8 Mosquito surveillance dataset. (data is not provided inside of this thesis, it will be publicly available when the paper is
published)

Article 4 (Accepted for publication) **Fernández Montoya L,** Marti-Soler H, Maquina M, Comiche K, Cumaba I, Alafo C et al. The mosquito vectors that sustained malaria transmission during the Magude project despite the combined deployment of indoor residual spraying, insecticide-treated nets and mass-drug administration.

Objectives addressed:

- Objective 1: To identify and describe the vectors that transmitted malaria during the Magude project and their relative important in malaria transmission.
- Objective 2: To evaluate whether local malaria vectors were amenable to the implemented vector control tools during the project
- Objective 8: To assess, from an entomological perspective, whether the deployment of IRS and ITN together added value compared to the deployment of one intervention alone.

The mosquito vectors that sustained malaria transmission during the Magude project despite the combined deployment of indoor residual spraying, insecticide-treated nets and mass-drug administration.

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Abstract

The “Magude project” aimed but failed to interrupt local malaria transmission in Magude district, southern Mozambique, by using a comprehensive package of interventions, including indoor residual spraying (IRS), pyrethroid-only long-lasting insecticide treated nets (LLINs) and mass-drug administration (MDA). Here we present detailed information on the vector species that sustained malaria transmission, their association with malaria incidence and behaviors, and their amenability to the implemented control interventions. Mosquitoes were collected monthly between May 2015 and October 2017 in six sentinel sites in Magude district, using CDC light traps both indoors and outdoors. *Anopheles arabiensis* was the main vector during the project, while *An. funestus s.s., An. merus, An. parensis* and *An. squamosus* likely played a secondary role. The latter two species have never previously been found positive for *Plasmodium falciparum* in southern Mozambique. The intervention package successfully reduced vector sporozoite rates in all species throughout the project. IRS was effective in controlling *An. funestus s.s.* and *An. parensis*, which virtually disappeared after its first implementation, but less effective at controlling *An. arabiensis*. Despite suboptimal use, LLINs likely provided significant protection against *An. arabiensis* and *An. merus* that sought their host largely indoors when people where in bed. Adding IRS on top of LLINs and MDA likely added value to the control of malaria vectors during the Magude project. Future malaria elimination attempts in the area could benefit from i) increasing the use of LLINs, ii) using longer-lasting IRS products to counteract the increase in vector densities observed towards the end of the high transmission season, and iii) a higher coverage with MDA to reduce the likelihood of human infection. However, additional interventions targeting vectors that survive IRS and LLINs by biting outdoors or indoors before people go to bed, will be likely needed to achieve local malaria elimination.

Introduction

Despite the remarkable reductions in the malaria burden in sub-Saharan Africa over the last two decades [1,2], no country in this region has managed to eliminate malaria. The lowest malaria burden of all sub-Saharan Africa is observed in its southern part, namely in Namibia, Botswana, South Africa and eSwatini. In 2016, the World Health Organization (WHO) determined that eSwatini and South Africa had the potential to achieve zero indigenous cases by 2020. Albeit several regional malaria elimination efforts over the last few years [3,4], neither country was able to reach this target [5]. The importation of malaria cases from neighboring Mozambique, a country with considerably higher malaria transmission levels, has been highlighted as one of the causes [6]. In South Africa and eSwatini, malaria transmission is primarily concentrated in areas bordering Mozambique [5] and is driven by cases among migrant populations. Reducing or eliminating malaria in Mozambique, especially in its southern provinces, is therefore crucial to achieve malaria elimination in Southern African and eSwatini. The southern part of Mozambique has been targeted by initiatives to stop malaria transmission since the 1960's, but, unfortunately, none have led to local malaria elimination [6–9].

The first attempt to eliminate malaria in southern Mozambique took place between 1960 and 1969 in the context of the Global Malaria Eradication Program (GMEP) [7]. The second initiative, the Lubombo Spatial Development Initiative (LSDI), was implemented between 1999 to 2011 [8–10]. Both were based on indoor residual spraying (IRS), which aims to kill mosquitoes resting on walls and ceilings with insecticides, although the second elimination attempt combined IRS with targeting the parasite reservoir using artemisinin combination therapies (ACTs). More recently, in 2015, the Mozambique, South Africa and eSwatini (MOSASWA) regional initiative [6] and the Mozambican Alliance Towards the Elimination of Malaria (MALTEM) [9] were established. MOSASWA aimed to strengthen regional collaboration and efforts to accelerate progress towards achieving malaria elimination in the region. MALTEM aimed, among other objectives, to create the necessary knowledge to inform an operational elimination plan

and roadmap for malaria elimination in Mozambique [9], which was piloted during the Magude project.

The Magude project was designed to evaluate the feasibility of eliminating malaria in southern Mozambique with a package of interventions available at the time, namely a combination of interventions targeting the vector (IRS and long-lasting insecticidal nets, or LLINs) and the parasite reservoir (mass drug administration, or MDA, and standard diagnosis and treatment) simultaneously. In addition, it implemented strong community engagement campaigns to maximize the acceptance and coverage of all interventions. The project was expected to reduce vector densities with a combination of the killing effects from IRS and LLINs, and then reduce transmission by surviving infectious vectors through the prophylactic effect of the MDA drugs alongside the prevention of vector-human contact by LLINs, thereby closing the gap towards elimination [9].

While all of the initiatives listed above were successful at reducing the local burden of malaria [6–8], none of them achieved malaria elimination. Learning from past experiences is critical to guide future malaria elimination efforts in Mozambique and hence, to achieve elimination in the region. All these initiatives relied heavily on vector control, as do current and future malaria control efforts [11]. Therefore, 1) identifying the vectors that sustained malaria transmission despite the implemented vector control interventions and 2) evaluating the vectors' amenability to the implemented vector control products, are crucial to understanding the shortcomings of the piloted approaches and, hence, to guide the design of future malaria elimination efforts in southern Africa.

The outcome of the two aims above are presented here. We first describe anopheline species composition, densities, host-seeking behavior (time and place) and *P. falciparum* sporozoite rates during the course of the project. We then evaluate relative vector importance by exploring the association between densities of different vector species and malaria incidence, accounting for the implemented interventions. We subsequently combine these findings with

previously published data on i) the efficacy of the three core interventions, LLINs, IRS and MDA [12], ii) susceptibility of the vectors to the used insecticides, and iii) the overlap between human and vector behaviors, to examine the ability of the implemented interventions to control the local vector populations. Finally, we use our new understanding to provide vector control recommendation for future malaria elimination efforts in the area.

Materials and methods

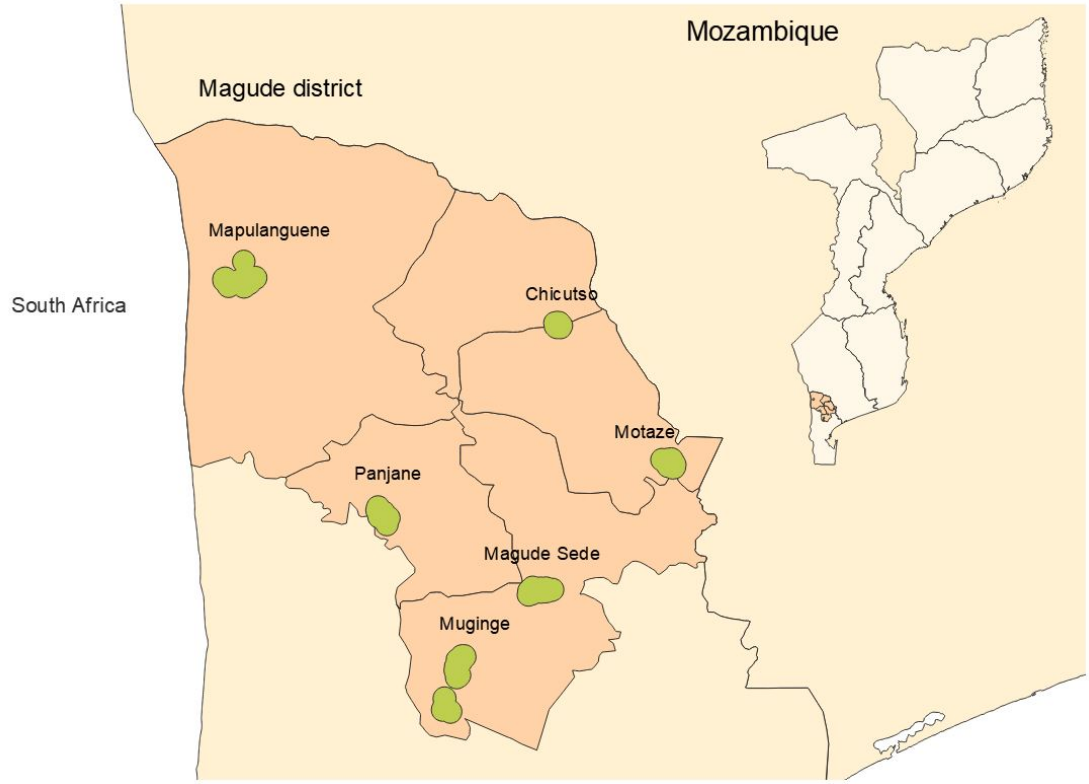
The Magude project in the Magude district

Magude district is a rural district located in Maputo province (southern Mozambique) that borders with Bilene district to the north and with Mpumalanga Province in South Africa to the West (Fig 1). The district has an area of 6,961 km^2 and is divided into five administrative posts, Magude Sede, Motaze, Panjane, Mahele and Mapulanguene. It had 48,448 residents in 2015 [13]. The vegetation is dominated by open forests and savannahs, and three main rivers cross the district. Most of the population relies on subsistence agriculture, fishing or working as sugar cane cutters in the sugar plantations in Magude, Xinavane or the neighboring Manhiça district. Houses are traditional round-shaped or rectangular-shaped huts constructed with cane, cement, mud brick or reeds and covered by adobe or cement. A comprehensive description of the district demographic, socio-economic and health characteristics, is provided elsewhere [13]. Two distinct climatological seasons are observed in southern Mozambique, a rainy season extending from October to March and a dry season from April to September. The high malaria incidence season occurs from November to April [13].

The Magude project started in 2015 by establishing a health and demographic platform to obtain the necessary information to guide the implementation of the core interventions during the project [13]. The district was covered (i.e. one net for every two people in a household) with LLINs that were distributed by the National Malaria Control Program (NMCP) through a mass distribution campaign in May 2014. Between August 2015 and December 2017, the project delivered an annual single-round of IRS with DDT and pirimiphos-methyl in the first year and only pirimiphos-methyl in the second and third year, and two annual rounds of MDA that were one

month apart. During each MDA round, the de-facto population of Magude (including visitors but excluding infants <6 months, women in the first trimester of pregnancy or severely ill individuals) received a full 3-day course of dihydroartemisinin–piperaquine (DHAp). Community mobilization campaigns were implemented to increase the uptake of the interventions. In parallel, the NMCP provided diagnosis (HRP2-based RDT and microscopy) and treatment (artemether–lumefantrine as first line drug for clinical cases) to patients presenting at health facilities or community health workers. To monitor malaria incidence and prevalence, the Magude project established a rapid case reporting system and conducted annual cross-sectional malaria prevalence surveys [9,12]. The epidemiological results of the project, including information on the MDA campaigns, have been published elsewhere [12], as well as detailed information on the implementation and coverage of IRS [14], access and use of LLINs [15], the susceptibility of the main local vector species to insecticides [14] and a detailed evaluation of the overlap between human and mosquito behaviors [16]. This paper combines novel and detailed data on mosquito bionomics with those data that are analyzed previously to improve our understanding of the effectiveness of the project's approach.

Fig 1. Map of Magude district highlighting the administrative posts and sentinel sites for entomological surveillance. Dots represent the houses where mosquitoes were collected. Map borders were obtained from the Humanitarian Data Exchange under license "Creative commons attribution for Intergovernmental organizations. (CC-BY-IGO). https://data.humdata.org/faqs/licenses



Entomological surveillance design

Mosquitoes were collected monthly between May 2015 and October 2017 in six sentinel sites in Magude district (Fig 1): Magude Sede, Muginge, Panjane, Mapulanguene, Chicutso and Motaze (Fig 2). These six sentinel sites were selected to represent the range of environmental and land use characteristics of the district.

In each sentinel site, mosquitoes were collected in fifteen households during two consecutive nights. They were collected indoors in 10 households and outdoors in another 5 households using CDC miniature light-trap (Model 512, John W Hock, Florida, USA). A Collection Bottle Rotator (Model 1512, John W Hock, Florida, USA) was added to six traps (three indoors and three outdoors, every night) to assess the time of mosquito host-seeking activity. The same houses were visited every month, but each month they were randomly assigned a trap type (i.e. CDC-light trap with or without a rotator) and a collection location (indoor or outdoor). Indoors, the CDC light-trap was hung at the foot-end of a bed with the trap opening approx. 1.5m above

the ground. One or two adult (>15 years old) volunteers from the selected household were asked to sleep in the bed under an LLIN during the night. Participants not owning a net were provided with a WHO-pre-qualified pyrethroid-only LLIN. Outdoors, CDC light traps were baited with a BG-Lure cartridge (Biogents AG, Germany) and CO_2 (generated through a mixture of 10g commercially available yeast (Instant yeast, Smart Chef), 100g white refined household sugar and 1L of regular tap water) to mimic indoor conditions (i.e. a human sleeping next to the trap). The outdoor traps were placed in the safest possible outdoor environment: under a tree close to the house, but away from animals and children. Due to suspicion of arboviral disease transmission in Mozambique, which was later confirmed [17,18], no comparisons against human landing catches (HLC) were done. Hence, we discuss here exposure to host-seeking mosquitoes rather than providing human biting rates.

Every morning after a collection night, the team visited the house to retrieve the collected mosquitoes and to record information on the quality of the collection using a digital structured questionnaire. This served to exclude collections that did not match our inclusion criteria (listed in S1). Data were collected with tablets (Huawei, Model S7-701u) using Open Data Kit. The collected mosquitoes were taken to the laboratory. *Anopheles* mosquitoes were selected and identified morphologically to species using a stereomicroscope and the keys of Gillies and Coetzee [19]. Individuals belonging to the *An. gambiae* and *An. funestus* species complex were transferred to the lab and identified to species level using the polymerase chain reaction (PCR) [20,21]. The presence of *P. falciparum* sporozoites in individual mosquito samples was analyzed through screening enzyme-linked immunosorbent assays (ELISA) conducted on mosquitoes' grinded head and thorax [22]. The presence of sporozoites of other *Plasmodium* species was not tested because *P. falciparum* is known to account for over 90% of all diagnosed malaria infections in Mozambique [23] and for almost all in the neighboring district of Manhiça [24], and because very low positivity rates were expected given the elimination context. Positive samples were confirmed through a second ELISA test. ELISA lysates were not heated before running the test and positive samples were not confirmed by PCR or gene sequencing.

Indoor resting vectors:

Indoor resting mosquitoes were not collected systematically, however, indoor resting blood-fed mosquitoes were collected for insecticide resistance monitoring purposes. Mosquitoes were collected from 6 am to 10 am using a mouth aspirator and a torch from April to September and in December of 2015, from February to August of 2016 and from August to November of 2017. A descriptive analysis is provided, as indoor resting behaviors are closely linked with the success of IRS campaigns (i.e. IRS products kill susceptible mosquitoes that rest on sprayed surfaces indoors).

Data analysis

The analysis aimed to 1) evaluate anopheline composition and densities over time; 2) quantify *P. falciparum* sporozoites per mosquito species and the impact of interventions on those sporozoite rates over time; 3) evaluate the association of different vector species with reported malaria cases, 4) evaluate the location and time of vector host-seeking activity and 5) identify the vector species that rested indoors. With these aims, the analysis makes use of the data collected through the surveillance system described in detail above as well of previously published datasets on malaria incidence, the efficacy of IRS, on ITN and MDA coverage and climatic data [12]. The results are discussed considering results from previously published analyses (see section below) to better understand the impact of the implemented interventions on local vector populations. Since the Magude project did not have a control area, nor a sufficiently long entomological baseline, this examination is mostly qualitative in nature, although we tried to quantify the impact where possible (e.g. the impact of interventions on sporozoite rates).

Evaluation of *Anopheles* composition and densities over time

We first evaluate *Anopheles* species compositions by calculating the relative abundance of each *Anopheles* species (i.e. the proportion of mosquitoes that belonged to each *Anopheles* species out of the total number of anophelines collected). We calculate each species' relative abundance for the period before the first intervention of the project (May to July 2015, before the first IRS campaign), and for the full intervention period (August 2015 to October 2017). For

the intervention period, we calculate the relative abundance of each species indoors and outdoors separately. We then calculate the ratio of mosquitoes collected indoors to those collected outdoors to evaluate the overall endophagy of each species during the intervention period. Since the number of collections indoors and outdoors per month was different, we normalized the number of mosquitoes collected indoors and outdoors in each month by dividing those numbers by the number of collections conducted indoors and outdoors, respectively, that month.

We calculate the number of host-seeking *Anopheles* mosquitoes per person per month as the mean value of the number of host-seeking *Anopheles* mosquitoes per person in each collection within that month, separated by species. The number of host-seeking *Anopheles* mosquitoes per person in each collection was collected as the number of *Anopheles* collected divided by the number of people who slept under a net next to the trap in the collection room that night. For outdoor collections, we assumed that our artificial lure mimicked a single person, and hence the number of mosquitoes that was collected is divided by 1. The number of monthly host-seeking mosquitoes per person for each *Anopheles* species from May 2015 until October 2017 is plotted alongside intervention coverage, use and/or efficacy, malaria incidence, as well as temperature and rainfall data, with the aim to visually explore potential associations between vector bionomics, interventions, climate and malaria incidence. Interventions coverage, use and efficacy data were obtained from previous publications [12,14,15]. Rainfall data were obtained from the Climate Hazards Group InfraRed Precipitation with Station data (CHIRPS). Data from every raster file per month were extracted for every household in Magude and aggregated to obtain monthly representative values [25]. Temperature data was obtained from the National Oceanic and Atmospheric Administration (NOAA) collected by the Maputo Weather Station (station ID 673410).

P. falciparum sporozoite rates and impact of interventions on these rates over time

Since very few mosquitoes were found carrying sporozoites throughout the entire project, we present the overall sporozoite rate (i.e. the number of *P. falciparum* positive mosquitoes over all mosquitoes analyzed) and the number of *Anopheles* mosquitoes of each

species that were found carrying *P. falciparum* sporozoites, separated by those that were collected indoors or outdoors. Overall sporozoite rates are subsequently provided for specific project periods related to the time of implementation of MDA and IRS, with the aim to understand the potential impact of these intervention on sporozoite rates. The impact of both MDA and IRS on sporozoite rates can already be observed two to three weeks after implementation. MDA with DHAp immediately eliminates gametocides from humans, which prevents feeding mosquitoes from ingesting gametocytes and becoming infective. The time between gametocyte ingestion and sporozoite migration to mosquito salivary glands can be two or three weeks depending on temperature. By then, a proportion of older infected mosquitoes will have died, only a few younger mosquitoes will be infected and hence sporozoite rates will be lower than before MDA. IRS immediately reduces vector densities through mortality-inducing effects, reducing parasite transmission success (from human to mosquitoes and vice versa). In addition, new mosquitoes that emerge during the two or three weeks after each IRS round are unlikely to become infected due to the lower levels of circulating parasites in the human population. As a result, sporozoite rates are expected to decrease. Since the temporal resolution of our data is monthly, we considered the following periods for sporozoite analysis to assess how both interventions may have impacted transmission: 1) prior to the first IRS campaign (May-July 2015), 2) between the start of the first IRS and the start of MDA 1 (August–October 2015), 3) during MDAs 1 and 2 (November 2015-February 2016), 4) at the end the high transmission season 2016 (March-July 2016), 5) between the start of second IRS and the start of third MDA (August to November 2016), 6) During MDAs 3 and 4 (December 2016 to March 2017), 7) at the end of the high transmission season 2017 (April to July 2017) and 8) from August to October 2017. Sporozoite rates are presented together with their 95% confidence intervals (CIs), calculated as confidence intervals of a population proportion assuming the sample meets the Central Limit Theorem (Table 1). Because we had low numbers of mosquitoes and low vector sporozoite rates, no further statistical analyses were undertaken.

