Monitoring and control of *Anopheles* vectors in the context of residual malaria transmission in Ulanga, Tanzania

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For my children Eddah, Naboth and Eleanor

In loving memory of my aunt Nereah, grandmother Catherine, and friend Dr. Terry O'Reilly

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Summary

The global burden of malaria is disproportionately high in sub Saharan Africa (SSA) where prolific cases and deaths affect some of the world's poorest populations. However, historically, malaria was widespread in virtually all habitable regions of the world. The current global map of malaria risk can be attributed to three factors: (i.) SSA was largely sidestepped during the Global Malaria Eradication Programme (GMEP), the first global attempt to interrupt completely widespread endemic malaria transmission. (ii.) Majority of SSA countries lack adequate resources and robust health systems that can support effective and consistent malaria control programmes necessary for malaria elimination. (iii.) A hot-humid tropical and sub-tropical climate supports breeding of some of the most effective malaria vector populations. In addition, designs of most traditional African huts particularly in the rural settings where malaria transmission tends to be high often allow easy entry of mosquitoes through openings on doors, windows and eaves. Such houses have often been associated with increased human-mosquito interactions and risk of malaria. Many African cultural traditions, perceptions, beliefs and practices have been found to be counter-effective to malaria control initiatives. For example, gatherings outdoors in the evenings during events such as funerals tend to expose people to mosquito bites and greatly undermine the effectiveness of ITNs.

Across many high burden areas, measures to protect people from malaria infection include use of insecticides treated nets (ITNs) and the indoor residual spraying (IRS) with insecticides. In areas where campaigns by the two core interventions have been implemented effectively, there has been dramatic reductions of malaria transmission and a general decline of the burden of disease. However, despite being highly effective, vector control faces some major challenges that primarily include resistance to insecticides by local vector populations and mosquito behavioural avoidance of the indoor-based insecticidal interventions typically manifested by increased outdoor biting. The World Health Organisation (WHO) warns of stagnated global

progress of malaria. Evidence suggests reduced effectiveness of ITNs and IRS across malarious parts. There is increased scepticism by malariologists regarding malaria elimination by the status quo majorly through relying heavily on ITNs and IRS. Going forward, the WHO encourages review and reassessments of the effectiveness of ITNs and IRS across malarious areas and recommends efforts to determine gaps in effective protection against malaria vectors in the context of universal coverage by the core vector control tools.

This PhD took advantage of population-wide ITN and IRS studies in Ulanga, a rural area in south-eastern Tanzania, one among the highest burden countries, and investigates, discusses and reflects on the effectiveness of the core vector control and potential of residual malaria transmission.

Findings of this PhD suggest that in a typical SSA setting where malaria transmission is endemic and where transmission is primarily by *An. funestus* and *An. arabiensis* that bite both indoors and outdoors, ITNs afford high protection against malaria transmission. Malaria control programmes should therefore ensure high household ownership and use of efficacious ITNs across all malarious areas. Strategies that promote high use of ITNs in the households by each member over all the times when they are in their sleeping spaces at night need to be encouraged to guarantee optimal protection by ITNs for everyone in the population.

IRS with the use of effective insecticides such as clothianidin and that function by a different mode of action from that of pyrethroids can be employed to mitigate the spread of *Anopheles* resistance to pyrethroids and help complement ITNs to drive higher effectiveness for vector control and greater impact on malaria transmission. Appropriate IRS deployment strategies need to be employed to ensure that pyrethroid insecticides are not used alongside ITNs, and that insecticides with similar modes of action are not used in the same locations but instead insecticides with dissimilar modes of action are used alternatingly or in combinations.

Sampling tools for estimating human biting by local malaria vector populations need to be considered on a case-by-case basis appreciating fundamental limitations of exposure-free mosquito traps for specific entomological survey tasks in different settings. HLC should be preferred over exposure-free traps where the purpose of surveying mosquitoes is to quantify absolute estimates of malaria risk more specifically the EIR. HLC-standardised entomological metrics estimated from catches by exposure-free mosquito traps may be used for evaluating malaria vector control interventions and for monitoring changes in behaviours in *Anopheles* populations including possible shifts in species composition, biting behaviours and occurrence or spread of insecticide resistance.

List of abbreviations

HLC	human landing catch
HDT	human/host decoy trap
CDC LT	United States of America centres for disease control and prevention light trap
EIR	entomological inoculation rate
EHT	experimental hut trial
RCT	randomised control community trial
WHO	World Health Organisation
ITN	insecticide treated net
IRS	indoor residual spraying
DDT	dichloro-diphenyl-trichloroethane
s.s.	sensu stricto
s.l.	sensu lato
An.	*Anopheles*
GMEP	Global Malaria Programme
DALYS	disability-adjusted life years
PBO	piperonyl butoxide
HBI	human blood index
USD	United States of American dollar

US CDC	United States of America Centres for Disease Control and Prevention
SSA	sub Saharan Africa
COVID-19	coronavirus disease 2019

1. Introduction

1.1 The global burden of malaria

Malaria remains a disease of leading global public health importance with an estimated 3.5 billion people at risk worldwide. According to the latest World Malaria Report, approximately 247 million cases and about 619,000 deaths due to malaria were reported across 84 countries where transmission was indigenous in 2021. These estimates went up compared to 2019 by an estimated 15 million malaria cases and about 51,000 malaria deaths attributed mainly to programme disruptions by COVID-19 during the 2020-21 pandemic peak transmission years [1]. The world's youngest and poorest are the most vulnerable to malaria with about two-thirds of deaths occurring in children below the age of five years, mostly from the poorest nations [2].

The global burden of malaria is highly concentrated in a few countries in sub Saharan Africa (SSA). Although nearly all of SSA population is at risk of malaria, accounting for over 95% of the global malaria cases and deaths, just 10 out of the region's 46 countries bear the greatest burden [3]. Nigeria, Niger, Mali, Ghana, Burkina Faso, Cameroon, Tanzania, the Democratic Republic of the Congo, Mozambique, Uganda, all in SSA, and India, contributed over 68% and 70% of the global malaria cases and deaths, respectively in 2021 with about half of all malaria cases and deaths reported from just four SSA countries [1]. Nigeria bore the greatest burden contributing over 26% and 30% to the global cases and deaths, respectively.

Malaria hits hardest at the most biologically susceptible in the at-risk population [4]. Usually, children <5 years and pregnant women face a higher risk of worse malaria health outcomes attributed to insufficient immunity that would normally be acquired from regular exposure to malaria under stable transmission. The most unsettling malaria burden statistic is a high child

mortality [5]. Over 400,000 children died from malaria in 2021, approximating to the death of a child every minute [1]. Over 80% of deaths in SSA were children <5 years. Between 2000 and 2019, malaria accounted for roughly a quarter of all deaths in children in the region with the highest country estimate being 27% in Burkina Faso [5].