Association between vector species densities and malaria incidence

To understand the relative importance of each potential vector in malaria transmission and given the fact that the number of sporozoite positive mosquitoes were too low to obtain accurate estimates of the entomological inoculation rates, the association between the number of host-seeking anophelines of each species collected per month per person and the monthly malaria incidence was explored through a negative binomial multivariate regression model. The results of this model are later combined with data on the relative vector abundance, vector behaviors and how those overlap with human behaviors, to understand which species were most likely the main malaria vectors during the project.

The model correlated monthly malaria cases, as diagnosed by RDTs, with the monthly number of mosquitoes collected per person per month of all *Anopheles* species that represented at least 1% of the total vector population. The analysis was not restricted to those vectors carrying sporozoites, as other anopheline species collected during the project are known vectors in surrounding countries [26], and the low number of mosquitoes collected of some of these species may have prevented us from detecting sporozoites in their population. Monthly LLIN use, IRS residual efficacy and MDA coverage were included in the analysis because these interventions confound the effect of vector densities on malaria cases, as they can prevent mosquito entry into houses, reduce contact between vectors and humans and reduce the proportion of infected vectors. Since our temporal resolution is a month but the effect of the implemented interventions on malaria cases can be observed within two-three weeks (given the biological cycle of malaria transmission and the vector's lifecycle) two models were fitted: one considering unlagged covariates and one considering covariates lagged one month. In addition, we fitted these two models for the entire vector surveillance period from May 2015 to October 2017 (i.e. including the baseline period May to July 2015), but also for the period August 2015 to October 2017 (intervention period only). The number of visits to a health facility was used as the offset, to account for variations in care seeking behaviors over time. Malaria incidence data were obtained from the DHIS2-based rapid case reporting system established in Magude district in January 2015 [12]. MDA coverage was assumed to be equal to the campaign coverage during the

two months that each campaign lasted and 0 for the other months. MDA coverage estimates were obtained from previously published analyses [12]. Monthly IRS efficacy estimates for the 2016 and 2017 campaigns were obtained by fitting a logistic binomial Bayesian model to the observed mosquito mortality data 24h-post exposure in WHO standard cones bioassays. More details on the collection of residual efficacy data, the residual efficacy data themselves, and the data analysis are provided elsewhere [14]. Since the residual efficacy of the products used during the 2015 IRS campaign was not monitored, we assumed that the residual efficacy of Actellic 300CS and DDT in 2015 was similar to the efficacy of Actellic 300CS alone in 2016. Similar residual efficacies for both products have been observed in other campaigns [27], and due to the overall high variability of the residual efficacy of IRS products across geographical locations [27–29], we refrained from including DDT residual efficacy data from other sites. To calculate the residual efficacy of the 2015 IRS campaign, the residual efficacy of Actellic 300CS in 2015 was adjusted for the observed pace of spraying and coverage (94.5%) of the campaign that year (following the exact same method described elsewhere for the 2016 and 2017 IRS campaigns [14]. For the models that included the baseline period (May-July 2015), the residual efficacy of the 2014 IRS with deltamethrin was considered zero, as only Motaze (that accounts for 13.5% of the Magude population) received IRS and because the optimal residual efficacy of deltamethrin has been observed to be between 3-6 months in other settings and is therefore expected to have waned by May 2015 when the project started [27]. LLIN use was measured several times during the Magude project (details on the methodology and data collection are provided elsewhere [15] and followed a seasonal pattern, which was modelled using a sinusoidal function,

$$f(x) = A \sin B(x - C) + D$$

where x is the month, A is the amplitude of the variation which we modeled as $amplitude = \frac{\max(ITN_{observed\ use}) - \min(ITN_{observed\ use})}{2}$, B is the period, which for months is $\frac{2\pi}{12}$, C was adjusted for the sinusoidal function to follow the seasonality of LLIN use and D is the minimum observed use (39.1%) plus the amplitude of the variation (the function is represented in S2). The goodness of fit was evaluated by checking the distribution and autocorrelation of the residuals. Models were compared using the Akaike Information Criterion. The model with the

lowest AIC was considered to be the best performing model, provided there was no autocorrelation in its residuals. Regression log transformed coefficients are reported together with their 95% confidence intervals. The predicted cases are shown along with true malaria cases. Detailed model results are provided in S4.

Vector host-seeking activity

We evaluated indoor and outdoor host-seeking times during the project's intervention period (August 2015 to October 2017) by calculating the number of host-seeking mosquitoes of each species collected per person for each 2 hour time interval (period of rotation of the CDC bottle rotator) from 18:00 to 06:00, before 18:00 and after 06:00, separating indoor and outdoor collected mosquitoes. Then we evaluated the composition of host-seeking vectors at the different collection time intervals by calculating the relative percentage of the total host-seeking mosquitoes per person that each species represented.

All data cleaning and analysis was conducted using R version 4.1.0.

Ethical Clearance

Ethical approval was obtained from the Manhiça Health Research Center's Institutional Bioethics Committee for Health (CIBS-CISM/043/2015 for our entomological surveillance) and local administrative authorities (52/SDSMASS/024.1). Verbal informed consent was obtained from an adult member of each household where a mosquito trap was placed indoors or outdoors. All participating households were free to withdraw from the studies at any given time. All other studies were approved by CISM's institutional ethics committee, Hospital Clinic of Barcelona's Ethics Committee, and the Mozambican Ministry of Health National Bioethics Committee. The study protocol to implement and evaluate the impact of MDAs was also approved by the pharmaceutical department of the Ministry of Health of Mozambique and registered as Clinical Trial NCT02914145. More details on the ethical considerations of the population census, household surveys, cross-sectional prevalence surveys and MDAs are provided elsewhere [12,13]

Results

Mosquito collections

A total of 5,361 trap-night collections were performed between May 2015 and October 2017. Of those, 513 collections were discarded for not complying with the inclusion criteria (S1). As a result, 4,848 trap-night collections were considered in the present analysis, 3,329 indoors (933 in CDC light traps with collection bottle rotators, and 2,396 in CDC light traps without rotators) and 1,519 outdoors (808 in CDC light traps with collection bottle rotators, and 711 in CDC light traps without). Only 18.4% of the trap-night collections yielded at least one female *Anopheles* mosquito, with 81.6% resulting in zero mosquitoes caught. A total of 4,655 *Anopheles* female mosquitoes were caught, 4,107 indoors (1,015 in CDC light traps with bottle rotators and 3,092 with CDC light traps without) and 548 outdoors (243 with CDC light traps with bottle rotators and 305 in CDC light traps without). Accounting for the differences in the number of sampling nights indoors and outdoors, these numbers indicate indoor-outdoor ratios of roughly 3.4 to 1 respectively.

Anopheline species composition and densities over time

Ninety-seven percent (97.5%, n=4,539) of all collected mosquitoes were identified morphologically. Of the indoor collected mosquitoes 1.9% (n=93) could not be identified; of the outdoor collected mosquitoes 5.9% (n=37) could not be identified because they were either too damaged or because the microscopists could not find a matching species in the dichotomous keys. Molecular identification was performed for 98% (n=3,364) of mosquitoes belonging to the *An. gambiae* complex, and 87.3% (n=332) of mosquitoes belonging to the *An. funestus* group.

Before the scale up of IRS (May-July 2015, i.e. the dry season), mosquitoes from the *An. gambiae* complex (all identified as *An. arabiensis*) accounted for 56.8% (n=225) of the *Anopheles* collected, and those from the *An. funestus* group accounted for 36.1% (n=143). Most mosquitoes from the latter species group were identified as *An. parensis* (44.8%, n=64), followed by *An. funestus s.s.* (23.8%, n=37), *An. leesoni* (1.4%, n=2) and *An. rivulorum* (4.2%, n=6). The other 23.8% (n=34) of the mosquitoes in this group could not be identified to species. *An. squamosus*

accounted for 2.3% (n=9) of the anopheline collected. Other *Anopheles* species accounted for 6.8% of the mosquito population, but less than six individuals of each of these other species were collected.

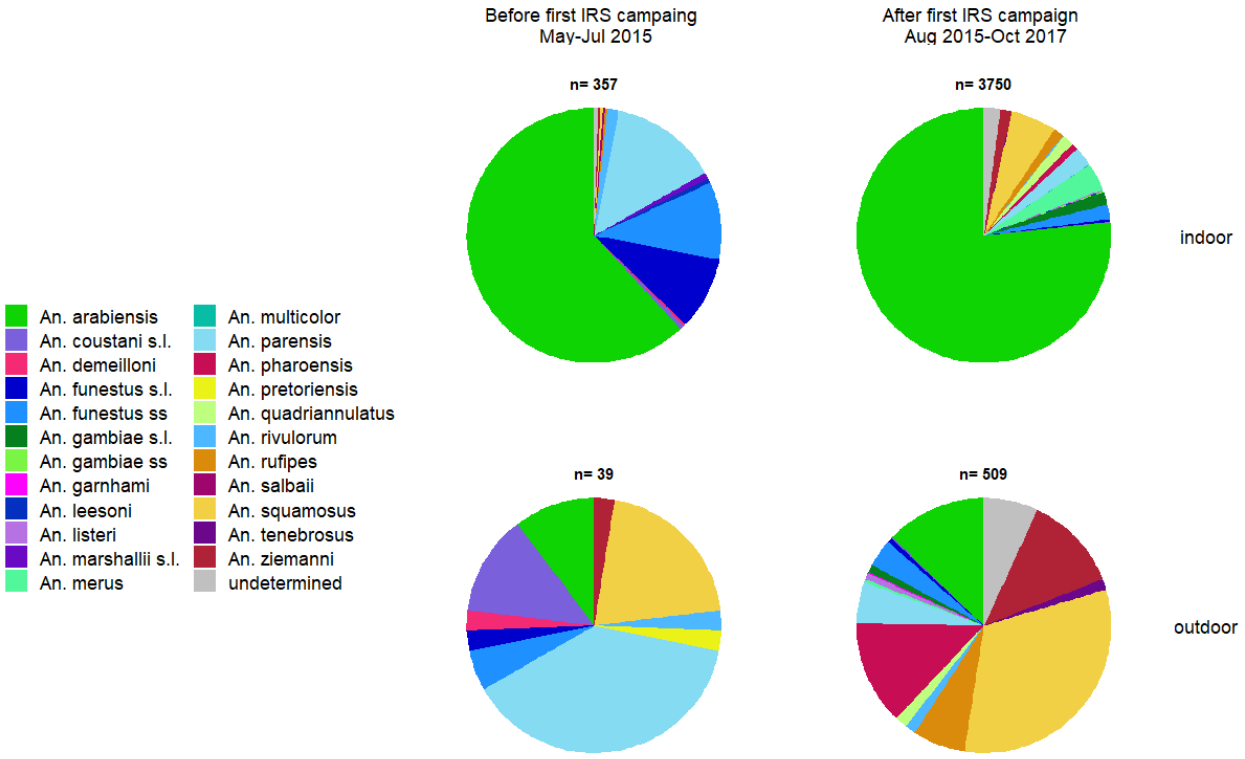
From the scale up of IRS (August 2015) onwards (i.e. intervention period), mosquitoes belonging to the *An. gambiae* complex continued to account for the majority of anophelines collected (75.3%, n=3,206). Molecular identification revealed the following composition: *An. arabiensis* (91.6%, n=2,938), *An. merus* (4.2%, n=135), *An. quadriannulatus* (2%, n=63), *An. gambiae s.s.* (0.1%, n=3) and 2.1% (n=67) could not be identified. *An. squamosus* accounted for 8.9% (n=380) of the *Anopheles* collected followed by the *An. funestus* group (5.6%, n=237). The species composition within this group was *An. parensis* (50.2%, n=119), *An. funestus s.s*. (37.6%, n=89), *An. rivulorum* (5.1%, n=12), *An. leesoni* (0.8%, n=2) and 6.5% (n=15) could not be identified. Finally, *An. ziemanni* accounted for 2.7% (n=114), *An. pharoensis* for 2.3% (n=99), *An. rufipes* for 1.9% (n=83) and several other vector species for 3.3%, with less than 12 mosquitoes of each of these other species collected.

The indoor vector composition during the intervention period was: *An. arabiensis* (76.6%, n=2,873), *An. squamosus* (5.8%, n=217), *An. merus* (3.5% , n=133), *An. parensis* (2.5%, n=92), *An. funestus s.s.* (1.9%, n=71), unidentified mosquitoes of the *An. gambiae* complex (1.7%, n=62), *An. quadriannulatus* (1.5%, n=55), *An. ziemanni* (1.4%, n=52), *An. rufipes* (1.3%, n=49), *An. pharoensis* (0.9%, n=32), unidentified mosquitoes of the *An. funestus* group (0.3%, n=12), *An. listeri* (0.2%, n=6), *An rivulorum* (0.1%, n=5), *An. coustani s.l.* (0.1%, n=4), *An. gambiae s.s.* (0.1%, n=3), *An. leesoni* (0.1%, n=2), *An. tenebrosus* (0.1%, n=2), *An. multicolor* (0%, n=1) and 2.1% (n=79) could not be identified (Fig 2).

The outdoor vector composition during the intervention period was: *An. squamosus* (32%, n=163), *An. pharoensis* (13.2%, n=67), *An. arabiensis* (12.8%, n=65), *An. ziemanni* (12.2%, n=62), *An. rufipes* (6.7%, n=34), *An. parensis* (5.3% , n=27), *An. funestus s.s*. (3.5% , n=18), *An. quadriannulatus* (1.6% , n=8), *An. rivulorum* (1.4% , n=7), *An. tenebrosus* (1.4% , n=7), unidentified

mosquitoes of the *An. gambiae* complex (1.0% , n=5), *An. listeri* (0.8% , n=4), unidentified mosquitoes of the *An. funestus* group (0.6%, n=3), *An. merus* (0.4%, n=2), *An. coustani s.l*. (0.2%, n=1), *An. garnhami* (0.2%, n=1) and 6.9% (n=34) could not be identified (Fig 2).

Fig 2. Indoor and outdoor anopheline species composition before and after the implementation of the first IRS campaign during the Magude project.



After normalizing the total number of mosquitoes collected indoors and outdoors by the number of collection nights indoors and outdoors, respectively, the ratio indoor to outdoor collected mosquitoes for the different species during the intervention period was as follows: *An. arabiensis* (20.0 indoors to 1 outdoors), *An. merus* (30.1:1), *An. quandriannulatus* (3.1:1), *An. coustani s.l.* (1.8:1), *An. funestus s.s.* (1.8:1), *An. parensis* (1.5:1), *An. listeri* (0.7:1), *An. rufipes* (0.7:1), *An. squamosus* (0.6:1), *An. ziemanni* (0.4: 1), *An. rivulorum* (0.3:1), *An. pharoensis* (0.2: 1) and *An. tenebrosus* (0.1:1). This suggests that *An. arabiensis* and *An. merus* were highly endophagic, that *An. coustani s.l., An. funestus s.s.,* and *An. parensis* were slightly more

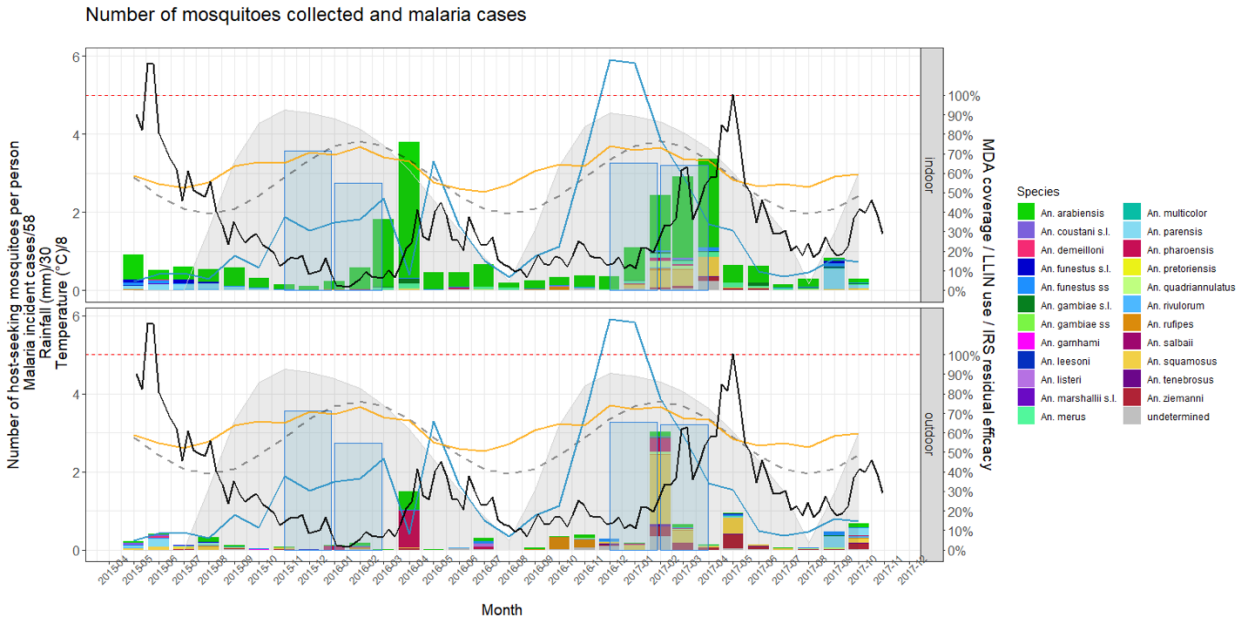
endophagic than exophagic and that *An. listeri*, *An. rufipes*, *An. squamosus*, *An. ziemanni*, *An. rivulorum*, *An. pharoensis* and *An. tenebrosus* were more exophagic.

The number of indoor host-seeking mosquitoes per person oscillated seasonally reaching a maximum in the month of April in both years, after the annual rainfall peak. More than 55% of the total host-seeking anopheline mosquitoes per person were collected during the months of February, March and April, with 27.7% of all mosquitoes being collected during the month of April (Fig 3). The greatest species richness was observed during the first half of 2017 (Fig 3), following the highest rainfall, and preceding the highest malaria incidence observed during the project.

Indoors, *An. arabiensis* was the predominant vector before and during the intervention period, accounting for between 48% and 100% of all host-seeking anophelines collected per person in any given month until September 2017. The proportion of host-seeking *An. funestus s.s*. and *An. parensis* decreased markedly after the first IRS campaign. *An. parensis* disappeared and was only collected again in November 2016 and from July 2017 onwards, accounting for 62.2% and 27.0% of host-seeking anophelines in September and October 2017, respectively. *Anopheles funestus s.s.* was occasionally present during the period when IRS was effective, but only accounted for 0.6% up to 12.1% of indoor host-seeking anophelines when it was found. *An. merus* was only collected after the first IRS campaign and accounted for between 1.2% and 26.0% of host-seeking anophelines when it was found. Indoor host-seeking *An. squamosus* accounted for between 0.2% and 4.0% of host-seeking anophelines before and after the first IRS campaign until February 2017, when it accounted for 17.5%. Its relative presence remained high in subsequent months (14.5% and 14.9% in March and April 2017, respectively). *An. ziemanni* was only present in larger numbers in 2017, but still accounted for less than 5% of the vectors collected indoors until May and June 2017, when its relative abundance increased to 7.2% and 7.8%, respectively (Fig 3).