In the high burden areas, malaria comprises an important component of maternal and child health [3, 6]. In pregnant women, the effects of malaria include maternal anaemia and death, intra-uterine growth retardation, preterm delivery, stillbirth and low birthweight [7-10]. These effects are usually worsened by co-infections with HIV/AIDS that is prevalent in low income settings [6]. Low birthweight neonates have been shown to face increased risk of death [11, 12], and higher probability of long-term developmental problems including subnormal growth, illnesses, neurodevelopmental challenges [13]. Roughly 880,000 still births [14] and 1.2 million neonatal deaths [15] occur each year in SSA. Between 75,000 to 200,000 infant deaths are associated with malaria infection in pregnancy in the same region [16]. Across 38 SSA countries with moderate to high malaria transmission but where malaria control interventions are also present, 13 million pregnancies had malaria that resulted in about half a million low birthweight neonates in 2021 [1]. Poor maternal and child health including infant deaths is exacerbated where there is failure to apply known effective antimalarial interventions through antenatal programmes [16].

Besides loss of lives due to malaria, clinical episodes directly affect the quality of life. A systematic review covering 10 years of 291 diseases across 21 regions found malaria to have been the seventh largest contributor to Disability Adjusted Life Years (DALYS) — the sum of years of life lost and years lived with disability, even though the global disease burden over the 10 year period had shifted dramatically from communicable to non-communicable diseases [17]. Malaria impedes attendance of school and physical activity in children and participation

in socio-economic endeavours by adults [18-20]. The problem caused by malaria in children living in areas with intense transmission was recently well demonstrated though the impact of transmission prevention. In a long-term study in Tanzania, increased survival to adulthood and better chance to education and general success in life was observed among children <5 years who used mosquito nets, the primary malaria intervention in the country [21, 22].

Arguably, malaria is a tropical disease because nowadays, nearly all global locally occurring cases are reported in the tropics and sub-tropics. However, historically, malaria was widespread in virtually all habitable regions of the world [23]. Resource limitations seem to play a bigger role in malaria transmission. This can be seen from high-income tropical countries like Dubai and Oman, which have both since eliminated malaria. Weak economies often have fragile health systems, under-resourced health personnel, poorly developed medical infrastructure, and are often unable to fund health expenditure sufficiently [19, 20, 24, 25]. The economic stress that malaria exerts on the affected populations often resulting in a vicious disease-poverty cycle makes disease control efforts difficult with limited access to primary health care being a major contributing factor [20, 26, 27]. Malaria certainly is a global disease that remains to be eliminated in the tropical and sub-tropical world due to poverty. Consequently, the global malaria fight agenda must also focus on economic empowerment starting from improving the standards of life of the affected communities [24]. To the extent that malaria control efforts are not completely crippled by funding constraints in the high burden areas, malaria control especially through vector control, the focus area of this thesis, can be improved through appropriate steps that reinforce existing measures while identifying new areas that need supplementary interventions.

1.2 The parasite of human malaria, its lifecycle and the vector

The parasite of human malaria

Malaria is caused by parasitic protozoa of the genus *Plasmodium*. Six *Plasmodium* species known to cause disease in humans are *Plasmodium falciparum, P. vivax, P. ovale curtisi, P. ovale wallikeri, P. malariae* and *P. knowlesi*. *P. falciparum* presents a substantial health threat for humans and presently contributes the highest proportion of malaria cases and deaths globally [1, 28]. *P. vivax* is a major cause of illness across large parts of the world being the most geographically spread [29]. It is increasingly thought that deaths due to vivax malaria have been underestimated previously [30, 31]. *P. ovale curtisi, P. ovale wallikeri*, and *P. malariae* are much less common but pose malaria risk in many parts of the world [28, 32]. *P. knowlesi*, naturally a simian parasite, has emerged as a geographically localised but important cause of human malaria [33-36]. Incidents of severe *P. knowlesi* malaria have recently been reported in Malaysia and other areas of Southeast Asia where the species is predominantly a zoonosis with no definite evidence of transmission within the human population [37, 38].

Plasmodial and plasmodium-like protozoa including members of the genus *Haemoproteus* and *Leucocytozoon* cause malaria as well in other vertebrates. *P. knowlesi* [39, 40], *P. relictum* [41], *P. berghei* [42] and *P. agamae* [43] cause monkey, bird, mice and lizard malarias respectively. Avian malaria, one of the most studied non-human malarias is caused mostly by *Haemoproteus columbae* [41] and *Leucocytozoon smithi* [44]. Non-human malarias are employed to help basic public health research as models to enhance understanding of biology and pathogenesis of the human *Plasmodium* parasites, and played a pivotal role in the history of malariology. For example, Ross's pioneering malaria work involved experiments with *P. relictum* in sparrows and crows [41, 45] whereas the first malaria parasite sexual stages to be demonstrated were of *H. columbae* [46].

Lifecycle of Plasmodium

Plasmodium spp. have a complex life cycle alternating between mosquito and vertebrate hosts in what is commonly also referred to as the extrinsic and intrinsic phases, respectively. Both lifecycle stages involve proliferation and increase in parasite numbers, sexually in the mosquito and asexually in the vertebrate host, an evolutionary feature that makes *Plasmodium* a highly successful parasite. The sexual phase requires simultaneous presence of the male and female gametes in the mosquito. The asexual phase requires formation of unique "*zoite"* forms to invade different host cell types at specific stages (Figure 1). The zoites are important for their role in clinical malaria disease and also bear distinguishing characteristics that help in species identification [45, 47].

Malaria infection in humans begins when (1) *sporozoites* are injected by a blood-feeding mosquito and are carried around the body until they invade liver hepatocytes where (2) they undergo a phase of asexual multiplication also known as exoerythrocytic schizogony, resulting in the production of many uninucleate *merozoites* from each sporozoite. Liver stage parasite of *P. vivax*, referred to as hypnozoites, hide and may remain quiescent for long in the liver cells. Hypnozoites present a critical challenge in *P. vivax* malaria case management as often cause replaces in infection if not cleared by specific medication [48, 49]. Merozoites flood out into the blood and invade red blood cells where (3) they initiate a second phase of asexual multiplication also called erythrocytic schizogony, resulting in the production of about 8-16 merozoites, depending on species, which invade new red blood cells. This process is repeated almost indefinitely and is responsible for clinical disease [50]. As the infection progresses, some young merozoites develop into male and female *gametocytes* that circulate in the peripheral blood until they are (4) taken up by the vector mosquito when it obtains a blood meal. Gametocytes are parasite gametes ensheathed in RBC membrane, this happens as a

mechanism to trick the host immune system. Within the mosquito, (5) the gametocytes unsheathe, and mature into male and female *gametes* referred to as micro- and macro-gametes, respectively [51]. Fertilization occurs when a female and male gametes fuse (6) and a motile *zygote* or *ookinete* is formed within the lumen of the mosquito gut, the beginning of a process known as sporogony. The motile ookinete, exflagellates and (7) penetrates the mosquito peritrophic membrane (outside layer of a blood meal in the mosquito gut) and gut wall and attaches under the mosquito gut basal lamina. The ookinete becomes a conspicuous (8) *oocyst* within which another phase of multiplication occurs resulting in the (9) formation of sporozoites that migrate to the salivary glands of a mosquito and are injected when the mosquito feeds on a new host [52].