Outdoors, different species dominated in different months. There was no single species that predominated over time during the intervention period, and several species were only collected outdoors in specific months. Two peaks in the number of outdoor host-seeking anophelines per person were observed. The first peak was in April 2016, the second in February 2017, with 15.1% and 31.0% of all mosquitoes collected after the first IRS campaign, respectively. The peak in April 2016 was dominated by *An. pharoensis* and preceded an increase in malaria cases. The peak in February 2017 was dominated by host-seeking *An. squamous* and was also followed by an increase in malaria cases. Outdoor host-seeking *An. arabiensis* were collected outdoors throughout the project. Outdoor host-seeking *An. parensis* accounted for a high proportion of outdoor host-seeking anophelines before the first IRS campaign (17.6% to 55.5%) and were absent until September and October 2017, when they accounted for 61.9% and 27.8%, respectively. Outdoor host-seeking *An. funestus s.s.* were sporadically collected throughout the study. Outdoor host-seeking *An. squamosus* accounted for between 15.4% and 37.5% of all outdoor host-seeking *Anopheles* before the first IRS campaign, but were mostly absent until December 2016. Between December 2016 and July 2017, they accounted for between 18.1% and 66.6% of the monthly outdoor host-seeking *Anopheles* collected. *An. ziemanni* was absent from outdoor collections from the first implementation of IRS until December 2016, when its relative abundance increased up to 42.8% in June 2017, preceding an increase in malaria incidence, and remained substantial until the end of the implementation period (October 2017) (Fig 3).

Fig 3. Anopheline densities over time during the Magude project in relation to malaria cases, climate data, and relevant malaria control intervention indicators. Number of anopheline mosquitoes collected (per person per month, colored bars), malaria cases (black line), modeled LLIN use over time (%, dashed grey line), modeled IRS efficacy over time (%, grey shaded areas), MDA coverage (%, blue shaded areas), amount of rainfall (mm, blue line) and temperature (°C, orange line).



Sporozoite rates

The presence/absence of *Plasmodium falciparum* sporozoites was determined for 3,656 specimens (78.5% of all mosquitoes collected). A total of 37 (0.8%) mosquitoes were sporozoite positive; 35 collected indoors and two collected outdoors. These belonged to five species: *An. arabiensis* (32 positive of 3,052 tested, 1%), *An. merus* (1/128, 0.8%), *An. parensis* (1/111, 0.9%), *An. funestus s.s.* (1/101, 1%), *An. squamosus* (1/17, 5.9%), and one unidentified mosquito from the *An. gambiae* complex (1/62, 1.6%). Of the 59 *An. quadriannulatus*, 46 *An. pharoensis*, 15 *An. rivulorum*, 8 *An. rufipes*, 6 *An. coustani s.l.*, 5 *An. ziemanni,* 3 *An. gambiae s.s.,* 3 *An. marshallii* complex, 2 *An. leesoni*, 1 *An. demeilloni*, 1 *An. garnhami*, 1 *An. pretoriensis* and 1 *An. tenebrosus* analyzed, none were positive. None of the *An. listeria*, *An. multicolor* or *An. salbaii* were tested for sporozoites.

Indoors, sporozoite rates were 1% for *An. arabiensis* (31/2987), 0.8% for *An. merus* (1/126), 1.1% for *An. parensis* (1/87) and *An. funestus s.s.* (1/88) and 33.3% for *An. squamosus* (1/3). The two outdoor sporozoite positive mosquitoes included one *An. arabiensis* (1/65, 1.5%) and one mosquito from the *An. gambiae* complex that could not be identified to species (1/5, 20%).

Sporozoite-positive *An. arabiensis* were detected for the duration of the project while other species only tested positive sporadically. After the first MDA campaign, only *An. arabiensis* and *An. merus* were found positive for *P. falciparum*. The sporozoite rates for the five vector species during specific points in time (described in the methods and related to the timing of our malaria and vector control interventions) are shown in Table 1. More details on sporozoite rates per species and month are provide in S3.

Overall, vector sporozoite rates decreased with the implementation of IRS and the two first rounds of MDA but increased again at the end of the high transmission season of 2017 when the highest malaria incidence of the entire project was observed (Table 1). An unexpected increase in sporozoite rates was subsequently observed in August 2017 (12.5% [5.2-25.9]) and September 2017 (20.0% [6.6-44.3]) (S3) and only *An. arabiensis* and one *An. merus* mosquito were sporozoite positive during those months.

Table 1 *Plasmodium falciparum* sporozoite rates of the five *Pf* positive malaria vector species during the Magude project at distinct relevant periods defined in relation to interventions. The proportions with 95% CI, and number of positive with respect to total mosquitoes collected in the period are shown.

Species	Before first IRS campaign (May-Jul 2015)	Between the start of the first IRS and the start of MDA 1 (Aug-Oct 2015)	During MDAs 1 and 2 (Nov-Feb 2016)	End the high transmission season 2016 (Mar-Jul 2016)	Between the start of second IRS and the start of third MDA (Aug-Nov 2016)	During MDAs 3 and 4 (Dec-Mar 2017)	End the high transmission season 2017 (Apr-Jul 2017)	From Aug 2017 to October 2017
An. arabiensis	5.4% [3-9.5] (12/222)	2.9% [1.1.-6.9] (5/174)	0.0% [0-2.8] (0/168)	0.2% [0-0.7] (2/1165)	0% [0-4.2] (0/109)	0.3% [0.1-1.2] (2/655)	1.3% [0.6-2.9] (27/525)	11.8% [3.8-28.4] (4/34)

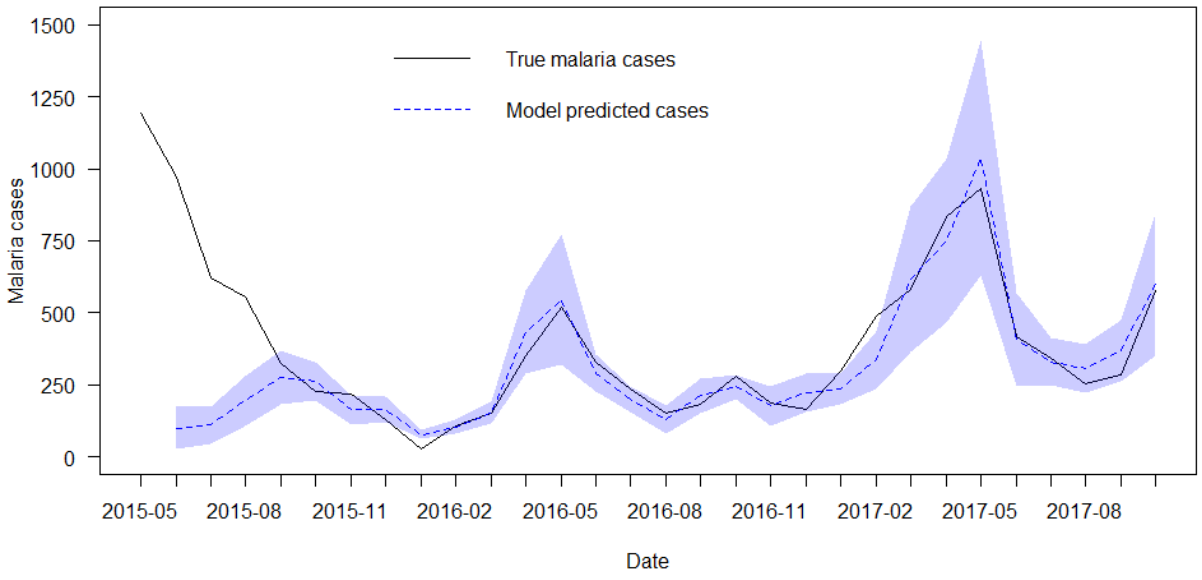
An. funestus s.s	2.7% [0.1-15.8] (1/37)	0% [0-37.1] (0/9)	Sporozoite presence not analyzed (n=3)	0.0% [0-28.3] (0/13)	0% [0-60.4] (0/4)	0.0% [0-37.1] (0/9)	0.0% [0-17.2] (0/24)	0.0% [0-53.7] (0/5)
An. parensis	1.6% [0.1-9.7] (1/63)	0% [0-12.6] (0/34)	No *An. parensis* collected	No *An. parensis* collected	0% [0-94.5] (0/1)	No *An. parensis* collected	0% [0-69] (0/3)	0.0% [0-34.5] (0/10)
An. squamosus	0% [0-40.2] (0/8)	20% [1.1-70.1] (1/5)	0% [0-80.2] (0/2)	0% [0-80.2] (0/2)	No *An. squamosus* collected	Sporozoite presence not analyzed (n=235)	Sporozoite presence not analyzed (n=121)	Sporozoite presence not analyzed (n=14)
An. merus	No *An. merus* collected	No *An. merus* collected	No *An. merus* collected	0.% [0-9.4] (0/47)	0.0% [0-94.5] (0/1)	0.0% [0-12.6] (0/36)	0.0% [0-10.7] (0/41)	20% [1.1-70.1] (1/5)
All five species combined	3.7% [2.1-6.2] (14/330)	2.5% [1-5.6] (6/222)	0% [0-2.5] (0/186)	0.2% [0-0.6] (2/1295)	0.0% [0-3.9] (0/120)	0.3% [0-1.1] (2/757)	1.3.% [0.6-2.7] (0/611)	7.5% [2.8-17.3] (5/67)

Association between host-seeking *Anopheles* per person and residual malaria incidence

Only species accounting for more than 1% of the anophelines collected were included in the analysis. Of the models used to correlate the number of host-seeking mosquitoes per person per month and per species, and controlling for MDA coverage, LLIN use and IRS efficacy, the best-performing model was the one including covariates lagged one month and which only considered the intervention period (August 2015 to October 2017). It had an AIC of 318.55 compared to 348.4, 377.2, 399.4 for the other models (detailed results from each model are presented in S4). In this best performing model, *An. arabiensis*, *An. funestus s.s*. *An. parensis, An. squamosus*, *An. merus*, *An. rufipes* and *An. quadriannulatus* were significantly associated with malaria incidence. *An. arabiensis* at <1% significance, *An. parensis* and *An. quamosus* at 1% significance, *An. funestus s.s.*, *An. merus* and *An. quadriannulatus* at 5% significance and *An. rufipes* at 10% significance.

The coefficients, reflecting the likely increase in malaria cases expected with the respective presence of each mosquito if positive, were: *An. arabiensis* 2.93 [95% CI: 2.06 - 4.19], *An. parensis* 23.4 [4.41 - 248.58], *An. squamosus* 30.21 [4.21 - 218.55], *An. funestus s.s.* 6.76×10^{-6} [3.64×10^{-10}- 0.11], *An. merus* 2.75×10^{-2} [1.14×10^{-3} - 0.74], *An. rufipes* 0.04 [1.42×10^{-3} - 1.26], *An. quadriannulatus* 4.31×10^{-7} [2.7×10^{-12} - 0.08]. Fig 4 shows the actual reported malaria cases and the cases predicted by the best performing model.

Fig 4 True malaria cases versus model predicted cases. Shaded areas represent 95% CI.

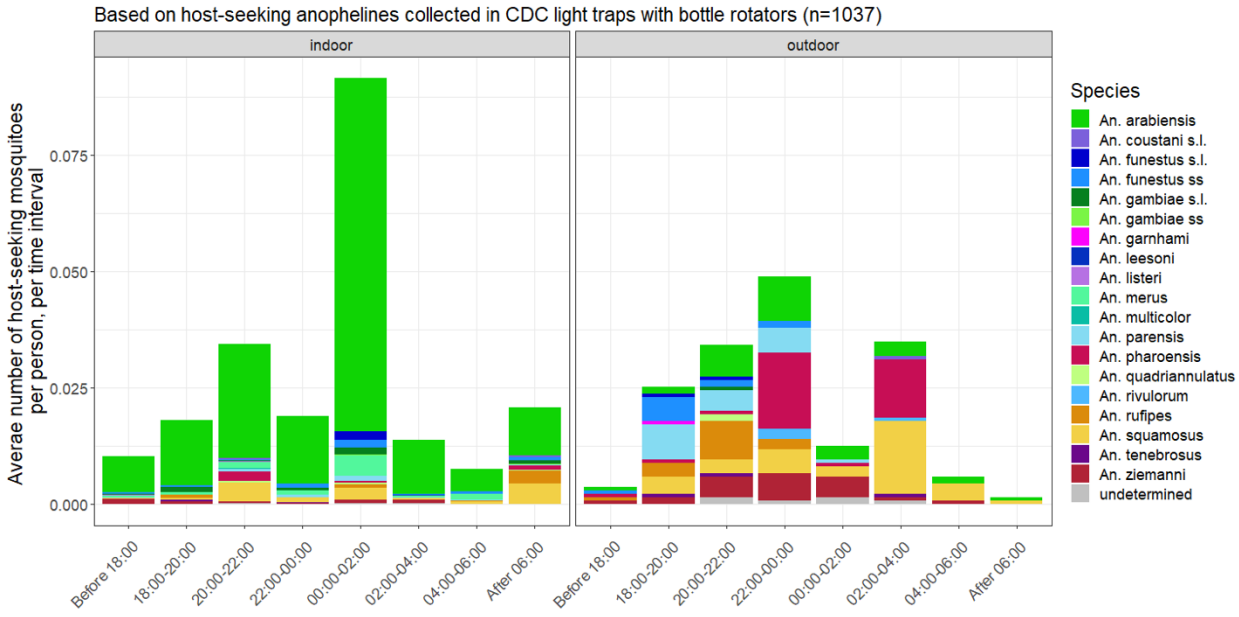


Place and time of host-seeking anopheline mosquitoes

Fig 5 shows both the composition of anopheline host-seeking mosquitoes and their densities for each 2h interval during the evening, night and early morning during the intervention period (August 2015 to October 2017). Indoors, most of the host seeking mosquitoes were *An. arabiensis*. Host seeking activity was concentrated in the evening and nighttime hours before 02:00 and showed the greatest peak between 00:00 and 02:00. Outdoors, host-seeking activity was concentrated between 18:00 and 00:00 and between 02:00 and 04:00. Early hours saw a marked presence of host-seeking mosquitoes of the *An. funestus* group and *An. squamosus*

mosquitoes. The peak between 02:00 to 04:00 was dominated by *An. squamosus* and *An. pharoensis*.

Fig 5 Density of host-seeking mosquitoes over different time intervals during the evening, night and early morning.



Identification of indoor resting vectors

A total of 1,042 blood-fed *An. funestus s.l.* mosquitoes were collected indoors in 2015, before the first IRS campaign was implemented. Very few mosquitoes of this group (insufficient for insecticide susceptibility testing) were collected indoors during the years following the first IRS campaign. Blood-fed mosquitoes of the *An. gambiae* complex were found resting indoors in sufficient numbers for resistance testing during the entire intervention period (1,024 in 2015, 3,753 in 2016 and 508 in 2017). A subset of *An. funestus s.l.* mosquitoes collected indoors in 2015 were identified, and the following species detected: *An. parensis*, *An. funestus s.s., An. rivolorum* and *An. vaneedeni.* Among the *An. gambiae* complex, most individuals were *An. arabiensis*, a few were *An. merus.* Although indoor resting behavior was not assessed through pyrethrum spray catches or similar methods, these data suggest that a part of the population of members of the *An. funestus* group and of *An. arabiensis* may rest indoors during a part of their gonotrophic cycle.

Discussion

The present study aimed to i) identify the anopheline species that sustained malaria transmission during the Magude project, ii) qualitatively evaluate the impact of the interventions on those species, and iii) identify the potential gaps in vector control during the project, to provide recommendations for future malaria elimination efforts in the area.

We believe that five species sustained transmission during the Magude project - *An. arabiensis, An. merus, An. funestus s.s., An. parensis* and *An. squamosus* - with others potentially playing a very minor role, if any. These five species were found positive for *P. falciparum* sporozoites and were significantly associated with malaria incidence. In southern Mozambique, *An. funestus s.s.* and *An. arabiensis* have historically been identified as the major malaria vectors [7,8,30]. *Anopheles merus* was first incriminated as a vector in year 2000 in Boane, located in Maputo province [31] and later during the Lubombo Spatial Development Initiative in the same province [8]. In contrast, and to our best knowledge, this is the first time that *An. squamosus* and *An. parensis* are found carrying *P. falciparum* sporozoites in southern Mozambique. *Anopheles squamosus* has been incriminated as a malaria vector in southern Zambia, but its exact role in malaria transmission in that area is unknown [32]. In 2017, *An. parensis* was identified as a minor vector species in Kwazulu-Natal, a province in South Africa that borders the southern part of Mozambique [33,34]. Although *P. falciparum* sporozoites were not detected in any of the *An. rufipes* (8 analyzed out of 83 identified) and *An. quadriannulatus* (59 analyzed out of 63 identified), these two species were significantly associated with malaria cases, albeit with the weakest associations and very small correlation coefficients. Although it has been demonstrated that *An. quadriannulatus* can carry *P. falciparum* sporozoites in laboratory studies [35], this species has never been incriminated as a malaria vector in nature and therefore unlikely played a role in malaria transmission during the Magude project. *Anopheles rufipes,* on the other hand, is a secondary malaria vector in equatorial countries of Africa (Burkina Faso, Cameroon, Gambia, Ghana, Kenya, Mali, Nigeria, Senegal and Togo) as well as in Zambia [26,36–44]. As such, this vector could have played a minor role during the project.

Other anopheline species that were found in Magude district are known vectors of malaria in southern Mozambique, i.e. *An. tenebrosus* [45], or elsewhere in Africa, namely *An. coustani s.l* [46–49], *An. ziemanni* [50–53], *An. rivulorum* [49,54,55], *An. leesoni* [44,56–58] and *An. pharoensis* [39,49,59–61]. However, none of the few analyzed specimens of these species were found carrying *P. falciparum* sporozoites. In addition, several non-vector *Anopheles* species were collected, namely *An. demeilloni, An. garnhami, An. listeri, An. marshalli s.l., An. multicolor* and *An. salbaii.* To our knowledge, this is the first time that *An. garnhami, An. multicolor* and *An. salbaii* are collected in Mozambique [26]. Of these, only *An. garnhami* has been detected in Mozambique's neighboring countries (i.e. South Africa, Tanzania and Zimbabwe) [26]. In contrast, *An. multicolor* and *An. salbaii* have been detected in equatorial Africa, namely Niger and Sudan (*An. multicolor*) and in Ethiopia, Kenya, Niger, Somalia and Djibouti [26]. The detection of these other vector and non-vector *Anopheles* species could, however, be an artifact of wrong morphological identification resulting from damaged mosquito specimens [49,62]. Unfortunately, none of these species were molecularly identified to species (e.g. using ITS2 and COI analysis [49]) and the presence of sporozoites was not assessed for several *Anopheles* species. Confirming the presence of these species and investigating their role in persistent malaria transmission, especially of those known to be vectors in other countries of Africa, should be a priority in future studies in southern Mozambique.

Three specimens of *An. gambiae s.s.,* a very competent vector of malaria transmission in sub-Saharan Africa, were found in Magude district. Although this vector is nowadays rarely found in southern Africa, it was found resting indoors in Magude's neighboring district of Chokwe back in 2000-2002 [63] and it was collected in 2017-2018 in the neighboring's province of Limpopo, South Africa [64]. The detection of this vector could have been the result of misidentifications. In 2018, Erlank et al. [62] showed that *An. squamosus*, *An rufipes* and *An. pretoriensis* showed amplicons similar to *An. gambiae s.s*. when the PCR protocol for identification of species of the *An. gambiae* complex was applied to them. Hence, if mosquitoes of any of these three species were wrongly identified as belonging to the *An. gambiae* complex, this could have led into false

identification of *An. gambiae s.s*. The current presence of *An. gambiae s.s*. in southern Mozambique and its potential role in transmission should be further investigated. It is recommended that if this species is reported in southern Africa in the future, the species identification be confirmed with sequencing of the ITS2 or COI regions.