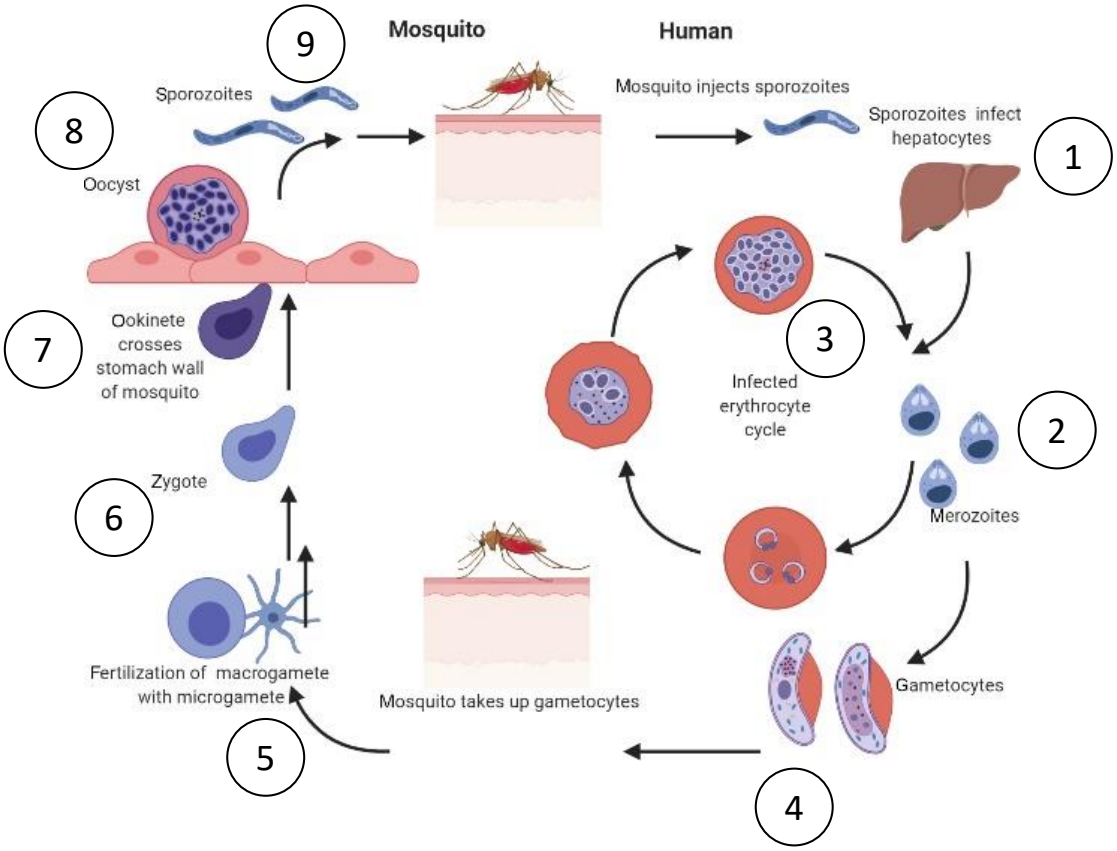

Figure 1. *Plasmodium* lifecycle (adapted from US CDC)

The malaria vector

Human malaria is naturally transmitted through the bite of a female *Anopheles* mosquito carrying malaria parasite sporozoites in her mouthparts. The term '*Anopheles*', Ancient Greek for 'un-' and 'profit'(able) or simply 'useless' first described by the German entomologist Johann Meigen in the early1800s, might have been meant to refer literally to the mosquito's 'uselessness' prior to the discovery of its public health importance. Other sources have since used the term 'harmful' as the better connotation. The role of *Anopheles* mosquitoes in the transmission of human malaria was first described in the late 1800s through the pioneering work of Ronald Ross who demonstrated malaria parasites in the mosquito while dissecting its gut tissue after feeding on a malaria patient. Malariologists would for the first time have a

scientific basis for malaria transmission and be able to explain the effects of traditional malaria control measures such as bed nets and drainage of swamps that had existed for decades before [53].

Known more universally as the 'malaria mosquito', the *Anopheles* mosquito can now be easily identified by the help of existing dichotomous keys of morphological features observable both by the naked eye and by magnifying lenses [54, 55]. Where the mosquito populations exist in species complexes, comprising closely related species that are not morphologically distinguishable, individual sibling species can be distinguished by molecular means [56]. Easily observable features unique to the anopheline eggs, juveniles and adults do not necessarily require an expert eye. The *Anopheles* eggs are boat-shaped and measure about 0.5 by 0.2 mm. They are often laid singly directly on water and are unique in having floats on either side. The juvenile *Anopheles* (larva and pupa) float under the surface of the water to breathe, with the worm-like larva known to lie typically parallel beneath the water surface. The adults can also be identified by their typical resting position, where they rest with their abdomens sticking up in the air at approximately 45° rather than parallel to the surface on which they are resting. The adults are also distinguishable by the palps (sensory organs near their mouthparts), which are as long as their proboscis (mouthparts). If examined carefully, their wings bear discrete blocks of black and white scales on the wings, giving the wings the general dotted appearance.

1.3 *Anopheles* lifecycle and bionomics

Understanding the *Anopheles* biology has played a crucial role in the control of malaria as well as several other mosquito-borne diseases. Today, favourable conditions of *Anopheles* breeding, growth, biting and resting are simulated to rear laboratory strains and to conduct experimental investigations that have contributed enormously to fighting disease transmission. Mosquitoes

breed sexually and their growth undergoes complete metamorphosis, which involves moulting through four discrete life cycle stages, namely, egg, larva, pupa and adult or imago (Figure 2). A complete mosquito lifecycle from egg to egg can be as fast as 10-14 days or may take several months in diapausing, hibernating or aestivating species. The specific features of the mosquito lifecycle vary between groups of mosquitoes but are largely similar in general.

Anopheles eggs are boat-shaped and measure about 0.5 by 0.2 mm. They are usually whitish immediately after oviposition, but gradually turn brown or blackish. The eggs are commonly laid on the surface of water singly, different from culicine eggs that are laid in rafts. Characteristic lateral floats on either sides of the anopheline eggs help keep them afloat. Depending on the species, breeding ground selection varies. Ovipisition sites are selected based on water chemistry and a circadian rhythm. Anopheline eggs are highly sensitive to desiccation and hatch within 2-3 days under conducive climate, typically the hot-humid tropical climate, although they can take up to 2-3 weeks in the cooler climate. Based on these features, high population densities of anophelines are always expected around the rainy seasons, these almost invariably coincide with peaks in disease transmission. In some mosquito species, for instance in the culine *Aedes*, the eggs can withstand desiccation on a damp substrate beside a water body, remain viable for months or even a few years, and hatch when it floods. Therefore breeding site management programmes like drainage of swamps, which have proven important for managing anophelines, may not achieve significant effects for *Aedes* populations. The female mosquito completes 4-5 ovipositions of 30-500 eggs each in a lifetime. Upon hatching, the mosquito egg produces a larva (plural larvae), commonly called the wriggler.

The mosquito larvae are worm-like and inhabit a wide range of aquatic habitats depending on species. With the exception of a few species, larvae come to the surface of water to breathe. The anopheline larvae lies parallel beneath the water surface where it breathes by a pair of

spiracular plates. Culicine larvae, on the other hand, normally have a breathing tube at the posterior end of their bodies. Anopheline larval habitats range from small water pools such as hoof-print collections, water-filled tree holes, leaf axils such as of bromeliad plants, and puddles, to rice-fields and marshy areas including saline marshes and mangrove swamps. Irrigated rice paddies are a common environmental hazard for disease such as malaria because they offer ample breeding spaces for major anopheline vectors. Larvae feed typically on algae and other miniscule organic sediments in water, although the food resources could be quite varied involving a wide range of microorganisms. A variety of insect, fish and bird species feed on mosquito larvae. The mosquito fish, *Gambusia* spp is a typical mosquito larvae predator that has been used in some instances for vector control. In the tropics, the mosquito larvae lasts between seven to ten days during which it develops via a series of four instar stages before finally moulting into the pupa (plural pupae). In cooler climates, larval development may take weeks to months while some species are known to overwinter.

The mosquito pupa, commonly called tumbler is a comma-shaped non-feeding stage. The pupae remain in the same aquatic habitat as larvae. They are commonly active and mostly hang below the surface of water where they breathe by help of a pair of thoracic trumpet-like structures. Pupal development takes two to three days in the tropical climate but may take up to a week in the cooler areas. The adult mosquito emerges as a teneral, possessing the full form of a mature adult except that the exoskeleton or cuticle is not hardened. The mosquito flies away soon as the cuticle hardens (is sclerotised). *Anopheles* mosquitoes naturally do not fly more than two kilometers from their larval habitats. *Anopheles* mosquitoes make their public health impact at the adult stage where they bite humans and transmit malaria.

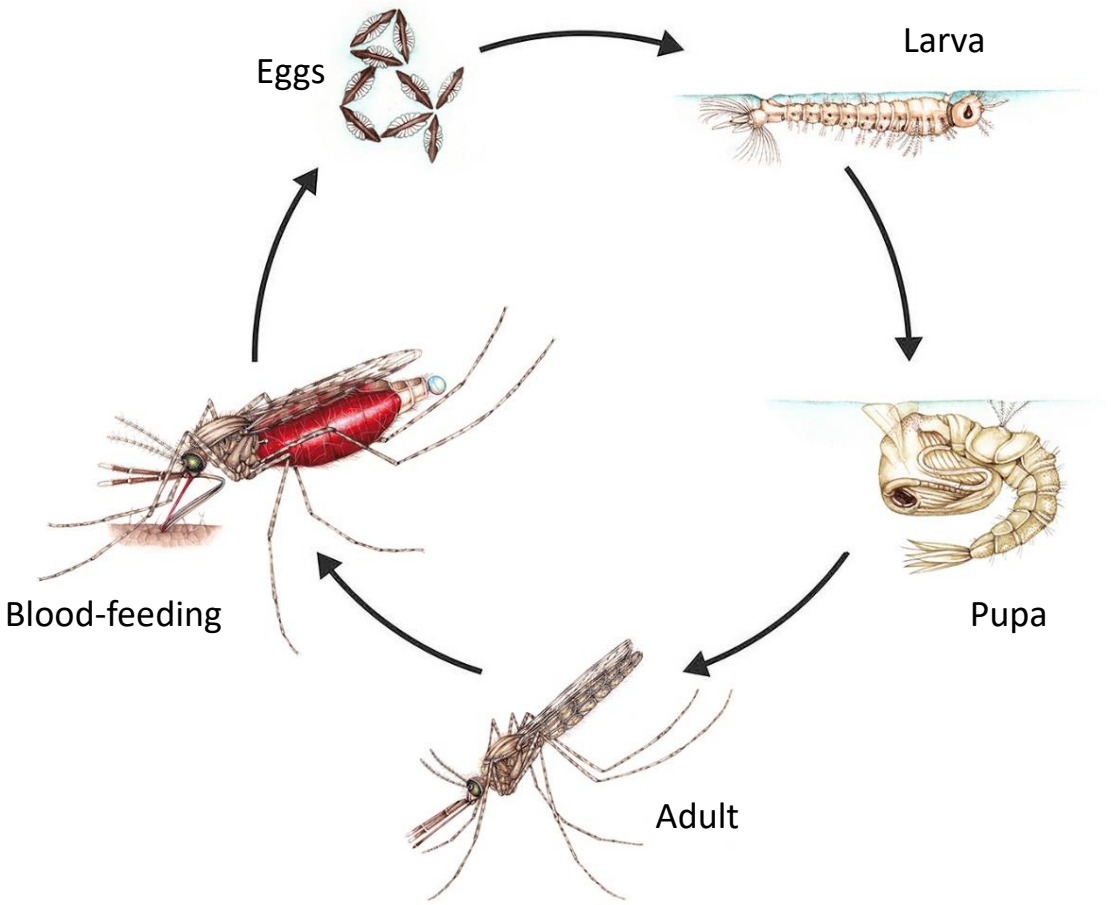

Figure 2. *Anopheles* lifecycle (Adapted from Lizzie Harper: Natural History Illustration for Books, Magazines and Packaging)

Besides malaria other pathogens of well-known human and zoonotic diseases such as lymphatic filariasis, yellow fever, dengue hemorrhagic fever, West Nile fever, Rift valley fever, chikungunya, heartworm disease, subcutaneous dirofilariosis and encephalitis are also transmitted by mosquitoes of other species [57, 58]. However, not all mosquitoes are capable of transmitting disease or inflicting nuisance bites to humans or animals. Just about 3% (120 species) of all known mosquito species have been studied in depth as being of global public health significance [59].