Although accurate estimates of sporozoite rates or entomological inoculation rates could not be established due to i) the low numbers of mosquitoes collected, and ii) the fact that the parasite reservoir was targeted by several interventions, *An. arabiensis* was most likely responsible for most of the residual malaria transmission during the Magude project. This is because it was the most abundant mosquito species, accounted for the highest proportion of human exposure to bites [16], it presented one of the strongest statistical associations with malaria incidence and it was the only vector found carrying sporozoites after the first IRS campaign, except for one *An. merus*. *Anopheles funestus s.s*. and *An. merus* may have played a minor but continuous role, based on their significant association with malaria incidence and consistent presence through the intervention period albeit with a low relative abundance. *An. squamosus* may have played a minor but more erratic role. While it was significantly associated with malaria incidence, it was only present in the months surrounding the observed high malaria incidence peak. *An. parensis* may have played a role sporadically. Despite its significant association with malaria incidence, it disappeared after the implementation of the first IRS campaign and practically didn't appear again until July 2017. If other anopheline species contributed to residual malaria transmission, such as the two other vectors that were significantly associated with malaria incidence (*An. rufipes* and *An. quadriannulatus*) or those that are known vectors elsewhere, they likely also played a very minor role due to their very low relative abundances and densities. Evaluating the exact role of each species will be critical to guide vector control strategies but is cumbersome in a project like the Magude project due to the high pressure exerted on both vector (LLINs, IRS) and parasite populations (MDAs). The relative importance of each species should be ideally assessed in similar nearby areas that are not subjected to intense control interventions.

The project's interventions managed to significantly reduce sporozoite rates from 3.7% before the first IRS campaign, to 0.1%, after the first MDA campaign before the second IRS campaign started, but sporozoite rates later increased to 1.1% after the MDA rounds in year 2. These patterns are in line with the decrease in malaria prevalence observed through the cross-sectional prevalence surveys conducted in May 2015 and May 2016, which revealed that malaria prevalence went from 9.1% to 1.5% after the implementation of the first IRS campaign and the two first MDA rounds, but later increased to 2.6% in May 2017 [12]. The fluctuation in sporozoite rates and malaria prevalence shows that, although the intervention package managed to reduce transmission to very low levels during the first year, it was not further reduced after the second year despite a similar coverage of all interventions. Only LLINs may have been less effective around that time (approx. two years after the mass campaign in 2014) as their integrity and insecticide bio-efficacy is known to decrease over time [65,66]. This, alongside the heavy rains in 2017, may be a reason for the slightly increased incidence rates observed that year.

IRS was likely very effective at controlling *An. parensis* and *An. funestus s.s.* because they were susceptible to the insecticides use in IRS (DDT and pirimiphos-methyl) [14] and the numbers in our CDC light trap collections decreased dramatically after the implementation of the first IRS campaign. *An. parensis* practically disappeared for approx. 2 years, whereas *An. funestus s.s*. remained present but in very low densities. In addition, very few (insufficient numbers to conduct insecticide susceptibility assays) blood-fed *An. funestus s.s*. and its sibling species were found resting indoors after the first IRS campaign, whereas mosquitoes of that species group were abundant prior to the first round of IRS. Our analysis suggests that *An. arabiensis* was not affected as much by IRS compared to *An. parensis* and *An. funestus s.s*., even though *An. arabiensis* was also susceptible to the IRS insecticides [14]. This is because the relative abundance of *An. arabiensis* increased after IRS, it remained the predominant species throughout the project, its population managed to increase rapidly every year when rains increased and as the effect of IRS waned off, and we continued to find large enough numbers of this species indoors for our insecticide susceptibility bioassays throughout the project. Elsewhere it has been observed that *An. arabiensis* is much less affected by IRS with pirimiphos-methyl than other important vector

species, such as *An. funestus s.s.* [67], possibly due to the fact that a proportion of its population rests outdoors after feeding avoiding contact with sprayed wall surfaces [68–70]. Actually, following intense implementation of IRS in southern Africa over the last eight decades, *An. arabiensis* has become the main malaria vector in South Africa and eSwatini [4,71]. Nonetheless, IRS presumably limited its population growth, as densities are expected to increase after the rains (because this vector readily breeds in small, temporary and shallow pools [72]), yet the population remained at similar densities after the heavy rains in 2017, compared to the drier preceding year. The effect of IRS on *An. merus* cannot be analyzed, as no mosquitoes of this species were collected before the first IRS campaign. However, studies conducted in Mozambique during the Global Malaria Eradication Campaign (1960-1969) showed that *An. merus* entered houses to feed, but rested outdoors, thereby avoiding contact with IRS [7]. A similar behavior is expected in Magude district, as very few *An. merus* (insufficient numbers for the insecticide susceptibility assays) were collected during indoor manual collections. The effect of IRS on *An. squamosus* cannot be properly examined due to their very low numbers, but the limited data suggest that it is likely little affected by IRS. The relative proportion of *An. squamosus* increased after the first IRS campaign and, although it maintained a very low relative abundance or was not detected during most months of the project, its population increased rapidly in February 2017, when the efficacy of IRS was waning but estimated to still be around 80%.

Looking at all species together, around 50% of mosquitoes were collected between February and April and *Anopheles* densities increased rapidly from January onwards, approximately five months after the start of each IRS campaign. This coincides with the duration of IRS's optimal residual efficacy, which was estimated to be between 3.5 months and 5.5 months, when considering mosquito mortality 24h post-exposure or delayed mortalities, respectively. It seems that IRS was effective at controlling mosquitoes during the initial months after implementation (and during the start of the rainy season), but that its residual efficacy did not prevent the growth of vector populations during the entire malaria transmission season [14]. A second round of IRS or using a product with longer residual efficacy will be needed to effectively cover the high transmission season. However, since a second round of IRS is unlikely to be

operationally feasible, given costs and the logistical challenges during a rainy season, products with a longer lasting residual efficacy will be preferred.

LLINs likely provided good protection against *An. arabiensis* (both in terms of killing susceptible mosquitoes, and reducing human-vector contact), as this species was largely endophagic, active when people were already sleeping [16] and susceptible to pyrethroids [14]. Our previous analysis showed that LLINs prevented 41.8% of human exposure to *An. arabiensis* and could have prevented 67.4% if all residents would have used a LLIN to sleep. LLINs likely provided lesser protection against *An. funestus s.s.* and *An. parensis*, compared to *An. arabiensis*. These two vector species were resistant to pyrethroids [14] and nets could only prevent 21.9% and 13.9% of exposure to host seeking mosquitoes of these species, respectively [16]. Although we could not evaluate the level of pyrethroid resistance, if any, in *An. squamosus* and *An. merus*, LLINs prevented less than half of the human exposure to these vectors (32.0% and 45.4%, respectively). As shown previously, LLIN use was suboptimal, especially during the low transmission season [15]. LLIN personal protection against host seeking mosquitoes of species that were *P. falciparum* positive during the project could have increased by between 18% to 30%, depending on the vector species, if all residents would have used the net to sleep [16]. We therefore conclude that LLINs did not achieve their full protection potential during the Magude project, but that they did complement the protection provided by IRS, mainly by providing a certain level of protection against *An. arabiensis*, *An. merus* and *An. squamosus*. Improving LLIN use will be especially important for controlling the *An. arabiensis* and *An. merus* that survive IRS, as they mainly bite indoors while people are in bed.

The results presented here, combined with previous analyses [16] show that there is a proportion of vectors that either seek their host outdoors, or seek their host indoors at times when people are not yet under a net. In addition, some species could not be found resting indoors whereas others were still found resting indoors after the implementation of IRS. This highlights that there are gaps in protection by both LLINs and IRS.

The complex changes in vector composition over time, the diversity in feeding behaviors, combined with suboptimal levels of LLIN use and short IRS realized efficacy, suggest that ITNs are IRS alone would not have been sufficient to fully control local vector populations. Additional interventions and stronger community engagement campaigns would likely have helped to achieve optimal vector control. House screening, eave tubes and lethal lures [73] could tackle those vectors that seek their hosts indoors before people are under a net. In addition, larviciding or other forms of environmental management could reduce local vector populations, including those that are not affected by current adult vector control interventions. Future interventions to kill or prevent outdoor host-seeking vectors from finding their host, including repel and lure devices and attractive targeted sugar baits, may become suitable options to increase human protection outdoors during elimination efforts in southern Mozambique.

The present study has several limitations that may have hampered our ability to fully understand the vectors that sustained malaria transmission during the project, and the impact of the interventions on those vectors. First, *P. falciparum* sporozoite rates were not assessed for all mosquito species collected, and, consequently, other species may have contributed to sustaining local malaria transmission. Second, the ELISA protocol that was followed has been observed to yield false positive results in some mosquito species, especially those that exhibit zoophilic tendencies [74]. Although we ran a confirmatory test for each positive specimen, since 1) *An. arabiensis*, *An. parensis*, *An. merus* and *An. squamosus* are all known to be partially or opportunistically zoophilic and 2) the presence of sporozoites was not confirmed by PCR, some species may have been falsely identified as carrying sporozoites. Third, the low number of mosquitoes one collects during an elimination campaign due to the high pressure on the vectors (LLINs and IRS), combined with high pressure on the parasite reservoirs (MDA), hamper the possibility to reliably estimate sporozoite rates. Fourth, the human blood index was not determined for any of the vector species identified as host-seeking mosquitoes, which were mostly unfed. Understanding this is important, as the sporozoite rate combined with the preference to feed on humans and overall human biting rates determine the entomological inoculation rate, the gold standard metric to understand the relative importance of each vector

species in malaria transmission. Fifth, since our CDC light traps started collecting mosquitoes several hours before people went to bed (i.e. while there was no human bait under the net next to the trap), the number of host-seeking mosquitoes per person reported for the time interval 18:00-20:00 may be an underestimation. Sixth, the residual efficacy of DDT was not measured after the 2015 campaign, which affects the accuracy of our IRS residual efficacy estimates for this campaign. Finally, due to the absence of a sufficiently long baseline of mosquito collections prior to the implementation of the first interventions, and/or the lack of a control district monitored simultaneously, the magnitude of the effect of vector control interventions on local vector population densities could not be quantified through robust statistical models. Future projects should include a baseline of at least one year, or a representative control district, to properly and quantitatively evaluate the effectiveness of the interventions on local vector populations. This is critical to identifying and subsequently addressing the gaps in the protection offered.

Conclusions

Anopheles arabiensis was the main vector species during the Magude project. *Anopheles merus*, *An. parensis*, *An. funestus s.s.* and *An. squamosus* likely played a secondary and minor role. Further investigation into the possible role of other collected vector species (i.e. *Anopheles* species that are known secondary vectors elsewhere in Africa) is needed. The deployment of MDA and IRS, in addition to LLINs, successfully reduced vector sporozoite rates during the first year of implementation but no further reduction in sporozoite rates was observed despite similar intervention coverages in the second year. IRS most likely controlled *An. funestus s.s.* and *An. parensis* and was also effective at controlling *An. arabiensis,* but its effect was limited by its short residual efficacy that went below optimal levels (80% mosquito mortality in WHO cone bioassays) before the end of the high transmission season. Its effect on *An. merus* and *An. squamosus* could not be assessed due to low mosquito numbers but should be investigated as these species were incriminated as malaria vectors in Magude. LLINs complemented the protection provided by IRS, especially by providing protection against the indoor and late-evening host-seeking *An. arabiensis* and *An. merus* vectors. Therefore, the combination of IRS and LLIN is likely to have brought added value to the control of malaria vectors during the Magude project, compared to

the implementation of one intervention alone. However, the effect of LLINs was compromised by their suboptimal use and the pyrethroid resistance in *An. funestus s.s.* and *An. parensis* populations. Future progress towards malaria elimination could be made by increasing LLIN use and distributing dual active ingredient LLINs to prevent transmission by the *An. arabiensis* and *An. merus* that survive IRS, by sustaining IRS to maintain control of *An. funestus s.s.* and *An. parensis*, and by using longer lasting residual insecticides for IRS to prevent the increase in vector densities observed at the end of the rainy season. Additional interventions will nevertheless be needed to tackle those vectors that transmit malaria outdoors and early indoors if we are to close the gap towards malaria elimination.

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Supporting information

S1 Box: Exclusions criteria for mosquito collections

Mosquito collections meeting the following criteria were excluded from the analyses.

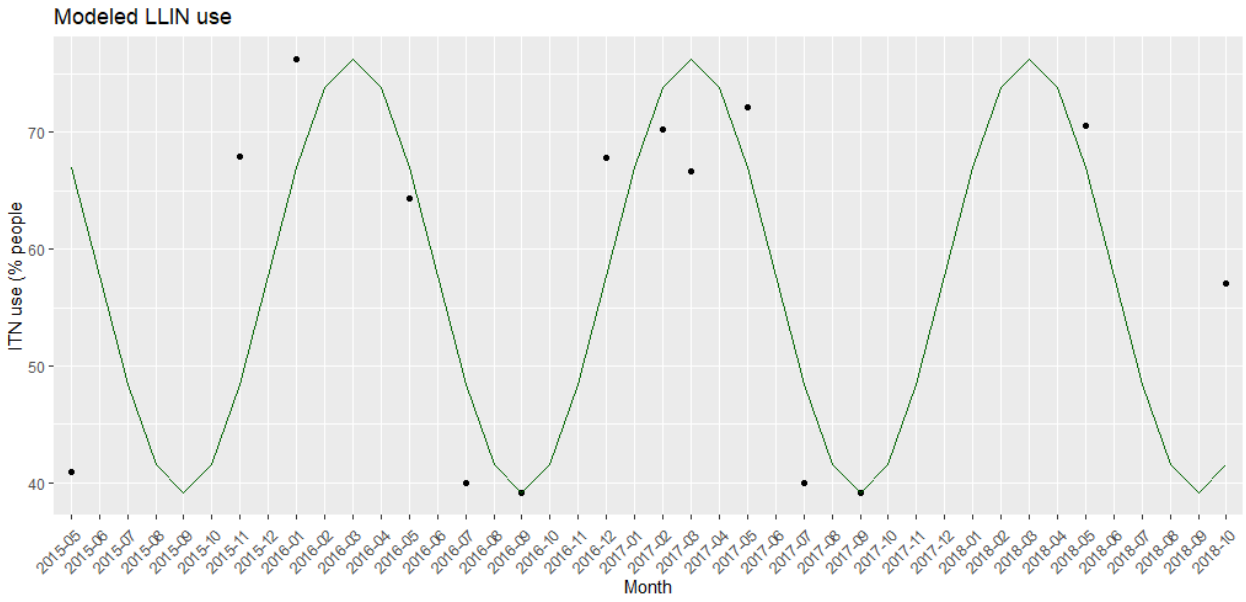
- The fan and/or light of the trap was not working;
- Collection bottle was not properly attached;
- Synthetic lure and/or artificial CO2 source not placed/connected properly.
- The household resident(s) did not sleep under their LLIN next to the trap,
- More than two people slept next to the trap;
- Ants found in a collection bottle;

- The bottle rotator had not completed all programmed rotations;
- Collections started before 4pm or extended beyond 8am.

S2 Sinusoidal function used to simulate ITN use based on observed values of ITN use

$$f(x) = A \sin B(x - C) + D$$

Where x is the month, A is the amplitude of the variation which we modeled as $amplitude = \frac{\max(ITN_{observed\ use}) - \min(ITN_{observed\ use})}{2}$, B is the period, which for months is $\frac{2\pi}{12}$, C was adjusted for the sinusoidal function to follow the seasonality of ITN use and D is the minimum observed use (39.1%) plus the amplitude of the variation.



S3 Detailed Sporozoite detection results

Table 1: Sporozoite rates per species during the entire study period (before and after intervention implementation)

Vector species	Sporozoite Negative n (%)	Sporozoite Positive n (%)	Not tested n (%)
An. arabiensis	3020 (95.5%)	32 (1%)	111 (3.5%)

An. quadriannulatus	59 (93.7%)	0 (0%)	4 (6.3%)
An. merus	127 (94.1%)	1 (0.7%)	7 (5.2%)
An. gambiae.ss	3 (100%)	0 (0%)	0 (0%)
Unidentified An..gambiae.s.l.	61 (91%)	1 (1.5%)	5 (7.5%)
An. funestus.ss	100 (79.4%)	1 (0.8%)	25 (19.8%)
An. parensis	110 (60.1%)	1 (0.5%)	72 (39.3%)
An. leesoni	2 (50%)	0 (0%)	2 (50%)
An. rivulorum	15 (83.3%)	0 (0%)	3 (16.7%)
Unidentified An..funestus.s.l.	34 (69.4%)	0 (0%)	15 (30.6%)
An. coustani	6 (50%)	0 (0%)	6 (50%)
An. demeilloni	1 (50%)	0 (0%)	1 (50%)
An. garnhami	1 (100%)	0 (0%)	0 (0%)
An. listeri	0 (0%)	0 (0%)	10 (100%)
An. marshallii	3 (100%)	0 (0%)	0 (0%)
An. multicolor	0 (0%)	0 (0%)	1 (100%)
An. pharoensis	46 (46.5%)	0 (0%)	53 (53.5%)
An. pretoriensis	1 (100%)	0 (0%)	0 (0%)
An. rufipes	8 (9.5%)	0 (0%)	76 (90.5%)
An. salbaii	0 (0%)	0 (0%)	1 (100%)
An. squamosus	16 (4.1%)	1 (0.3%)	372 (95.6%)
An. tenebrosus	1 (11.1%)	0 (0%)	8 (88.9%)
An. ziemani	5 (4.3%)	0 (0%)	111 (95.7%)

Fig 1 Monthly proportion of collected mosquitoes that underwent sporozoite detection

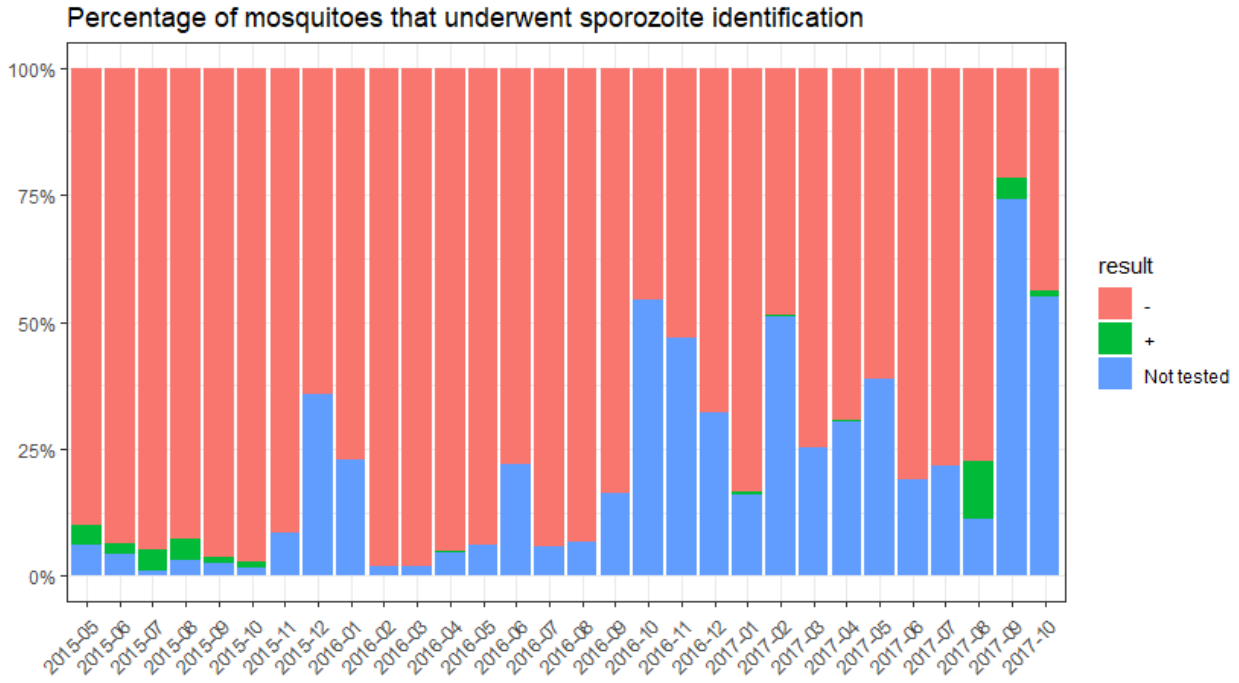


Table 2: Monthly percentage of sporozoite positive *Anopheles* mosquitos

Month	Number sporozoite positive	Number tested	Positivity rate (%)
May-15	171	7	4.09
Jun-15	91	2	2.2
Jul-15	118	5	4.24
Aug-15	94	4	4.26
Sep-15	78	1	1.28
Oct-15	68	1	1.47
Nov-15	32	0	0
Dec-15	9	0	0
Jan-16	44	0	0
Feb-16	101	0	0
Mar-16	335	0	0
Apr-16	657	2	0.3
May-16	78	0	0
Jun-16	64	0	0
Jul-16	133	0	0