About 400 species of *Anopheles* mosquitoes have been described of which roughly 100 are known malaria vectors. Just about 30-40 *Anopheles* species carry the greatest malaria transmission importance. Across most high burden countries, members of the *Anopheles gambiae* (*An. gambiae* sensu lato [s.l.]) and *An. funestus* (*An. funestus* sensu lato [s.l.]) complexes cause the greatest magnitude of disease transmission. *An. gambiae* s.l. comprises of seven sibling species, namely *An. gambiae* sensu stricto (s.s.), *An. arabiensis*, *An. merus*, *An. melas*, *An. bwambae*, *An. quadriannalatus* A and *An. quadrianalatus* B. *An. funestus* s.l. comprises of the *An. funestus* s.s., *An. rivulorum* and *An. leesoni* [60] .

Anopheles populations can be characterised based on breeding, biting and resting behaviours. Breeding sites are associated with water sources due to the aquatic lifestyles of mosquito juveniles. Permanent fresh water sources such as rivers, lakes and perennial floodplains are preferred by *An. funestus* s.l., *An. gambiae* s.l. except *An. melas* and *An. merus*, which breed in salt water and *An. arabiensis* that prefers transient water sources such as rice paddies and seasonal floodplains. *An. arabiensis* breeding in small water sources such as water collections in bromeliad leaves, litter cans, disposed car tires and animal hoof prints. Most *Anopheles* species predominantly bite humans at night and rest indoors (endophilic) on house walls and roofs and places like hanging clothes. Other species such as *An. arabiensis* have feeding and resting habits that are more dynamic. They prefer outdoor spaces (exophilic) and bite animals (zoophagic) in addition to humans. Usually, *An. arabiensis* bites around dusk (crepuscular biting) when humans are still outdoors, or bites indoors and exits immediately to rest outdoor (post-prandial exophilic behaviour).

1.4 The epidemiology of malaria

The risk of malaria infection is generally spread across all areas with a warm-humid climate and ground water sources ideal for breeding of the vector mosquitoes and a parasite pool in the

human and mosquito populations. The tropical and sub-tropical regions of the world normally carry a high malaria burden due to optimal temperatures and rainfall that support mosquito breeding and a high capacity of the mosquito vector to sustain parasite development. Within these limits, malaria transmission intensity is determined by frequency of contact between infected mosquitoes and humans. Therefore, changes in mosquito population densities with weather conditions directly influence the transmission intensity of disease. Differences in geological features such as topography that influence rainfall and temperature affect the risk of malaria. Higher altitude commonly has reduced risk of malaria due to cooler temperatures whereas higher transmission may be experienced with lower elevation, for instance in the often warmer floodplains. Similarly, temporal changes in weather across the year equally influence the risk of malaria. Seasonality of malaria transmission is a critical forecasting component of malaria control planning.

Malaria vectors across many high burden areas mostly bite humans at night indoors when they are sleeping [61, 62]. The mosquitoes have evolutionarily adapted a nocturnal lifestyle primarily to protect their delicate cuticle from desiccation by the typically hot diurnal weather. In daytime, the mosquitoes exhibit reduced activity and often rest in cooler areas inside and around human shelters, mostly on inner walls of the house. Increased mosquito activity including host-seeking and biting begins at dusk when day temperatures start to cool off and extend for most of the night stopping at dawn with onset of hostile day weather, this often corresponds well with availability of human hosts inside and around houses [63]. The primary malaria prevention tools exploit the typical nightlife of the vectors by protecting sleeping humans using mosquito nets and by spraying walls and inner surfaces of houses with insecticides. Whether or not the mosquitoes also exhibit diurnal biting during cooler days or inside houses under shelter is little known, but a recent finding of up to a third of malaria vector biting activity during daytime in the Capital of the Central African Republic [64] has sparked

debate [65]. Outdoor biting by malaria mosquitoes on the other hand is common and its link to malaria risk has been well-elaborated [66-71]. Mosquito biting activity outside the limits suitable for protection by the core indoor-based vector control interventions creates a gap in the effective protection against malaria.

Human exposure to malaria is not limited to the behaviours of mosquitoes, but of humans as well. Within a population living in an endemic area, it is common to identify groups of people with increased risk of malaria episodes. A major component of the anthropological risk factors of malaria involves use of vector control interventions. Habitual practises such as sleeping outdoors at night or staying longer indoors before retreating to sleeping spaces for the night's sleep often impede effective use of mosquito nets and may lead to increased contacts with mosquitoes [72]. In many African cultures, ceremonies such as funerals and weddings involve outdoor gatherings at night. Incidental exposure to malaria mosquitoes during socio-cultural activities is often a major challenge for malaria control due to lack of effective protection applicable outdoors and the almost inevitable nature of such traditional practises. However, sometimes the ITNs are not sufficient to cover all members of a household. For instance, ITN access in the household is recently shown to be biased to the disadvantage of school-aged children in many areas, likely due to a focus on pregnant women and children <5 years in turn resulting in increased risk in this group.

The intensity of malaria transmission is critical in determining the clinical picture of disease in at-risk populations. In areas where the risk of infectious bites is low and unpredictable, malaria transmission is said to be unstable. Under such settings, all ages are susceptible to worse infection outcomes, and in general, all infective episodes will result in clinical disease. However, if the intensity of malaria-infective bites is consistently moderate or high, transmission is termed stable and human exposure becomes more frequent. In the long-term

transmission becomes more consistent from year-to-year and exposed individuals begin to develop a degree of immunity. Infant and maternal malaria burden is commonly higher in areas of intense stable transmission because these groups often lack sufficient immunological protection compared to others. Under low unstable transmission settings, the incidence of febrile malaria is often higher as the bulk of the population often has low malaria immunity [73]. The risk of severe malaria and death is highest in children <5 years and declines rapidly with age [47].

1.5 Malaria control

The malaria control strategy could be broadly classified based on whether the goal is control or elimination. The WHO defines Malaria Control as the "Reduction of disease incidence, prevalence, morbidity or mortality to a locally acceptable level as a result of deliberate efforts" while elimination is defined as the "Interruption of local transmission (reduction to zero incidence of indigenous cases) of a specified malaria parasite in a defined geographical area as a result of deliberate activities" [74]. This thesis focuses on intervention approaches under malaria control settings.

Key malaria control interventions

The Global Technical Strategy (GTS) for Malaria 2016-2030 outlines three key pillars of malaria control: (i.) Universal coverage of malaria prevention, diagnosis and treatment. (ii.) Acceleration of malaria elimination and attainment of malaria free state. (iii.) Elevation of surveillance as a key malaria intervention [75]. The primary components of malaria control are (i.) case management and (ii.) prevention. Here, several approaches that target to interrupt disease transmission from both ends of the *Plasmodium* lifecycle in the human and vector hosts are employed. Malaria prevention involves (i.) vector control, (ii.) chemoprophylaxis, (iii.) preventive chemotherapies and most recently (iv.) vaccine. Malaria case management

comprises two primary components: (i.) diagnosis and (ii.) treatment. Malaria elimination primarily involves malaria vector surveillance in addition to the malaria control components deployed at more settings-specific scales and implemented in two phases: (i.) pre-elimination and (ii.) prevention of reintroduction [76].