Aug-16	28	0	0
Sep-16	26	0	0
Oct-16	37	0	0
Nov-16	57	0	0
Dec-16	40	0	0
Jan-17	111	1	0.9
Feb-17	236	1	0.42
Mar-17	370	0	0
Apr-17	369	1	0.27
May-17	101	0	0
Jun-17	72	0	0
Jul-17	18	0	0
Aug-17	55	7	12.73
Sep-17	26	4	15.38
Oct-17	37	1	2.7

Table 3: List of sporozoite positive *Anopheles* mosquitos

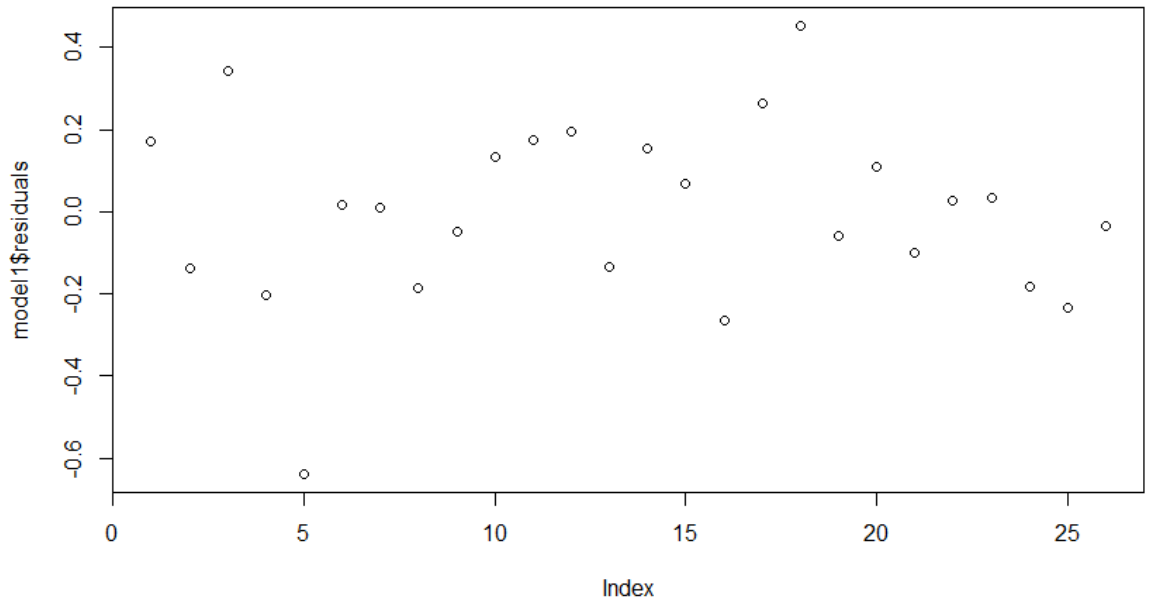
Date of mosquito collection	ELISA result	Species	Sentinel site	Trap location
2015-05-26	+	An. arabiensis	Mapulanguene	indoor
2015-05-21	+	An. arabiensis	Muginge	indoor
2015-05-21	+	An. parensis	Muginge	indoor
2015-05-19	+	An. arabiensis	Panjane	indoor
2015-05-06	+	An. arabiensis	Chicutso	indoor
2015-05-28	+	An. arabiensis	Chicutso	indoor
2015-05-28	+	An. arabiensis	Chicutso	indoor
2015-06-10	+	An. arabiensis	Motaze	indoor
2015-06-19	+	An. funestus ss	Muginge	indoor
2015-08-07	+	An. arabiensis	Magude Sede	indoor
2015-07-23	+	An. arabiensis	Muginge	indoor
2015-07-23	+	An. arabiensis	Muginge	indoor
2015-07-23	+	An. arabiensis	Muginge	indoor
2015-07-23	+	An. arabiensis	Muginge	indoor
2015-07-30	+	An. arabiensis	Chicutso	indoor
2015-08-27	+	An. arabiensis	Chicutso	indoor
2015-08-27	+	An. arabiensis	Chicutso	indoor
2015-08-27	+	An. arabiensis	Chicutso	indoor
2015-09-10	+	An. arabiensis	Chicutso	indoor

2015-10-16	+	An. squamosus	Motaze	indoor
2016-04-28	+	An. arabiensis	Muginge	indoor
2016-04-29	+	An. arabiensis	Muginge	indoor
2017-01-27	+	An. arabiensis	Muginge	indoor
2017-02-24	+	An. arabiensis	Muginge	indoor
2017-04-28	+	An. arabiensis	Muginge	indoor
2017-08-01	+	An. gambiae s.l.	Chicutso	outdoor
2017-08-02	+	An. arabiensis	Chicutso	indoor
2017-08-02	+	An. arabiensis	Chicutso	indoor
2017-08-11	+	An. arabiensis	Magude Sede	indoor
2017-09-13	+	An. arabiensis	Muginge	indoor
2017-09-13	+	An. merus	Muginge	indoor
2017-08-15	+	An. arabiensis	Muginge	indoor
2017-08-15	+	An. arabiensis	Muginge	indoor
2017-08-15	+	An. arabiensis	Muginge	indoor
2017-09-22	+	An. arabiensis	Magude Sede	indoor
2017-09-21	+	An. arabiensis	Magude Sede	indoor
2017-10-24	+	An. arabiensis	Muginge	outdoor

S4 Detailed model results

a) Best model: With covariates lagged one month and considering only the intervention period

```
Call:
glm.nb(formula = cases ~ time_factor + lag(arabiensis, +1) +
    lag(funestus, 1) + lag(parensis, 1) + lag(squamosus, +1) +
    lag(ziemanni, +1) + lag(merus, +1) + lag(rufipes, +1) + lag(quadriannulatus,
    +1) + lag(pharoensis, +1) + lag(llin_use, 1) + lag(mda_cov,
    1) + lag(irs_efficacy, 1) + offset(log(totvis)), data = data_model[which(!data_model$year_month %in%
    c("2015-05", "2015-06", "2015-07")), ], init.theta = 22.81567064,
    link = log)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-3.4792  -0.6535   0.0608   0.6545   1.8489

Coefficients:
                           Estimate Std. Error z value Pr(>|z|)
(Intercept)               -3.285962   0.347898  -9.445  < 2e-16 ***
time_factor                0.042781   0.009525   4.491 7.08e-06 ***
lag(arabiensis, +1)        1.076596   0.185339   5.809 6.29e-09 ***
lag(funestus, 1)         -11.904121   5.296617  -2.247  0.02461 *
lag(parensis, 1)           3.481200   1.090508   3.192  0.00141 **
lag(squamosus, +1)         3.408163   1.039069   3.280  0.00104 **
lag(ziemanni, +1)          0.567082   2.556487   0.222  0.82445
lag(merus, +1)            -8.199055   4.059577  -2.020  0.04342 *
lag(rufipes, +1)          -3.241006   1.762009  -1.839  0.06586 .
lag(quadriannulatus, +1) -14.655650   6.406453  -2.288  0.02216 *
lag(pharoensis, +1)       -2.135676   1.428702  -1.495  0.13496
lag(llin_use, 1)          -2.330517   0.851215  -2.738  0.00618 **
lag(mda_cov, 1)           -0.148302   0.291704  -0.508  0.61117
lag(irs_efficacy, 1)       0.631902   0.352458   1.793  0.07300 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Negative Binomial(22.8157) family taken to be 1)

    Null deviance: 178.43  on 25  degrees of freedom
Residual deviance:  28.68  on 12  degrees of freedom
  (1 observation deleted due to missingness)
AIC: 318.56

Number of Fisher Scoring iterations: 1


              Theta:  22.82
          Std. Err.:  7.51

 2 x log-likelihood:  -288.555
```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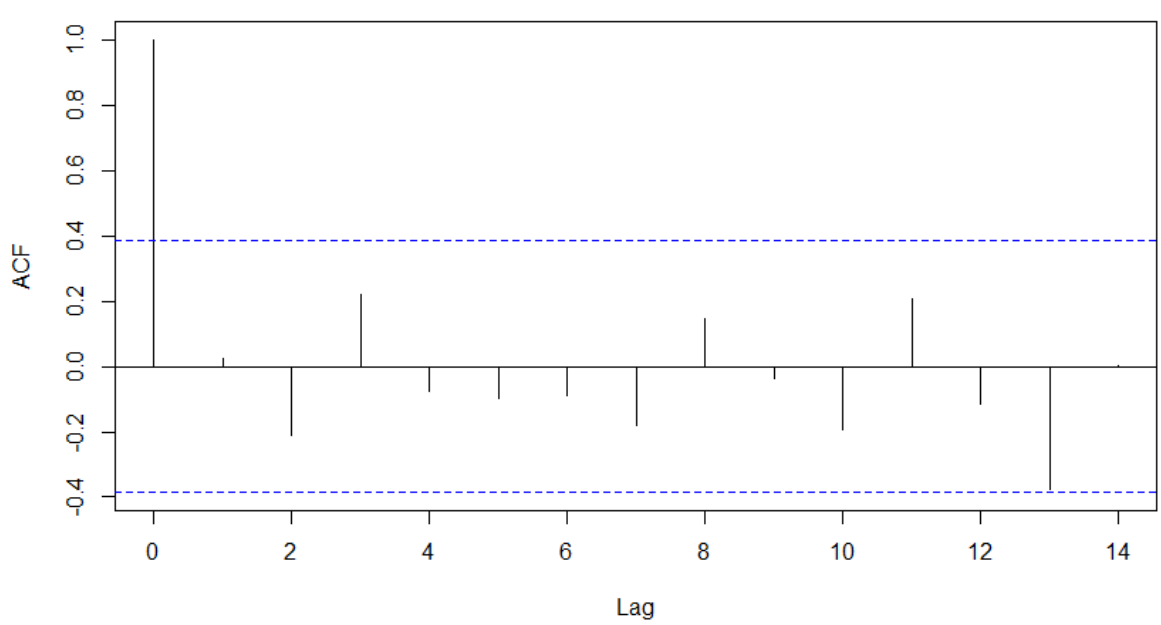
15
20
25
Index


Series model1$residuals
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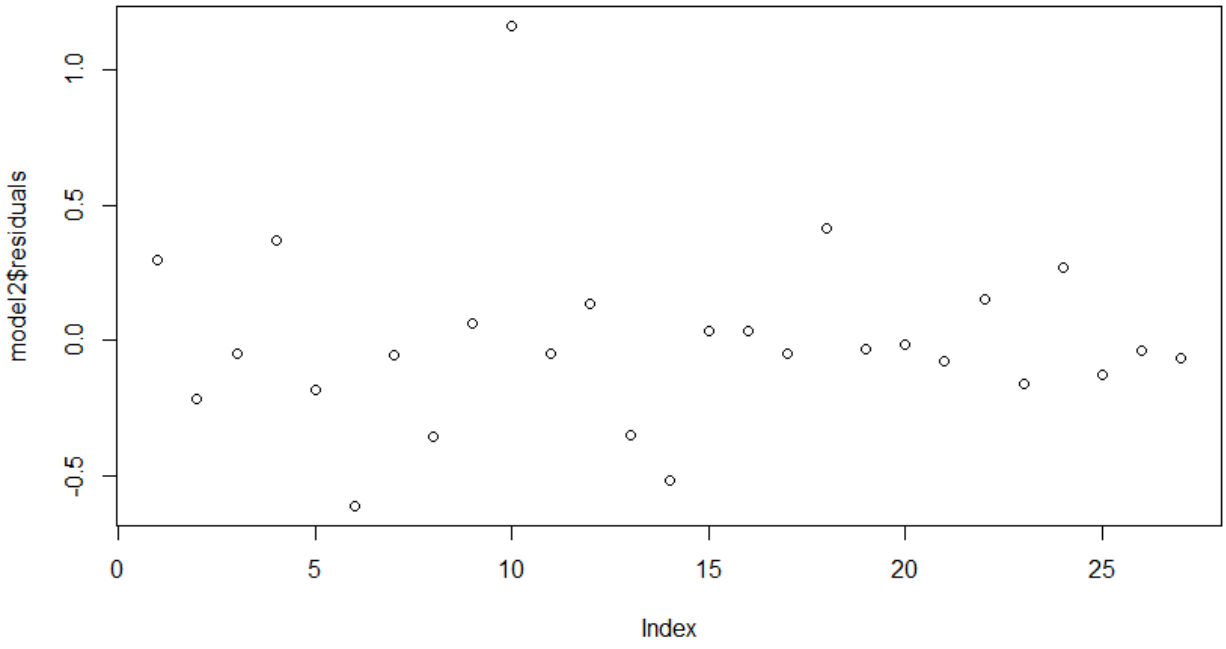
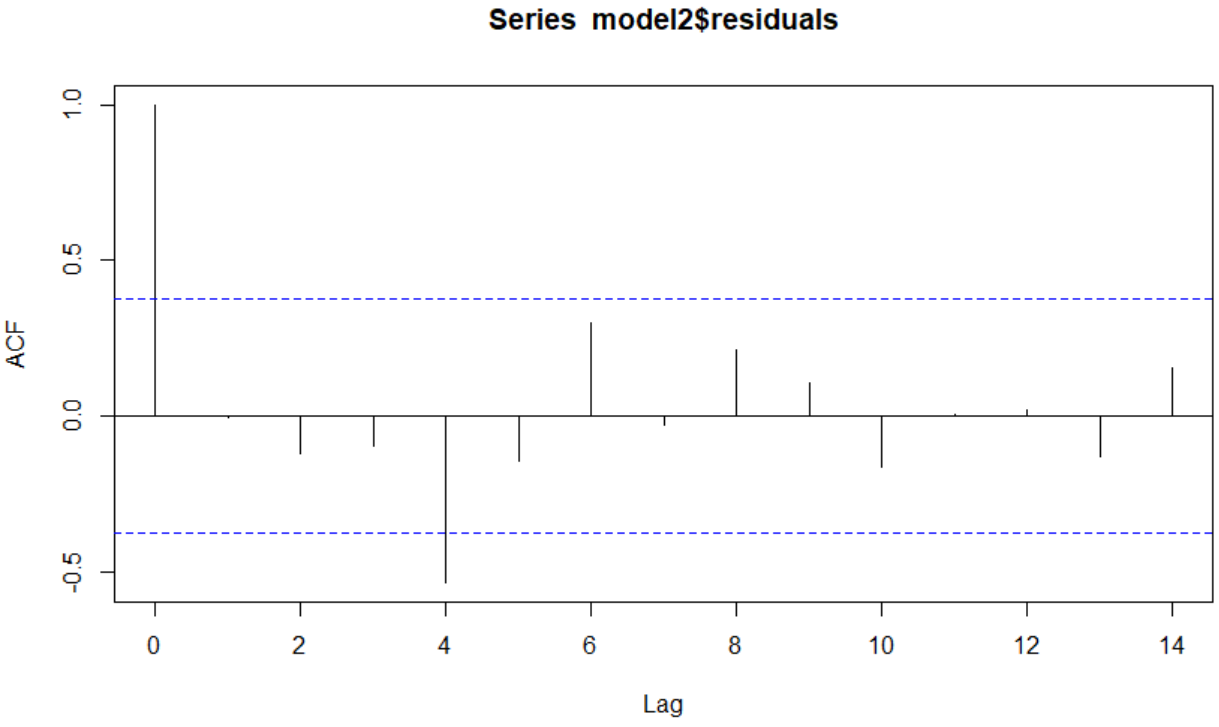
2
4
6
8
10
12
14
Lag

b) Model 2: With unlagged covariates and considering only the intervention period

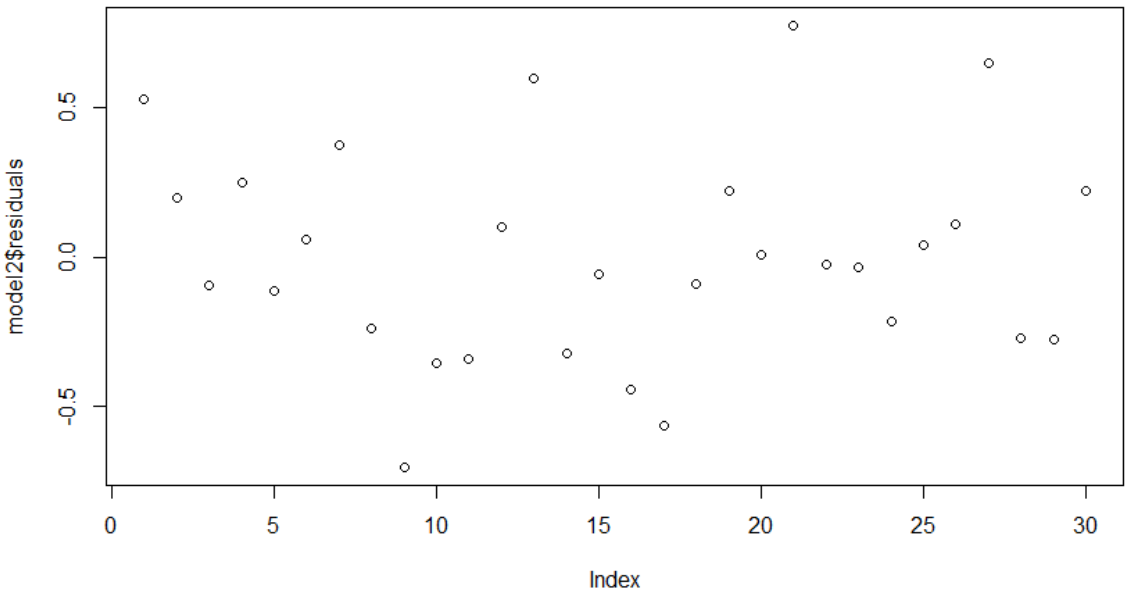
```
Call:
glm.nb(formula = cases ~ time_factor + lag(arabiensis, 0) + lag(funestus,
    0) + lag(parensis, 0) + lag(squamosus, 0) + lag(ziemanni,
    0) + lag(merus, 0) + lag(rufipes, 0) + lag(quadriannulatus,
    0) + lag(pharoensis, 0) + lag(llin_use, 0) + lag(mda_cov,
    0) + lag(irs_efficacy, 0) + offset(log(totvis)), data = data_model[which(!data_model$year_month %in%
    c("2015-05", "2015-06", "2015-07")), ], init.theta = 10.76633527,
    link = log)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-2.4467  -0.4892  -0.1614   0.3076   2.8515

Coefficients:
                        Estimate Std. Error z value Pr(>|z|)
(Intercept)             -2.038685   0.491253  -4.150 3.33e-05 ***
time_factor              0.004153   0.011574   0.359   0.7197
lag(arabiensis, 0)       0.606561   0.254990   2.379   0.0174 *
lag(funestus, 0)       -10.519312   6.904754  -1.523   0.1276
lag(parensis, 0)         1.750713   1.458993   1.200   0.2302
lag(squamosus, 0)        2.738434   1.395407   1.962   0.0497 *
lag(ziemanni, 0)         8.440506   3.613981   2.336   0.0195 *
lag(merus, 0)           -4.531404   5.079116  -0.892   0.3723
lag(rufipes, 0)         -0.270067   2.378252  -0.114   0.9096
lag(quadriannulatus, 0) -14.184541   8.391366  -1.690   0.0910 .
lag(pharoensis, 0)       -1.591980   1.947906  -0.817   0.4138
lag(llin_use, 0)         -2.835500   1.180433  -2.402   0.0163 *
lag(mda_cov, 0)          -0.157152   0.411013  -0.382   0.7022
lag(irs_efficacy, 0)      0.170199   0.483469   0.352   0.7248
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Negative Binomial(10.7663) family taken to be 1)

    Null deviance: 92.156  on 26  degrees of freedom
Residual deviance: 28.003  on 13  degrees of freedom
AIC: 348.38

Number of Fisher Scoring iterations: 1


              Theta:  10.77
          Std. Err.:  3.08

 2 x log-likelihood:  -318.382
```