A. Malaria case management

a) Diagnosis

The WHO advises early diagnosis and treatment of malaria cases in order to reduce the incidence of clinical malaria, prevent deaths and interrupt onward transmission. Malaria treatment best practice requires that all the suspected cases of malaria be (i.) confirmed using parasite-based diagnostic testing by either microscopy or a rapid diagnostic test, ideally from finger prick blood and (ii.) only confirmed cases be treated with an efficacious antimalarial. Malaria infection advances a debilitating disease that if left untreated could cause death. Hence, effective treatment of all confirmed malaria cases is a highly valuable life-saving measure.

b) Treatment

Several factors guide the choice and dosage of antimalarial for treatment. These include (i.) the malaria parasite species, (ii.) whether a malaria parasite is resistant to a medicine, (iii.) the weight of the person infected with malaria to decide on dosage and (iv.) whether the person is pregnant. For *P. falciparum* especially, but also *P. vivax* and *P. knowelsi* infections, in addition the severity of a malaria episode is taken into account [30, 77, 78]. The WHO-recommended antimalarials [79] are: (i.) Artemesinin-based combination therapy (ACT) drugs such as artemether-lumefantrine, which comprise the first-line treatment of uncomplicated malaria for most endemic countries, and have been shown in a meta-analysis to be highly effective in clearing systemic infection and gametocyte carriage, causes of clinical malaria and mosquito

infection, respectively [80]. (ii.) Quinine with clindamycin is used for treatment of malaria in the first trimester of pregnancy. If unavailable or fails, an ACT or oral artesunate with clindamycin may be used (iii.) Chloroquine is recommended for treatment of *P. vivax* infection but only in places where the parasite is still susceptible to the drug. (iv.) Primaquine should be added to the main treatment to prevent relapses of infection with *P. vivax* and *P. ovale* parasites. Single low-dose primaquine is also added to *P. falciparum* treatment course to clear late-stage gametocytes. Antimalarials are mostly in a pill form for uncomplicated malaria, and in injectable form (parenteral) for severe malaria episodes, which requires hospital attendance with close medical supervision [79].

A severe malaria case is considered a health emergency deserving a robust response [81]. Management of severe malaria involves more than treatment with antimalarials. A comprehensive set of procedures are outlined depending on the presentation of the severe malaria episode [81]. The treatment of *P. falciparum* severe malaria cases, common in children <5 years, takes the form of parenteral antimalarial chemotherapy, which include: (i.) parenteral artesunate administered intramuscularly or intravenously and is the first-line severe malaria treatment. (ii.) In the absence of parenteral artesunate, artemether is used as an alternative treatment in preference to (iii.) quinine. Follow-on oral treatment is effected with a full course of effective antimalarials, ideally ACT to prevent parenteral monotherapy, as soon as the patient is able to take oral medication but not less than 24 hours of parenteral treatment. Due to the high fatality rate of severe malaria, pre-referral treatment is advised by (i.) intramuscular artesunate, artemether or quinine, or (ii.) rectal artesunate for children <6 years of age [81], especially if the patient cannot reach a health facility quickly where full treatment can be offered.

Resistance to antimalarials is a critical limitation to malaria case management, hence adhering to the WHO guideline [82] and staying updated and strictly implementing proper mitigation steps is highly recommended.

B. Malaria prevention

a) Preventive chemotherapies

Preventive chemotherapies comprise the use of antimalarial drugs, either alone or in combination, to prevent malaria infections and related health outcomes. They also help in clearing existing blood-stage infections in the human host and preventing resulting clinical episodes and other effects for instance, on the unborn child [79]. It requires administration of a full course of an antimalarial to vulnerable groups at designated times when the greatest malaria risk occurs indiscriminate of whether the individuals have a detectable malaria infection. The common forms of preventive malaria chemotherapies are (i.) perennial malaria chemoprevention (PMC), (ii.) seasonal malaria chemoprevention (SMC), (iii.) intermittent preventive treatment in pregnancy (IPTp) and school-aged children (IPTsc), (iv.) post-discharge malaria chemoprevention (PDMC) and (v.) mass drug administration (MDA). Preventive chemotherapy ideally is intended to complement ongoing malaria control activities, most importantly vector control, proper and prompt diagnosis of suspected malaria, and treatment of confirmed cases with effective antimalarials [79].

b) Chemoprophylaxis

Chemoprophylaxis is the principal malaria prevention tool for people visiting endemic areas who do not normally live in malarious areas and are there without malaria immunity [79]. Chemoprophylaxis seeks to arrest progression of infection to malaria disease by interrupting *Plasmodium* lifecycle at the early stages. Malaria chemoprophylactic drugs target liver and blood stage schizonts or hypnozoites. The intervention does not prevent mosquito bites hence

malaria infection may still occur. Therefore, proper, highly efficacious drugs are required for the intervention to function effectively. The three most commonly prescribed chemoprophylaxis medications include atovaquone-proguanil, doxycycline and mefloquine. As a best practise, the WHO advises travellers of endemic areas to consult their doctor several weeks before departure. The specific medications depend on malaria health policies of countries visited [83].

c) Vaccines

Vaccines are the latest addition to the list of preventive interventions of malaria. Starting October 2021, WHO recommends wide-scale use of the RTS,S/AS01 (RTS/S) malaria vaccine among children living in regions with moderate to high *P. falciparum* malaria transmission. This came after a long history of searching [84] and successful and promising results from clinical trials across seven countries including four high burden countries [85]. The trials showed that among children receiving four doses, the vaccine reduced one in every four cases of malaria episodes and one in each three severe malaria cases over a four-year period. A follow-up of the children who participated in the RTS,S vaccine trials intended to assess long-term effects showed that the incidence of severe malaria decreased as children got older, regardless of whether children received the vaccine and that there was no evidence of rebound of severe malaria following the recommended four doses of the vaccine [86]. The greatest challenge of vaccines is a high cost of production. Going forward, hopefully access to malaria vaccines can be improved by addressing initial production costs with lower-budget technologies for example, for low-income countries [87]. The R21, PfSPZ and *P. vivax* are at various research and development, and WHO approval stages and will hopefully in the near future help reduce the malaria vaccine gap further.

d) Malaria vector control

Malaria vector control primarily takes the form of mosquito bite prevention by insecticide treated nets (ITNs) and indoor residual spraying (IRS) of insecticides. Use of insecticides aims to reduce the number of *Anopheles* mosquitoes carrying sporozoites by ensuring high enough mortality to suppress population average age of mosquitoes below the parasite extrinsic incubation period or to weaken surviving/resistant mosquitoes to the extent that successful parasite development in the mosquito is impeded [88-90]. Campaigns with the two interventions are usually informed from behaviours of local malaria vector populations, particularly insecticide resistance, and whether a programme is targeting control or elimination [75, 91, 92]. Although the actual level of protection offered by the combination of ITNs and IRS is unclear [93], the two interventions are proven to offer substantial malaria protection where the majority of the vector population feeds and rests inside houses, the vectors are susceptible to the insecticide that is being deployed and people mainly sleep indoors at night. In addition, IRS success depends on conditions that the malaria transmission pattern is such that the population can be protected by one or two rounds of IRS per year, the majority of structures are suitable for spraying and structures are not scattered over a wide area, resulting in high transportation and other logistical costs [94].

However, even with complete coverage by ITNs and IRS, occurrence of vector activity outside the limits of the indoor-based measures causes risk of residual malaria transmission that must be addressed by supplementary tools addressed later in this thesis.

i. Insecticide treated nets

An insecticide treated net (ITN) is a mosquito net whose fabric is impregnated by a chemical designed to kill mosquitoes. Standard ITN treatment consists primarily of pyrethroids, commonly known as standard pyrethroids-only ITNs. An ITN may have treatment with an

additional chemical to the pyrethroids, these are called dual-active ingredient ITNs or new generation ITNs. Three agents have been commonly used so far for the new generation ITN treatment. They include: (i.) piperonyl butoxide, a pyrethroids synergist, (ii.) chlorfenapyr, a pro-insecticide and (iii.) pyriproxyfen, an insect growth regulator. There are two broad categories of ITNs used for protection against malaria presently, based on treatment longevity. The first category consists of ITNs with temporary insecticide impregnation that requires repeated treatment over its lifespan. The second category of ITNs known as long-lasting insecticidal nets (LLINs) contain treatment that lasts its lifespan, ideally three years, and requires no re-treatment by the user.

Use of ITNs is the WHO-recommended first-line intervention for malaria prevention across all endemic areas. ITNs have significant benefits as has been increasingly demonstrated by trials and descriptive studies across high burden areas [21, 22, 93, 95]. A meta-analysis including five randomised-controlled trials found an overall reduction in all-cause child mortality of 17%, with six lives saved per year for every 1000 children protected [95]. The study showed that ITNs protected 60% of severe malaria cases in children using them compared to those using untreated nets. A high impact of ITNs stems from their capacity to prevent potentially infective mosquito bites by physically blocking attempted attacks on users as well as killing susceptible mosquitoes that interact with them. Under wide-coverage, the killing effect of ITNs has a great potential to reduce mosquito numbers drastically if the mosquitoes mostly bite indoors. With reduced mosquito numbers and potentially mosquito infection, so that the infectious bite risk is substantially reduced, the ITNs attain their communal protective effect by protecting users at times when their use is impractical as well as protecting individuals who do not sleep under them. The communal protective effect of ITNs is the underlying basis for the push for their universal coverage. ITNs may also directly prevent other vector-borne diseases where they occur in malarious areas particularly the lymphatic filariases, which are common malaria

comorbidities with a similar epidemiology to malaria, as was shown in Papua New Guinea [96]. The substantial impact of ITNs is also seen through indirect health benefits. ITNs are associated with reductions in the child all-cause mortality [97]. A recent long-term study found association between ITN use in early childhood and improved survival to adulthood [21], including a higher probability of marriage, child-bearing and a better education [22].

Usually ITN distribution takes the form of national campaigns, ideally implemented over 3-year cycles targeting the entire population at risk. To maintain high access between campaigns, catch-up and keep-up strategies are often necessary [98] as attrition and loss of ITNs from households is expected [99]. These are commonly implemented via school-based and health-facility-based antenatal care (ANC) channels or during expanded programme on children immunisation (EPI) campaigns [100]. A large multi-country study reported that ITN distribution via ANC and EPI can not only assist countries in maintaining ITN ownership and use, but can be extremely effective at increasing ITN ownership and use and that an additional benefit is expected while combining ANC and EPI-based ITN distribution, compared to ANC distribution alone [101].

However, the effectiveness of ITNs is primarily dependent upon two primary conditions: (a.) The quality of ITNs and (b.) effectiveness of their use. ITNs of good quality (i.) Are made of a fabric of good physical integrity, are durable and less prone to tear and wear for instance due to washing, so that they do not require regular replacement, preferably able to last three years [102]. (ii.) bio-efficacious, meaning that the impregnated insecticides are able to kill mosquitoes that interact with them. (iii.) They have a sufficient insecticidal effect that lasts long, preferably long enough to cover their lifespan or of a fabric that permits retreatment with no or minimal loss of bio-efficacy and physical integrity. A good ITN should ideally have a lifespan of three years. In addition, they are of (iv.) appropriate designs and available in variable

sizes for the varieties of sleeping spaces in the communities. People are more likely to accept ITNs if their physical appearance and colour is desirable [103].

Nevertheless, a good quality ITN has certainly to be used well to afford protection to capacity. Proper ITN use entails use of a proper quality ITN properly installed in the sleeping space preferably by suspending it directly above the sleeping area and firmly tucking its edges to avoid mosquito access. WHO-recommended use of a single regular size ITN for a maximum of two adult individuals should be adhered to in order to ensure no or minimal contacts with the fabric of the ITN as mosquitoes can bite through the pores of the net. The ITN only protects against mosquito bites occurring at the time when it is in use. Using an efficacious ITN at all times of the night when asleep ensures maximum protection an ITN can confer. Lastly, the impact of ITNs is massively enhanced under high coverage. The WHO advises that at least 80% of the population sleep under ITNs of proper physical integrity and bio-efficacy for there to be effective protection against malaria in the whole community [104].

ii. Indoor residual spraying

Indoor residual spraying (IRS) of insecticides is a vector control tool that targets vector populations that mostly rest indoors. Traditionally, IRS mainly employed dichloro-diphenyl-trichloroethane (DDT) application as the primary insecticide. This was after the discovery in the 1940s of the insecticide that proved to have a long insecticidal residual effect on house walls [105]. Prior to DDT, insecticide spraying was hardly ‘residual’, as programmes mainly employed pyrethrum-extracts that naturally had a short-term effect lasting just a couple of weeks. IRS with DDT found application as predominant malaria control intervention in the first global attempt of malaria eradication [106]. Due to high risk of adverse environmental effects, such as those to wildlife, as well as its potential human health risks wide-scale DDT use was globally banned at the Stockholm Convention on Persistent Organic Pollutants [107].