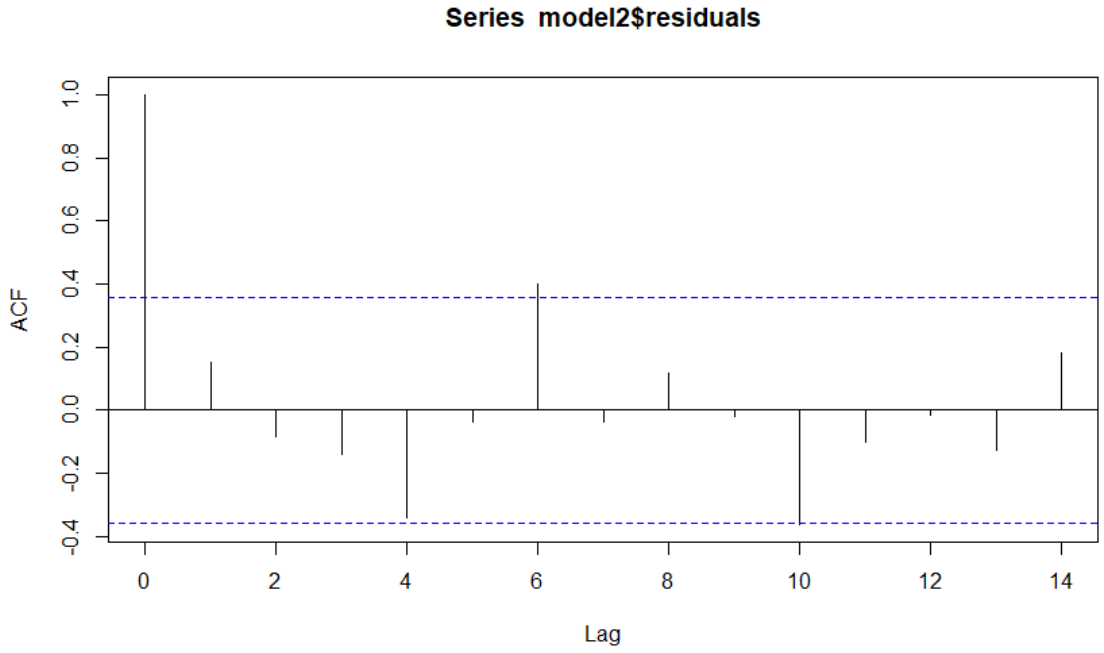




c) Model 3: With covariates lagged one month and considering the intervention and baseline period

```
Call:
glm.nb(formula = cases ~ time_factor + lag(arabiensis, +1) +
    lag(funestus, 1) + lag(parensis, 1) + lag(squamosus, +1) +
    lag(ziemanni, +1) + lag(merus, +1) + lag(rufipes, +1) + lag(quadriannulatus,
    +1) + lag(pharoensis, +1) + lag(llin_use, 1) + lag(mda_cov,
    1) + lag(irs_efficacy, 1) + offset(log(totvis)), data = data_model,
    init.theta = 10.37239326, link = log)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-2.8087  -0.7287  -0.1734   0.5315   2.3044

Coefficients:
                          Estimate Std. Error z value Pr(>|z|)
(Intercept)              -3.467487   0.459559  -7.545 4.51e-14 ***
time_factor               0.006466   0.009796   0.660  0.50919
lag(arabiensis, +1)       0.873379   0.250525   3.486  0.00049 ***
lag(funestus, 1)          0.831176   5.794446   0.143  0.88594
lag(parensis, 1)          2.274164   1.355615   1.678  0.09343 .
lag(squamosus, +1)        2.215822   1.349258   1.642  0.10054
lag(ziemanni, +1)         4.526824   3.513529   1.288  0.19761
lag(merus, +1)           -9.129091   5.723251  -1.595  0.11069
lag(rufipes, +1)          0.310958   2.344407   0.133  0.89448
lag(quadriannulatus, +1) -10.670210   8.355400  -1.277  0.20159
lag(pharoensis, +1)       -2.529123   2.073499  -1.220  0.22256
lag(llin_use, 1)          0.483596   0.989230   0.489  0.62494
lag(mda_cov, 1)          -0.000491   0.411117  -0.001  0.99905
lag(irs_efficacy, 1)     -0.863043   0.335089  -2.576  0.01001 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Negative Binomial(10.3724) family taken to be 1)

    Null deviance: 106.042  on 28  degrees of freedom
Residual deviance:  30.226  on 15  degrees of freedom
  (1 observation deleted due to missingness)
AIC: 377.25

Number of Fisher Scoring iterations: 1


              Theta:  10.37
          Std. Err.:  2.87

 2 x log-likelihood:  -347.253
```




Series model2$residuals
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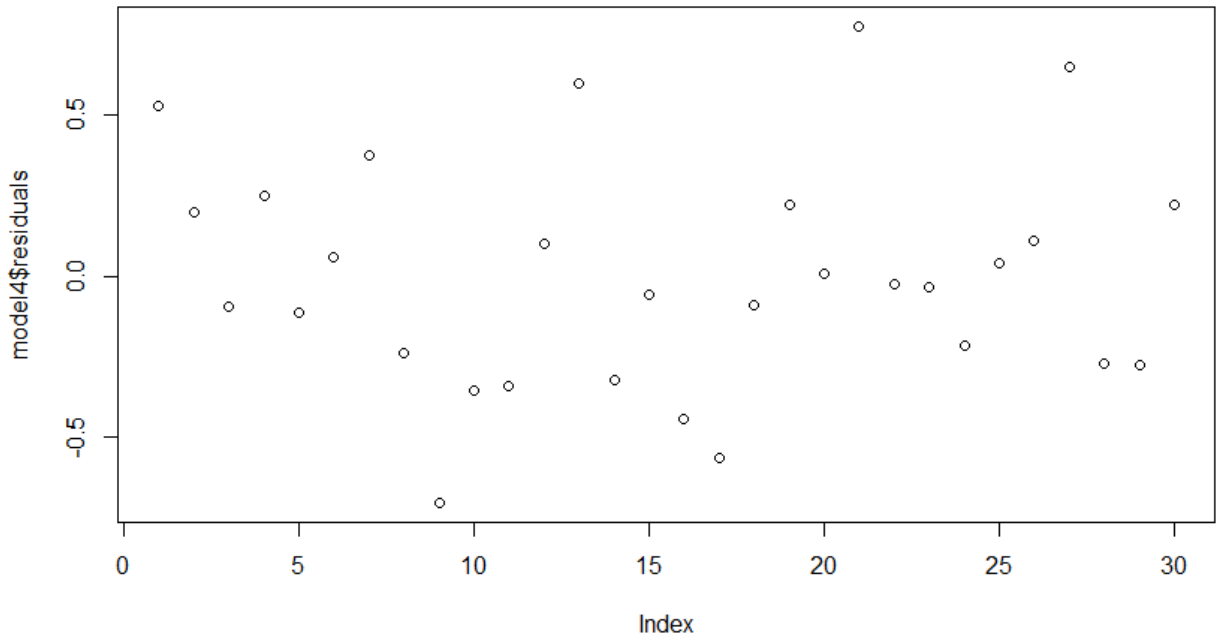
0.6
0.4
0.2
0.0
-0.2
-0.4
0
2
4
6
8
10
12
14
Lag

a) Model 4: With unlagged covariates and considering the intervention and baseline period

```
Call:
glm.nb(formula = cases ~ time_factor + lag(arabiensis, 0) + lag(funestus,
    0) + lag(parensis, 0) + lag(squamosus, 0) + lag(ziemanni,
    0) + lag(merus, 0) + lag(rufipes, 0) + lag(quadriannulatus,
    0) + lag(pharoensis, 0) + lag(llin_use, 0) + lag(mda_cov,
    0) + lag(irs_efficacy, 0) + offset(log(totvis)), data = data_model,
    init.theta = 8.022668053, link = log)

Deviance Residuals:
     Min        1Q    Median        3Q       Max
-2.67919  -0.80676  -0.08142   0.55519   1.75889

Coefficients:
                          Estimate Std. Error z value Pr(>|z|)
(Intercept)              -2.367044   0.517266  -4.576 4.74e-06 ***
time_factor              -0.018459   0.009954  -1.854  0.06367 .
lag(arabiensis, 0)        0.420790   0.279790   1.504  0.13259
lag(funestus, 0)          1.106189   6.368322   0.174  0.86210
lag(parensis, 0)          0.654883   1.492135   0.439  0.66074
lag(squamosus, 0)         1.777829   1.487797   1.195  0.23211
lag(ziemanni, 0)         12.391464   3.833489   3.232  0.00123 **
lag(merus, 0)            -8.185209   5.611187  -1.459  0.14464
lag(rufipes, 0)           2.363642   2.593103   0.912  0.36203
lag(quadriannulatus, 0) -12.177749   8.972561  -1.357  0.17471
lag(pharoensis, 0)       -0.775865   2.236203  -0.347  0.72862
lag(llin_use, 0)         -0.458332   1.121626  -0.409  0.68281
lag(mda_cov, 0)          -0.103605   0.460207  -0.225  0.82188
lag(irs_efficacy, 0)     -0.973023   0.377057  -2.581  0.00986 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Negative Binomial(8.0227) family taken to be 1)

    Null deviance: 96.882  on 29  degrees of freedom
Residual deviance: 31.158  on 16  degrees of freedom
AIC: 399.43

Number of Fisher Scoring iterations: 1


              Theta:  8.02
          Std. Err.:  2.13

 2 x log-likelihood:  -369.428
```


model4$residuals
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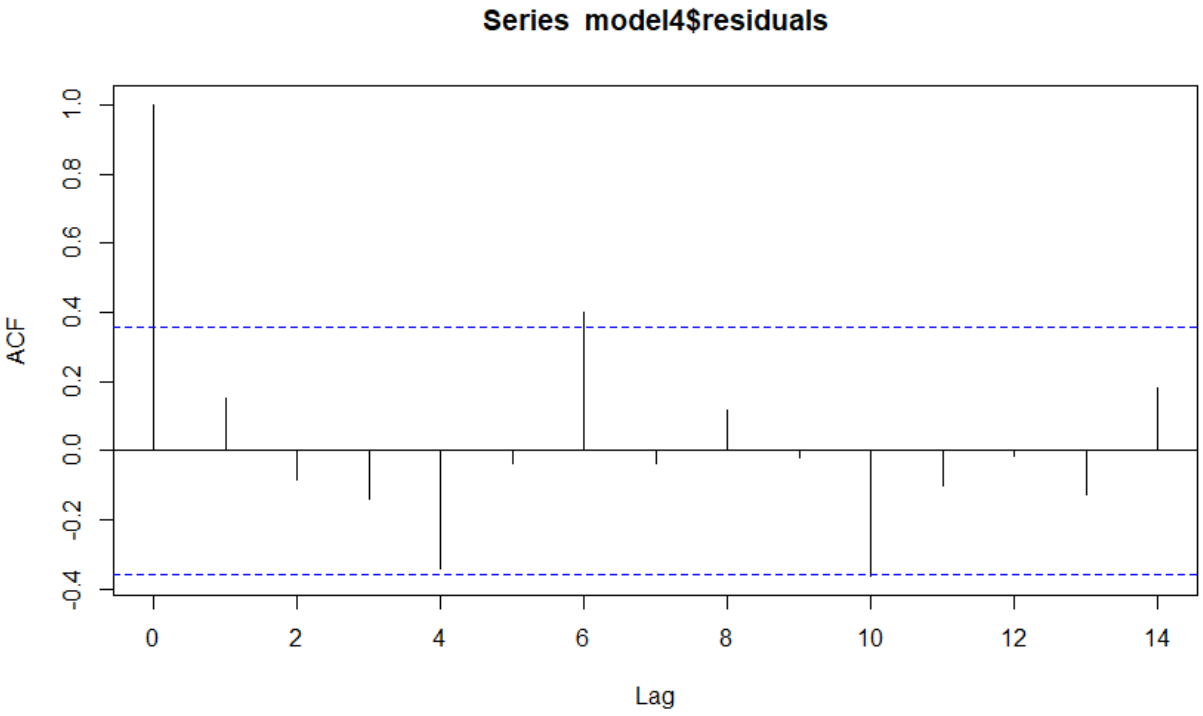
0.0
-0.5
0
5
10
15
20
25
30
Index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7. Discussion

The present thesis contributes to an improved understanding of 1) the challenges with current vector control when implemented with the aim to eliminate malaria in areas of low but stable malaria transmission in southern Africa, and 2) the benefits and limitations of using ITNs and IRS in combination. It identifies and describes the vectors that sustained malaria transmission during an attempt to eliminate malaria in southern Mozambique with a comprehensive intervention package (using IRS, ITNs, MDAs and routine testing and treatment), evaluates the implementation and efficacy of ITNs and IRS, and characterizes residual host-seeking mosquito populations. Its results can be used to improve existing vector control strategies in southern-Africa and to inform the development of new interventions that complement the protection provided by ITNs and IRS. Although the results should be extrapolated with care, many of the findings presented here are applicable to settings where *An. arabiensis* is the main vector of transmission and exhibits similar behaviors to the ones presented in this thesis, and where other important vector of transmission (e.g. *An. funestus s.s.*) are also present.

7.1 The deficiencies in IRS and ITN implementation and opportunities for improving their impact

This part of the discuss addresses research question 4 and 5.

IRS protection during the Magude project

IRS acceptability was high as only 6-10% of surveyed individuals reported rejecting IRS as the reason for their household not being sprayed. The IRS campaigns implemented during the

Magude project achieved high coverage, as observed by the fact that the percentage of structures sprayed (as reported by the IRS campaign implementers), the percentage of households sprayed (as measured through MDAs) and percentage of people protected were all above 83% in all three campaigns. In addition, coverage was consistent across district sub-areas. This refutes hypothesis H7 and suggest that IRS protection, based on coverage, was high. In addition, the fact that the mosquito mortality in WHO cone bioassay conducted 24h after spraying was 100% suggest that the quality of IRS application was good. IRS residual efficacy, on the contrary, did not last for the entire high transmission season, which confirms hypothesis 8. The novel method developed in article 1 to estimate the real duration of optimal IRS efficacy (i.e. including the pace of spraying and differences in residual efficacies between wall types) showed that, although the efficacy as measured through traditional WHO cone bioassays (110,111) seemed sufficiently long to cover the entire high transmission reason (6-7 months depending on wall type), the realized efficacy was much shorter, namely 3 months and 20 days and was achieved between mid-November and early March. Delayed mosquito mortalities could have extended this by an additional month, prolonging optimal efficacy until April, but as shown by article 4, this was not sufficient to prevent the increase in mosquito densities, and to effectively suppress transmission, at the end of the traditional high transmission season. The highest malaria incidence during the project was observed between April and June. Mosquito densities also reached their peak precisely in April, likely due to mosquito populations building up towards the end of the rainy season as larval habitats continue to be formed by the rainfall while the effect of IRS weakened. By then, the prophylactic effect among recipients of MDA had faded away, allowing the proliferating vectors to drive the increase in transmission. The residual efficacy of

IRS could have been further affected by wall replastering or modifications, as such behaviors have been observed in Mozambique (112) and are known to reduce the efficacy of IRS (113), but those relevant human behaviors were not assessed during the Magude project.

IRS possible improvements

Findings from article 4 suggest that achieving longer IRS efficacy could have extended protection and prevented -or at least lowered- the incidence peaks observed towards April-June. The duration of Actellic® 300CS's residual efficacy, as measured through WHO cone bioassays, have been observed to varied significantly across countries and wall types, ranging anywhere from 3 to 11 (114–121) and great variations have been observed for other IRS products too (122). Nonetheless, a recent review of the residual efficacy of different IRS products in experimental hut trials has shown that pyrimiphos-methyl, compared to pyrethroids, bendiocarb and clothianidin, killed a greater proportion of mosquitoes in the first two months post spraying and that pyrimiphos-methyl and clothianidin exhibited the longest residual efficacies compared to the other two products (123). Experiment hut trials in Tanzania have shown that pyrimiphos-methyl had the greatest toxicity against *An. arabiensis* (the main vector in Magude) when deployed on top of LLINs compared two other products available at the time of the Magude project, i.e. DDT and lambda-cyhalothrin (124). Since newer IRS products such as ShumiShield®, Fludora Fusion® or 2GARD® had not yet been prequalified by WHO at the time of the Magude project, I conclude that the Magude project used the best IRS product of all products pre-qualified by WHO at the time of the project implementation. In addition, the quality of IRS implementation was good.

Extending IRS residual efficacy would require longer lasting IRS product or implementing another IRS round. Implementing a second IRS campaign would have prevented IRS residual efficacy from falling below optimal levels before the end of the high transmission season. However, the high rains still occurring in these months would have likely made it logistically unfeasible. A better alternative would therefore be to use a longer lasting IRS product. Several semi-field trials suggest that newer IRS products, which contain clothianidin, may have a longer residual efficacy than pirmiphos-methyl and are effective against pyrethroids resistance mosquitoes, such as *An. funestus s.s.* and *An. parensis* in Magude (125–130). They should be therefore considered for future IRS campaign in Magude, but evidence on their residual efficacy under local field conditions should be generated to inform their use. Finally, another way to increase the overall effectiveness of IRS would be to spray animal sheds, as several of the *Anopheles* species tentatively identified as vectors are known to be zoophilic (see discussion on vectors below). Nonetheless, further entomological investigations on local vector resting places and blood means would be needed to evaluate the potential impact of this strategy.

ITN protection during the Magude project

The fact that most of the residents (80-91% depending on the year) that used a net, used one impregnated during the last 12 months or an LLIN suggests that nets provided both personal and community protection. This is further reinforced by the fact that 77.1% of the nets in the district were Olyset® Nets and this net brand has been observed to induce high mortalities in pyrethroid-susceptible *An. arabiensis* (the main susceptible vector in Magude) (131). However,

part of their community protection was likely compromised by the fact that two of the five main vector species were resistance to pyrethroids, i.e. *An. funestus s.s.* and *An. parensis,* and therefore unlikely to die upon contact with ITNs. ITN protection was further compromised by suboptimal access, which remained below 73.6% during the project despite the implementation of two ITN mass distribution campaigns, one before and one during the course of the project. It was further compromised by ITN use which, although fairly high in the high transmission season (73.6%), dropped to 40% in the low transmission season. This confirms hypothesis 3 and 4. Beyond ITN access and use, vector behaviors further compromised ITN protection. The analysis of mosquito and human behaviors shows that the personal protection of ITNs averted less than half (i.e. 39.2%) of human exposure to the host-seeking vectors that survived the combined deployment of IRS and ITNs. This was partially due to the suboptimal ITN use but also due to the fact that a proportion of the vector population sought their host during the early evening times and outdoors. Together, all these results show that the protection conferred by ITNs during the Magude project was suboptimal, especially during the low transmission season.

In addition, ITN protection was not homogeneous across the population in the district. ITN protection was lower in harder to reach localities, in poorer and larger households, compared to easier to reach localities, richer and smaller households. This was due to poorer access and use in these households compared to the others. ITNs prevented a higher proportion of exposure to vector bites in children <5 years of age (45.4%) than in school-aged children (32.5%) or adults (38.9). This was mainly due to their higher ITN use and the greater time they spent indoor and in bed. ITN use was also lower in males, especially young males, compared to females. Low ITN use in school age children and young males has been observed elsewhere in Africa (132–136) and

poses challenges for malaria elimination in Magude as, like in other Africa settings (137,138) young adults were observed to be important parasite reservoirs during the Magude project.

Optimization of ITN efficacy

Given that hypothesis H3 and H4 were confirmed, there is a need to understand how the efficacy of ITNs could have been improved during the Magude project to inform future similar projects and ITN campaigns in Mozambique.

It appears that ITN access limited use. This statement is grounded in the facts that 1) the most frequently reported reason for not using the net to sleep was not having one (56-78% of respondents, 2) the percentage of interviewees that reported such reason increased over time as ITN access decreased due to the rate of net loss observed in the district (>30% lost per year), 3) ITN use exceeded ITN access during the high transmission season of the first implementation year (76.3% use when access was between 73.7% and 68.2%), showing residents willingness to use the nets and 4) ITN use was higher in households that had a least one net for every two people, showing that people use the net more when they have better access. In addition, access seems to have contributed to the differences in use across localities and households, as the localities and types of households (wealth and size) where use was lower were also those presenting the lowest access. Hence, my results suggest that ITN use could have improved by improving access.

Article 2 strongly suggests that improving access will require revising the ITN allocation strategies currently used during mass distribution, both the numbers of nets distributed and the distribution across different households, and implementing behavioral change campaigns to encourage net sharing. This is evidence by 1) the fact that the 2017 campaign, despite distributing 25% more nets than the 2014 campaign, did not improve ITN access; 2) the inequalities observed in net access and use across different types of households and areas and 3) the fact that a portion of the population was not willing to share their net, as shown by the fact that 25% of nets located in households with more than two people per net were used by only one person. This last fact also suggests that the common campaign target of distributing a net for every two people does not guarantee that everyone will have access to sleeping under a net in the absence of behavioral change campaigns. Efforts should therefore focus on 1) improving the calculation of how many nets should be distributed in an area and to each household, considering factors such as household size, wealth, and people behaviors 2) not missing hard to reach areas during the campaign and, 3) implementing behavioral change campaigns to increase net sharing and, hence, access to sleeping under a net.

The high rates or net loss (approx. 30% annual loss) compared to those of other provinces in Mozambique (139) and the fact that inequalities in ITN access exacerbated over time, suggest that conducting more frequent ITN campaigns or implementing top-up schemes will help to sustain high levels of ITN access. Although top-ups campaigns are not recommended by the World Health Organization (WHO) for programmatic settings because of the costs of quantifying the number of nets required (7), top-up campaigns could have been implemented in Magude

district as gaps in access were regularly being recorded during the health and demographic surveys. Given that inequalities in ITN access across household wealth level exacerbated over time top-up campaigns or continuous distribution channels targeting poor households may help to sustain ITN access in this disadvantages group.

Attention should be paid to existing continuous distribution channels, because despite the theoretical continuous distribution of ITN through antenatal care services and immunization services, access was lower among children than among adults and lower in young women (below 30 years of age) than older women. Since use of antenatal care services was between 60 and 75% , this could be due to ITN stock-outs in health facilities and should be further investigated to ensure that continuous distribution channels contribute to improving ITN access among vulnerable groups.

ITNs only provide protection when they are used. Although improving ITN access would have likely increase ITN use, this will not necessarily happen during the low transmission season (May to October), which contributed to a significant proportion of transmission during the Magude project (109), and when only 66.1% of people with access used their net. Improving ITN protection would have also required improving ITN use among those with access, especially during this season. Article 3 shows that if all residents would have used the net to sleep (assuming they all had access to it), the proportion of human exposure to host-seeking mosquitos that nets could have prevented would have risen to 63.3%. Article 2 shows that community awareness campaigns were likely effective at increasing use. ITN used increase from 25.4% at the

start of the Magude project (January-June 2015) to 64.4% and 76.3% in January and May 2016 and, since no nets were distributed during the project, such increase can be attributed to the community engagement campaigns implemented during the project. The reduction in net use during the low transmission or dry season is commonly observed across Africa and is frequently attributed to low vector abundance or to heat (140–145). This shows that it is important for community awareness campaign to raise awareness of the risk of contracting malaria during the low transmission season, especially when transmission patterns are altered through intense malaria programs like the Magude project. In addition, the inequalities observed in ITN use across population groups (lower use in men than in women and in school-age children (5-15 years of age) than in younger children or adults) suggests that specific behavioral change campaigns may be needed for certain population sub-groups, especially targeting young males. Like in other settings (137,138), this age group (5-15 years of age) was an important reservoir of malaria parasites in Magude (109). The low levels of net use in this age group may have contributed toward sustaining malaria transmission during the project and hence it is important to target community engagement campaigns to this group.

7.2 The vectors that sustained malaria transmission during the Magude project and their amenability to control by IRS and ITNs

This part of the discussion addresses research question 1 and 2 and draws from the results of all articles.

The vectors identified during the Magude project

Twenty-one *Anopheles* species were identified during the project, some known to be vectors or present in Mozambique, others never found before. Unfortunately, our ability to confirm the presence of these species and determine which of these species were vectors of malaria during the project was jeopardize by the fact that 1) CDC light traps damage individuals, which made morphological identification challenging at times, 2) molecular species identification was only conducted in mosquitoes that belonged to the *An. gambiae* complex or the *An. funestus* group as morphologically identified, 3) the sporozoite detection ELISA protocol (which did not include a sample boiling step) has been observed to yield false positive results in zoophilic mosquitos (146); 4) the low numbers of mosquitoes collected of certain species hampered the detection of positive individuals and 5) *P. falciparum* sporozoite detection was not conducted in all mosquitoes collected, but rather focused on the known vectors in the region given the limited capacity in the laboratory. Because of these limitations, the list of vectors identified during the Magude project, more specifically those that were detected in very low numbers and had never been detected in Mozambique before, and the list of species incriminated here as vectors, should be taken as tentative. Whether all these species were identified correctly and were actual vectors

should be confirmed by further studies including detailed molecular analyses (using e.g. ITS2 and COI analysis (147)).

At least five *Anopheles* species likely transmitted malaria during the Magude project, namely *An. arabiensis, An. merus, An. funestus s.s., An. parensis* and *An. squamosus.* These species were the only ones found carrying sporozoites and were all significantly associated with malaria incidence. Of them, *An. funestus s.s.* and *An. arabiensis* are well known malaria vectors in southern Mozambique (104,148,149) and *An. merus* was already incriminated as a vector in 2000 in the nearby Boane district, southern Mozambique (150) and later identified as a vector during the LSDI project (104). In contrast, this is the first time that *An. squamosus* and *An. parensis* are found carrying *P. falciparum* sporozoites in Mozambique. *An. squamosus* was previously found carrying *P. falciparum* sporozoite in southern Zambia in 2016 (151) and *An. parensis* in 2017 in Kwazulu-Natal, a province in South Africa that borders with the southern part of Mozambique (152–154), and has more recently been found positive for malaria in Tanzania (155). Two additional species were statistically associated with malaria incidence, *An. rufipes* and *An. quadriannulatus*, albeit with a weak association, but none of the *An. rufipes* and *An. quadriannulatus* specimens were found carrying sporozoites during the project. For *An. rufipes*, sporozoites may have been missed due to the low number of specimens analyzed for presence of sporozoites (8 out of 83 identified). In contract, most of the *An. quadriannulatus* collected were analyzed (59 of 63 identified). *An. quadriannulatus* has been demonstrated to transmit malaria in controlled laboratory conditions (156), but never in nature. Hence it likely did not play a role in transmission during the Magude project. *Anopheles rufipes* is a secondary malaria vector

in equatorial Africa (Burkina Faso, Cameroon, Gambia, Ghana, Kenya, Mali, Nigeria, Senegal, Togo) as well as southern Africa (Zambia) (157–166) and therefore could have played a minor role in Magude. Nonetheless, the total numbers of mosquitoes that were collected of both of these species during the project were low.

Other *Anopheles* vector species were identified that are known to be malaria vectors in Mozambique (*An. gambiae s.s.* and *An. tenebrosus* (167)) or elsewhere in Africa (i.e. *An. coustani* (147,168–170)*, An. ziemanni* (171–174) *An. rivulorun* (147,175,176), *An. leesoni* (166,177–179) and *An. pharoensis* (147,160,180–182)*),* but none was found carrying sporozoites or found associated with malaria incidence and hence, if they did play a role, it was likely very minor. Most of these vectors did not undergo sporozoite detection, and, even if correctly identified, it is not possible to tentatively point to them as vectors of transmission during the project. Further studies are needed to determine whether they are vector of transmission in Magude. In addition to these vector species some none vector species were identified too, namely *An. demeilloni, An. garnhami, An. listeri, An. marshalli s.l., An. multicolor* and *An. salbaii.* Some of them have never been detected in Mozambique before, like *An. garnhami, An. multicolor* and *An. salbaii* (162) but have been detected elsewhere in Africa. Because none of these vector and non-vector species were molecularly identified, as mentioned before, their detection could also be an artifact of wrong morphological identification, potentially caused by specimens being damaged by the CDC light traps (147,183).

Identifying the main vector of malaria transmission

The relative importance of various vector species in malaria transmission is normally evaluated using the entomological inoculation rate (184) because it integrates vector abundance, anthropophilic feeding preference, and infection rate in one metric. However, in malaria elimination settings with intense vector control, like the situation in Magude district, this indicator is hard to calculate, mainly due to low mosquito numbers and low sporozoite rates resulting in high confidence intervals on this indicator. In addition, mosquito blood meal source was not analysed, as the mosquitoes collected in CDC light traps were mostly unfed. Hence, the evaluation of vector relative importance had to be supported by other evidence, namely the results of the negative binomial model of association between vector densities and malaria incidence and a detailed analysis of the vector population abundance, sporozoite rates and behaviors. Several findings lead to conclude that *An. arabiensis* was the main vector of transmission in Magude, as this species 1) was the most abundant species during the entire project, 2) was responsible for the majority (74%) of the human exposure to host-seeking mosquitoes both in the high (78.5%) and low transmission season (64.2%), 3) presented the strongest statistical association with malaria incidence and 4) was the only vector found carrying sporozoites after the first IRS campaign, except for one *An. merus* found infected towards the end of the second year of the project. The other tentative vectors, i.e. *An. funestus s.s.*, *An. parensis*, *An. merus* and *An. squamosus,* and possibly *An. rufipes*, likely played a secondary role.

An. arabiensis and its amenability to control by ITNs and IRS

An. arabiensis is known to be an important malaria vector in eastern and southern Africa (88,104,162,185–189) This vector exhibits an opportunistic feeding behaviors, biting both human and animals, feeding both indoors or outdoors, during dusk, dawn or the night depending on host availability (155,190–192), and it is known for its ability to escape contact with indoor insecticide-based interventions by resting outdoors (193,194). As a consequence of broad ITN and IRS implementation, this vector is playing an increasingly important role in malaria transmission in Africa, especially as ITNs or IRS have reduced the abundance of other major vector species that are more amenable to these interventions, such as *An. funestus s.s.* or *An. gambiae s.s.* (193). In Boane district, around 100km south of Magude district, *An. arabieniss* has been observed to play a similar role in malaria transmission as *An. funestus s.s.* (148). In South Africa, after years of IRS implementation, *An. arabiensis* became the main vector of residual malaria transmission (193). In Kenya, following scale up of ITN distribution, the relative abundance of *An. arabiensis* increased as that of *An. gambiae s.s.* decreased (195). A similar pattern was observed in Tanzania, following high coverage of LLINs (196).

During the Magude project, *An. arabiensis* sought their host throughout the evening and night, indoors and outdoors, but the highest percentage of human exposure to host-seeking *An. arabiensis* occurred indoor while people were in bed (63%-73.4% depending on the season). This behavior allowed ITNs to prevent a significant (although still limited) proportion of human exposure to *An. arabiensis* at the observed levels of ITN use (41.8%) and would have allowed ITNs to prevent 67.4% of human exposure to host-seeking *An. arabiensis* if all residents would have

used an ITN to sleep. In addition, and although *An. arabiensis* has become resistant to pyrethroids across eastern Africa (197), there are no previous reports of resistance to pyrethroids in Mozambique (197) and *An. arabiensis* was susceptible to pyrethroids in Magude. This suggest that ITNs could have been able to reduce *An. arabiensis* densities too. Although we did not measure the capacity of ITNs to kill *An. arabiensis*, experimental hut trials conducted in Tanzania with brand new nets and *An. arabiensis* that were mainly susceptible to pyrethroids, showed that only between 11.8% (dry season) and 14.8% (wet season) of the *An. arabiensis* caught in huts with these nets died within 24h (124). Such low mortality was attributed to the fact that mosquitoes were not spending enough time in the huts to receive fatal exposure to insecticides. The proposed hypothesis was that *An. arabiensis* entered houses, could not find a host to bite because occupants were well protected under new nets, gave up on searching and exited early. If the same is true for Magude, this would mean that ITN were not very effective at killing *An. arabiensis* mosquitoes, a fact that can partially justify that *An. arabiensis* continued to be found in significant numbers during the Magude project.

Although my attempts to establish a statistically significant relationship between IRS and vector densities were not successful, article 1 shows that *An. arabiensis* was susceptible to the two insecticides used for IRS in Magude (DDT and pirimiphos-methyl) and article 4 suggests that IRS did manage to contain the increase of *An. arabiensis* population in the rainy season. IRS optimal residual efficacy lasted from mid-November to mid-March. *An. arabiensis* breeds in small, temporary and shallow pools (198) which can easily be formed by rains and, hence, *An. arabiensis* densities are expected to increase following the rains. In both project years, *An.*

arabiensis densities remained very low during most part of the rainy season and increased rapidly around the time that IRS was losing its optimal residual efficacy (~14th March). In addition, although rainfall patterns were different between the two project years, *An. arabiensis* densities remained similar. The rains started around October in both years, but the first year of the project was unusually dry and presented a peak in rainfall in May, while the second was unusually wet with a peak in December (109), more consistent with the traditionally described rainy season (108). The fact that *An. arabiensis* densities were not higher in the second year compared to the first, suggest that IRS managed to prevent the expected population growth during the time it remained optimally efficacious. Nonetheless, despite IRS, *An. arabiensis* continued to be collected in significant numbers resting indoors and in CDC light trap, suggesting that although IRS probably contributed to the control of this vector, its effect was likely suboptimal. This may be due to inherent traits of *An. arabiensis,* such as its ability to rest outdoors. Studies in Tanzania have shown that *An. arabiensis* is much less affected by IRS with pirimiphos-metyl (the insecticide primarily used for IRS in Magude) than other important vector species such as *An. funestus s.s.* (196,199).

An. funestus s.s. and its amenability to control by ITNs and IRS

An. funestus s.s, is known to be a highly anthropophilic and a very competent vector of malaria in Mozambique (148) and in Africa in general (200), and likely played a minor role in transmission during the project. As shown in article 4, one *An. funestus s.s*. was found carrying *P. falciparum* sporozoites before the implementation of the first IRS campaign, and although no sporozoite positive specimens were found during the project, *An. funestus s.s*. was associated

with malaria cases. The absence of sporozoite positive *An. funestus s.s.* could have been an artifact of the low the numbers of mosquitos collected of this species which jeopardized the detection of positive mosquitoes of this species.

IRS seems to have been effective at controlling *An. funestus*. Article 1 shows that *An. funestus* was susceptible to the insecticide used for IRS (DDT and pirimiphos methyl) and was found resting indoors before the project started. Article 4 shows that the density and relative abundance of this vector decreased dramatically after the 2015 IRS campaign and that numbers remained very low until September 2017. In contrast, ITNs did not seem very effective at reducing *An. funestus s.s*. densities nor at preventing bites form the *An. funestus* s.s. that survived the implementation of IRS and ITNs. Article 1 shows that *An. funestus s.s.* was highly resistant to pyrethroids and evidence from the neighboring district of Manhiça shows that new LLNs no longer effectively kill *An. funestus s.l.* mosquitoes (201). Article 3 shows that the personal protection of ITNs only prevented 21.9% of the human exposure to host-seeking *An. funestus s.s.* compared to 41.8% of prevented exposure to *An. arabiensis.* Beyond the suboptimal bednet use that reduced ITN protection against all vector species, the lower protection against *An. funestus s.s.* was due to its host-seeking behaviors. As shown in article 4, the host-seeking activity of this vector was more pronounced during the early evening hours (before people went to bed), both indoors and outdoors. Article 4 shows that this activity pattern, combined with human activity patterns, resulted in most of the human exposure to host-seeking *An. funestus s.s*. (60% in the low transmission season and 66% in the high transmission season) occurring before people went

to bed. Due to its host-seeking behavior, even if all residents would have used the net to sleep, ITNs would have only prevented less than half of the human exposure to this vector (42.6%).

An. parensis and its amenability to control by ITNs and IRS

An. parensis was found carrying *P. falciparum* sporozoites before the start of the project, suggesting it was a vector in the district. During the project, it likely played a secondary role because it practically disappeared after the first IRS campaign for approx. 2 years and was never found carrying *P. falciparum* sporozoites again during the project. It was however significantly associated with malaria incidence.

Article 1 shows that members of the *An. funestus* group in Magude were fully susceptible to DDT and pyrimiphos-methyl before the project started, and that some *An. parensis* were collected resting indoors in Magude. This behavior is unexpected for *An. parensis,* a vector believed to rest mainly outdoors (152), but has been observed previously in Kenya (202). This suggests that IRS could have been effective to some extent at controlling this vector in Magude but, as shown in article 4, the overall low densities of this vector during the project prevented us from describing the effect of IRS on its densities. Article 1 shows that *An. parensis* was resistant to pyrethroids although the frequency of its resistance to pyrethroids seemed lower than that of *An. funestus s.s.* This suggests that ITNs were likely not effective at reducing its densities. In addition, articles 3 and 4 show that *An. parensis* sought their host during the early evening times, indoors and outdoors, and that ITN -considering the human activity patterns in the district- could only prevent 13.9% of the human exposure to host-seeking *An. parensis*. Hence, I conclude that ITNs were likely little effective at controlling *An. parensis.* This vector is now believed to be a

secondary malaria vector in South Africa (152), it is suspected to be a vector in Tanzania (178) and it is also present in Kenya, Malawi, Uganda, Zambia and Zimbabwe (162), hence, effective tools are urgently needed to control this.

An. merus and its amenability to control by ITNs and IRS

An. merus likely played a minor role in sustaining transmission. *An. merus* was, beyond *An. arabiensis*, the only other vector species found carrying sporozoites after the first IRS campaign and its densities were significantly associated with malaria incidence, however the association was weak compared to that of other species. It was also collected in small numbers throughout the project. The susceptibility of *An. merus* to the insecticides used in IRS and ITNs was not evaluated because practically all of the *An. gambiae s.l.* mosquitoes that were tested for insecticide susceptibility were molecularly identified as *An. arabiensis.* The fact that no *An. merus* were collected before the first IRS campaign jeopardizes our ability to understand how IRS affected this vector. Nonetheless, three facts suggest that IRS may have had an effect, even if small, on this vector: 1) the density of the few *An. merus* collected oscillated similarly over time compared to the patterns observed for *An. arabiensis*, 2) most *An. merus* collected from 2016 were collected indoors and 3) a few *An. merus* were found resting indoors during the early morning mosquito collections for insecticide resistance testing. Evidence from the 1960-1969 global eradication program in Mozambique shows that this vector entered houses to bite but rested outdoors (149), which suggest that, if any, the effect of IRS on this species was likely small. In contrast, ITNs provided significant personal protection against *An. merus*. Article 3 shows that all *An. merus* sought their host indoors and mainly during the night at times when people were

in bed. Combining human and *An. merus* activity patterns, ITNs prevented 45% of human exposure to this vector. Although believed to have been a minor vector of transmission in Magude, the importance of this species should not be underestimated since its relative abundance has been observed to increase over the years in Mpumalanga Province in South Africa, where it is nowadays suspected to play an important role in sustaining malaria transmission (203).

An. squamosus and its amenability to ITNs and IRS

An. squamosus was found carrying sporozoites and was associated with malaria incidence, but is likely to have played a secondary role in malaria transmission due to its very low densities. However, as shown in article 4, this vector may have played a role during the high malaria incidence peaks observed during the project (February and June 2017). Two facts support this hypothesis: 1) unlike most other vector species, the relative abundance of this vector increased greatly just before and during the highest peaks of malaria incidence (from 0.2%-4.0% to 16.8%) and 2) the largest peak in outdoor vector densities observed in February 2017 was dominated by *An. squamosus*, which in general accounted for approx. 50% of all outdoor anophelines collected per person per night between January 2017 and March 2017. This is however just a hypothesis, as sporozoite detection in the *An. squamous* mosquitoes collected during this period was not conducted.

We cannot draw any firm conclusion about the effect of IRS on *An. squamosus* due to the low densities of this species and the lack of insecticide resistance data. However, two facts suggest that IRS did not affect this vector, 1) no *An. squamosus* were found resting indoors during

the early morning collection of mosquitos that were used for insecticide resistance monitoring, suggesting that *An. squamosus* did no exhibit the vector behaviors targeted by IRS and 2) *An. squamosus* densities did not follow a seasonal pattern and only increased significantly around the high malaria incidence peak. Regarding ITNs, the susceptibility of *An. squamosus* to pyrethroids could not be evaluated, which hampers our understanding of ITN's ability to reduce *An. squamosus* densities. Articles 3 and 4 suggest however that ITN provided some personal protection against *An*. *squamosus*, as ITNs prevented 32.0% of human exposure to this vector. This is because this vector sought its host both indoors and outdoors.

7.3 Where and when did persistent malaria transmission occurred?

This part of the discussion addresses research question RQ3.

The overlap of human and vector behaviors, considering the observed rates of ITN use, showed that most of residual exposure to host-seeking mosquitoes in Magude occurred when residents were in bed (64%, 95% CI: 55.3-1.9), followed by indoors before going to bed (21.9%, 95% CI: 15.5-29.9), outdoor in the evening (12.5%, 95% CI: 7.7-19.5), indoors after getting up (1.4%, 95% CI: 0.2-5.6) and outdoors during the morning (0.2%, 95% CI: 0-3.8). Parous *An. arabiensis*, the portion of *An. arabiensis* population that can be infective as they have blood fed at least once already, has been observed to be actively host-seeking at later times during the night, compared to non-parous *An. arabiensis* (204,205). This adds further importance to the biting occurring during the night while people are in bed. The high proportion of bites still occurring while people were in bed was due to 1) the local vector behaviors, especially that of *An. arabiensis*, which

sought its hosts mainly indoors while people were asleep and was the main vector in the district; 2) the suboptimal bednet use, and 3) the imperfect feeding inhibition of ITNs, which we assumed here to be 18.9% based on experimental hut trials conducted in Tanzania with domestically used Olyset Nets.

Article 4 shows that about a third of the human exposure to host-seeking vectors (32.4%) happened in the low transmission season. From the differences in the distribution of exposure to host-seeking mosquitoes between the low and high transmission season compared to the high transmission season alone, it can be observed that the exposure during the low transmission season is not only a result from lower ITN use (40% compared to 76.3% in the high transmission season) but is also due to 1) the observed earlier biting behavior of *An. arabiensis* indoors and outdoors and 2) the fact that people went indoors earlier and spent more time indoors not under the net during this season compared to the high transmission season.

7.4 Combining IRS and ITN or implementing a single intervention in the future?

This part of the discussion responds to research question RQ6.

The epidemiological value of adding IRS on top of ITNs could not be assessed during the Magude project due to the lack of a control district. The global epidemiological evidence to-date on the value of adding IRS on top of ITNs in settings where *An. arabiensis* is the primary vector is contradictory, and was generated in settings where *An. arabiensis* was resistance to pyrethroids

(206). Because *An. arabiensis* was susceptible to pyrethroids in Magude, such evidence cannot be extrapolated to Magude.

My entomological research suggests that, given the diversity of vectors, their behaviors and insecticide susceptibility levels, deploying ITNs and IRS together provided an increased protection against exposure to vector bites compared to deploying one intervention alone. ITNs and IRS likely both contributed to reducing the densities of *An. arabiensis*, as this vector was susceptible to pyrethroids, sought their host mainly indoors and rested, at least to some extent, indoors, but at the other hand *An. arabiensis* continued to be present and the most abundant vector during the entire project, even after the second IRS campaign and a new ITN campaign. This suggests that none of the two interventions, or their combination, were fully effective at reducing its densities.

An experimental hut trial in Tanzania has indeed shown that the mortality induced by ITNs and IRS, either alone or in combination, in susceptible *An. arabiensis* is <30% and that adding IRS on top of LLINs caused no statistically significant increase in mosquito mortality, except when adding IRS with pirimiphos-methyl to LLINs (124). This experimental hut trial in Tanzania showed that adding IRS with pirimiphos-methyl to Olyset Nets increased mosquito mortality from 11.8% to 16.4% in the dry season and from 14.8% to 20.3% in the wet season. It further showed that adding LLINs (practically any brand except Olyset) on top of IRS provided greater value than adding IRS on top of LLINs. This suggests that ITNs in good conditions would provide better value in controlling *An. arabiensis* than IRS, but that adding IRS will have some added value and that neither IRS, nor ITNs or their combination, would be optimally effective at reducing the densities of susceptible *An. arabiensis.*

The added value of deploying IRS and ITNs together is more obvious when looking at *An. funestus s.l.* species. ITNs likely had a very limited success in controlling of *An. funestus s.s.* (a very competent vector) and *An. parensis.* (a secondary vector in the region) because these vectors were resistance to pyrethroids and hence ITNs could not effectively reduce their densities, and because more than half of their population sought their host outdoors or at times before people were under the net, jeopardizing nets' ability to prevent them from biting humans. Due to such behaviors, as well as to the suboptimal ITN use, ITNs only prevented 21.9% and 13.9% of human exposure to *An. funestus s.s. and An. parensis,* respectively, and could only have prevented 42.6% and 31.7%, respectively, if everyone would have used an ITN to sleep. IRS, on the other hand, played an important role in controlling *An. funestus s.s.* and *An. parensis* which, as mentioned before, were susceptible to the insecticide used in IRS and practically disappeared after the first IRS campaign of the project. The value of adding IRS on top of LLINs, in terms of reducing exposure to *An. funestus s.l.*, in an area where *An. funestus s.l.* vectors are also resistant to pyrethroids, has recently been demonstrated in Mopeia, another district of Mozambique (207).

Finally, it is worth highlighting another benefit of deploying the two interventions together, which is that ITN use was higher among people living in sprayed (IRS) households than among those living in unsprayed households. This suggests that deploying IRS in combination with ITNs had a positive impact on ITN use. Such synergistic associations has been observed

elsewhere (208,209) and highlights a potential added value of deploying these two interventions together.

All in all, all my evidence suggests that ITNs should be the core intervention for Magude given that *An. arabiensis* is the main vector but that adding IRS will be important to control secondary vectors and may have added benefits such as increased ITN use. Ultimately the decision to deploy ITNs and IRS together in Magude, and elsewhere, should be based on the evaluation of their epidemiological impact and cost-effectiveness compared to other malaria control interventions, and not on entomological data alone. Clinical trials evaluating the added value of ITNs and IRS in areas where *An. arabiensis* is susceptible to pyrethroids are needed to inform such decision.

7.5 Suitable additional vector control intervention for the future

This part of the discussion responds to research question RQ7.

As discussed before, IRS and pyrethroid-only ITNs, could not prevent 1) the outdoor exposure to host-seeking vectors, driven primarily by outdoor biting *An. funestus s.s*. and *An. parensis*, or 2) the residual exposure occurring indoors when people were not yet under the net, which was mainly driven by *An. arabiensis*, *An. merus* and, to a lesser extent, *An. squamosus*. Article 3 shows that, even if everyone would have used an ITN to sleep and provided that nets inhibited 81.9% of the bites, 78.9% of the residual exposure to host seeking mosquitos would have occurred indoors and 21.1% outdoors. This residual exposure, amidst suboptimal MDA coverage and limited prophylactic effect (i.e. one month) sustained malaria transmission. Supplementary

interventions would therefore be needed to prevent transmission by the vectors that survived or were not affected by the combined deployment of IRS and ITNs, and should prioritize targeting the indoor environment.

Although several new interventions and products are currently under evaluation by WHO (210–212) PBO-nets, larviciding and house screening with untreated materials are the only two that have so far received a positive, although for some conditional, WHO recommendation for public health use (7).

PBO-nets only bring value against pyrethroid-resistant mosquitoes whose resistance is caused, at least partially, by the increased activity of cytochrome P450 enzymes, which are involved in metabolic detoxification of insecticides. This is the case for *An. funestus* s.s. in southern Mozambique. However, the main malaria vector in Magude, *An. arabiensis,* is susceptible to pyrethroids. In addition, studies conducted in Manhiça, a district neighboring Magude, have shown that *An. funestus s.s.* with the aforementioned P450 resistance mechanism, survives exposure to PBO nets in WHO cone bioassay and that pre-exposure to the PBO synergist in synergist insecticide bioassay only moderately increases *An. funestus* s.s. mortality (213). Therefore PBO-nets may not be effective at controlling this species in Magude district.

The success of larviciding relies on larval habitats being few, fixed and findable (7). *An. arabiensis* prefers temporary, sunlit, clear, and shallow aquatic breeding habitats (214), which can be numerous during the rainy season, but will be few during the dry season. As mentioned

before, almost a third of malaria transmission during the Magude project occurred in the dry season and hence larviciding could be an effective tool to complete protection by ITNs and IRS during this season. In addition, larviciding can target other vector species whose behaviors (e.g. outdoor biting or resting) cannot be effectively target by IRS and ITNs, such as *An. squamosus, An. merus* or *An. quadriannalus*, provided that their main larval habitats can be found.

Given the precarity of housing in Magude, house screening with untreated materials could have provided great benefits in the district. In contrast to ITNs, this intervention does not depend on human use and, if well maintained, its efficacy will not decay over time as that of ITNs or IRS. As such, it could have provided high and longer lasting protection, also covering the low transmission season, a season neglected during the project as efforts focused on implementing MDA and IRS on the high transmission, and a season during which ITN protection was low due to poor ITN use.

There are several other interventions that are currently under development or evaluation and that could have increased protection against malaria infection during the Magude project. One such intervention that can reduce residual exposure to vector bites, especially indoors, are lethal house lures. A clinical trial conducted in Côte d'Ivoire showed that the combination of house screening and eave tubes reduced malaria clinical cases in an areas where *An. coluzzii, An. gambie s.s.* and *An. funestus* were the main vectors and are resistance to almost all insecticides commonly used for vector control (215,216). Such results cannot be extrapolated to Magude because the main vector is *An. arabiensis* and it is susceptible to pyrethroids, but a new clinical

trial is planned for Tanzania, which could provide more applicable evidence (211). Another intervention that could potentially reduce the number of mosquitoes of several species that feed both indoors and outdoors are attractive targeted sugar baits (ATSB). This intervention has demonstrated to reduce *An. coluzzii* and *An. gambiae s.s.* densities, survivorship and infection (217), but no clinical trials have yet been conducted to prove their epidemiological impact. Like lethal house lures, the evidence cannot be extrapolated to Magude due to the differences in vector species composition but two planned clinical trials, one in Kenya and one in Zambia, could provide more applicable evidence. Another relevant intervention is the release of sterile *An. arabiensis* males. This intervention could provide protection against transmission both indoors and outdoors and is in the process of being piloted in KwaZulu-Natal, South Africa (bordering with southern Mozambique) where *An. arabiensis* is also the main vector of transmission, malaria incidence is low and IRS is otherwise used for *An. arabiensis* control (218). If the results are positive, it could be tested in Magude given the similarities between the two settings. Finally, zoo-prophylaxis is likely not applicable to this district as less than a third of the households owns cows and less than 10% pigs, although ongoing evaluation of this intervention in Mozambique could provide more insights into its potential use.

7.6 Comparison between the Magude project and it closest relative, the Garki project

This part of the discussion responds to research question RQ8.

Compared to the Garki project (99), the Magude project presented several advantages to achieve malaria elimination: 1) it was implemented in a low transmission area, 2) the area had free diagnosis and treatment for all malaria cases presenting at health facilities or to community health workers, 3) the project interventions (IRS and MDA) where implemented on top of mass distributed ITNs, an intervention that was not available at the time of the Garki project, 4) the IRS insecticides used in the Magude project (i.e. DDT and pirimiphos-methly) had a much longer residual efficacy (as measure through WHO cone bioassay) than propoxur used in the Garki project (6 months compared to 2-4 months). The Magude project also had some disadvantages: 1) the prophylactic drug used in the Magude project (DHA-PQP) required three doses compared to sulfalene-pyrimethamine used in the Garki project, which only required one dose, and only the first dose was humanly supervised.

The epidemiological results of both projects cannot be properly compared because the Magude project did not have a control area, whereas the Garki project did, and as such the methods to evaluate the projects' epidemiological impacts were not the same. However, both projects achieved significant reductions in malaria transmission but did not completely interrupt it. In both projects, transmission continued during the dry season and there was an increase in *P. falciparum* prevalence during the wet season of the second year, despite MDA coverage not

being much different than in the first year. This was attributed in both Garki and Magude to an increase in vector densities due to the favorable conditions for vector breeding.

An. arabiensis was the main vector in both projects, but although *An. gambiae s.s* was present in Garki this species was virtually absent in Magude. *An. funestus s.s.* was a vector in both projects. *An. funestus* s.s. bit earlier in the Magude project (early evening times) compared to the Garki project, but *An. arabiensis* bit mainly during the night in both projects. IRS had a stronger effect on *An. funestus* than *An. gambiae s.l.* and a similar effect during both intervention years in both projects.

The present thesis and previously published analysis suggest that there are several similar reasons that can explain the failure to interrupt local transmission in both projects, but that others reasons differ (109). The Garki project attributed failure to interrupt transmission to the high levels of vectorial capacity, to the outdoor resting nature of *An. gambiae s.s.* and *An. arabiensis* and to that fact that full effective intervention coverage could not be achieved. The fact that the Magude project was implemented in an area of low transmission excludes the high levels of vectorial capacity as a reason for failure. Of the other two reasons provided by the Garki project, the ability of a proportion of vectors to evade interventions seems to be a common challenge in both projects. In the Garki project, a significant proportion of *An. gambie s.l*., specially of *An. arabiensis*, were found resting indoor. In the Magude project, a significant proportion of residual human exposure to vector bites occurred outdoors (12.7%) or indoors at early times when people were not under the net (23.3%). Regarding the last reason, i.e.

attainable total coverage, the Magude project achieved similar IRS coverage but lower MDA coverage than the Garki project. In addition, the fact that the prophylactic drug of the Magude project required 3 doses and that only one was supervised may have further hampered MDA efficacy due to the need for adherence to treatment. In addition, MDA during the Magude project was only conducted during the rainy season and twice per season, while it was conducted at much higher frequency and during the dry season during the Garki project. It can be therefore expected that the suboptimal MDA coverage was a stronger reason for failure in the Magude project compared to the Garki project.

Entomological surveillance methods were very different in both projects. As the main vector of transmission in both projects was *An. arabiensis,* the entomological surveillance method used in Garki were more appropriate than those used in the Magude project. As discussed before, *An. arabiensis* has been observed to enter houses to feed but to frequently rest outdoors (193,194), it presents varying degrees of zoophagy and outdoor biting behavior in different areas (155,190–192), which allows it to partially evade the current core vector control intervention (i.e. ITNs and IRS). Therefore, local measurements of its biting behavior, resting behavior and blood preference are important to estimate the impact that different vector control interventions have on *An. arabiensis* and to understand reasons for unexpectedly low impact when it happens. While the Garki project measured vectors' indoor resting behavior (using PSC), biting behaviour (using HLC), house existing behavior (using Exit traps), feeding preferences (using precipitin tests) and tried to measure outdoor resting behaviour (using outdoor resting collections), the Magude project only measure biting behavior and using CDC light traps instead

of HLC due to local risk of arbovirus transmission (219,220) The use of other collection methods during the Magude project would have highly helped understanding the amenability of *An. arabiensis* to control by the interventions implemented during the Magude project.

The Garki project had one control arm, one arm with IRS and two arms with IRS+MDA at different administration regimes, allowing for the comparison of the efficacy of one intervention alone versus their combination. In contrast, the Magude project had only one arm with IRS+MDA+ITNs, which prevented the evaluation of the impact of individual interventions. In contrast to the Garki project, the Magude project did not have a control, which made the quantification of entomological and epidemiological impacts more challenging than in the Garki project. The long epidemiological baseline available for the Magude project made it possible to quantify the epidemiological impact of the project through an interrupted time series analysis (109). In contrast, the entomological baseline covered only three months of the dry season, making it impossible to accurately quantify the entomological impact of the implemented intervention during the Magude project.

8. Conclusions

1. *An. arabiensis* was the main vector of transmission during the Magude project. *An. funestus s.s. An. parensis*, *An. merus*, *An. squamosus* and *An. rufipes* likely played a secondary or sporadic role. Other species known to be vectors in Africa were also identified. Confirming the role of these and of the secondary vectors should be prioritized in future studies.
2. *An. arabiensis* was amenable to control by IRS and ITNs, *An. funestus* and *An. parensis* were amenable to control by IRS but not to ITNs. The amenability of other tentative vectors such as *An. merus* and *An. squamosus* could not be evaluated due of the low numbers of mosquitoes collected.
3. More than 85% of residual exposure to host-seeking vectors during the Magude project occurred indoors and a third occur during the traditional low transmission season, which is often ignored in malaria control and elimination programs.
4. ITN protection during the Magude project was suboptimal, especially during the low transmission season, and unequal across Magude residents. People living in hard-to-reach areas, poorer and larger households, and young males presented poorer access and use. Access decreased rapidly over time due to high attrition rates.
5. ITN distribution strategies for ITN campaign should be revised to ensure that all household are reached and provide equal ITN access for all, regardless of their sex, age, wealth and the place where they live.

6. Increasing ITN access would have likely increased ITN use, but community mobilization campaigns would have been needed to increase ITN use, especially in the low transmissions season, and to encourage people to share nets. These campaigns should especially target young male and school age children, as these population groups presented the lowest ITN but are important reservoirs of malaria transmission.
7. The coverage of the IRS campaigns implemented during the Magude project met the WHO recommended targets. However, its realized residual efficacy did not remain above optimal levels (80% mosquito mortality in cone bioassays) for the entire high transmission season. A second round, or a longer lasting IRS formulation would have been needed to cover this season.
8. The current methods to estimate IRS residual efficacy in the field should be revised, as our novel method suggest that the realized efficacy of IRS in the field is much shorter than that estimated by WHO cone bioassays alone.
9. Deploying ITN and IRS had benefits on vector control during the project, especially on the control of *An. funestus* s.s. and *An. parensis* and deploying IRS in combination with ITNs can improve ITN use.
10. Additional vector control intervention would have been needed to prevent the o 36.7% of human exposure to host-seeking vectors that was left unprevented. These interventions should prioritize targeting the indoor environment and human exposure to *An. arabiensis.* Larviciding, ATSBs, house screening, lethal house lures and release of sterile *An. arabiensis* may be suitable interventions for Magude, but more evidence

on their epidemiological impacts needs to be gather before deploying them programmatically in the district.

11. The ability of vectors to evade interventions due to their outdoor or early biting and resting behaviors and suboptimal MDA coverage was a common challenge for the interruption of transmission in both projects, but MDA coverage was lower in the Magude project and its dose regime more vulnerable to population adherence.
12. Sporozoite rates or entomological inoculation rates are hard to calculate in low transmission or elimination settings due to the low numbers of mosquitoes collected, and due to the decreased chances of finding infectious mosquitoes after several rounds of MDA. However, calculating biting rates adjusted for human behavior and the use of longitudinal time series analysis to correlate vector densities and malaria incidence can help identifying vectors that drive residual malaria transmission.
13. The Magude project did not have a sufficiently long preparatory phase, baseline or control district. Projects like the Magude project should include a control district or a long-enough baseline to properly quantify the epidemiological and entomological impact of interventions.

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