Its use for IRS is now only on restricted special-case basis where use of other insecticides is not ideal. Use of pyrethroids has commonly been employed in many IRS campaigns in malarious areas. However, with the advent of widespread ITN use, which largely employ pyrethroids, IRS with pyrethroids is now increasingly shown to have limited benefits, and is not recommended as it increases selective pressure and can contribute to spread of insecticide resistance [108]. A meta-analysis including four randomised-controlled trials in highly endemic areas where people were using ITNs found IRS with 'non-pyrethroid-like' insecticides to have been associated with lower malaria prevalence compared to IRS with pyrethroids [93]

Presently, IRS mainly takes the form of spraying with pirimiphos-methyl, an organophosphate insecticide. Organophosphates employ a different mode of action from pyrethroids and are highly effective when used in combination with pyrethroids, but presently pirimiphos-methyl IRS programmes do not provide sufficient longevity of effect [109]. There are a number of compounds in the research and development pipeline, some of which have already shown promising results in trials. A good profile for IRS includes a compound with (i.) a different insecticidal mode of action to pyrethroids and (ii) a longer-lasting insecticidal residual effect. Pyriproxyfen, chlorfenapyr and clothianidin, a neonicotinoid insecticide have shown consistently good promise in experimental huts and cluster-randomised village trials. The WHO recommends that programmes must target spraying above 80% of structures in the community for the effective IRS protection against malaria. Different deployment strategies can be employed to implement an IRS programme, ideally with insecticides using different modes of action to mitigate resistance. These include (i.) mosaics, spraying different insecticides in geographically adjacent areas, (ii.) mixtures, involving IRS formulations of mixed insecticides, (iii.) combination, where different insecticides are used in the same geographical area, and rotation, where different insecticides are applied in the same locations but at alternating time intervals [110].

e) Malaria surveillance

In addition to malaria intervention commodities, the elevation of malaria surveillance as a core malaria intervention has been reiterated by the WHO [75]. Surveillance constitutes the "Continuous, systematic collection, analysis and interpretation of disease-specific data and use in planning, implementing and evaluating public health practice" [74]. Good quality data of malaria cases and deaths enhances proper stratification of malaria risk at the national and sub-national levels and allows effective monitoring of changes in the disease patterns induced by interventions. This helps in identify gaps in coverage of interventions, which is necessary to stimulate targeted responses to reach those gaps. Agile surveillance systems are required to respond to the population dynamics of mosquitoes related to vector control, in particular population densities, species composition, biting behaviours, parasite infection and insecticide resistance.

Intervention approach under malaria elimination settings

The WHO recommends that once the prevalence of malaria parasite infection in the human population as determined by microscopy or rapid diagnostic tests drops below 5%, programme aim should change from malaria control to pre-elimination by re-scaling control interventions and changing the strategy accordingly [76]. Whereas the aim of programmes in a control phase is achieving universal population coverage with preventive and treatment interventions to reduce morbidity and mortality, the essential components of a programme aiming at malaria elimination are as follows: (i.) Detection of all malaria cases, where surveillance becomes a critical intervention tool. (ii.) Prevention of onward transmission. (iii.) Management of malaria foci. (vi.) Management of importation of malaria parasites. Malaria elimination programmes require more technical malaria expertise than general control programmes, especially also at sub-national levels, and are driven by national expertise in malaria epidemiology and

entomology [111]. With reductions of the incidence to <1 case per 1000 population at risk per year, the state of malaria elimination is approached. Imported malaria becomes the greater risk than the dwindling local parasite pool. Accordingly, surveillance and prevention of re-introduction should be stepped-up. Finally, when no locally contracted cases are reported for three consecutive years, malaria elimination certificate can be requested [76, 111].

1.6 Significance of malaria vector control

High public health impact

Vector control has been a primary malaria control component in all areas where malaria has been eliminated [112]. The global malaria eradication programme (GMEP) that primarily targeted malaria vectors by aggressive spraying of habitats with insecticides, eliminated malaria across most of the Americas, Europe and northern Africa [106]. Vector control with ITNs and IRS drives a high impact against the disease burden in malarious areas. Between 2000 and 2015, ITNs were estimated to have averted 68% of over 660 million *Plasmodium falciparum* malaria cases prevented by malaria control efforts in SSA [113]. A 6-year study in Burkina Faso that assessed the effects on malaria of insecticide-treated curtains reported that between 19–24% reduction in child mortality was achieved and that the protection of insecticide-treated curtains was sustained over time [114], disapproving fears that preventing malaria early in life may lead to delayed mortality as a consequence of poor immunity development. The study further found that the probability of a child dying before its fifth birthday was reduced from 240 per 1000 to 170 per, representing 29% gain in averted mortality. Few other child survival interventions are currently as effective. More recently, a 22-year prospective cohort in Tanzania, that examined long-term effects of ITN use among children <5 years of age found that users compared to non-users were more likely to survive to adulthood

and to have better success in life, including attaining a better education, marriage and child-bearing [21, 22].

Cost-effectiveness of interventions

An LLIN, the commonly used ITN in malarious areas is estimated to cost roughly USD 2 [115]. Assuming one net is used by two people and the net is functional for three years, the overall annual cost of protecting an individual by an LLIN is roughly 30 cents, excluding distribution costs [115-117]. Scates et al. estimated in a study in Ghana and Tanzania that overall, campaigns (keep-up) and continuous (catch-up) systems delivered ITNs at overlapping economic costs per net distributed of between USD 4.4-4.6 and USD 3.6-9.9 [118]. For IRS, the average total cost of a community-wide programme is estimated to cost roughly USD 3.5 per person protected per year [91]. Vector control value for money is reasonably high considering the health benefits. A model-based analysis projected that distributing replenishment ITNs each year in addition to the replacement of all nets every 3-4 years increased the averted number of deaths of children <5 years by 5-14% at an annual cost of USD 17-25 per additional child protected or USD 1080-1610 per additional child death averted [119]. The benefits of malaria prevention besides reductions of illness and death also include saving on case management costs [113]. Cibulskis et al. estimated that malaria interventions, with a significant vector control component, saved about USD 900 million in malaria case management costs to public providers in SSA between 2000 and 2014 [113]. Malaria prevention can as well transform the well-being and livelihoods of some of the poorest communities across the globe because of ameliorating the burden caused by DALYs [21, 22].

1.7 Opportunities for expanding choices of vector control tools

Attacking vectors on multiple fronts

There are various mechanical, biological, and chemical vector control options to attack the *Anopheles* mosquito along the continuum of its lifecycle stages ranging from her emergence from breeding sites, to mating, sugar feeding, host-seeking for a blood meal resource, biting humans or animals, resting and oviposition [120]. Man biting prevention has been associated with reductions in malaria across many areas by use of ITNs [121], repellents [122, 123], protective clothing [124], house mosquito-proofing modifications [125-127], mosquito repellent coils [128], and by use of IRS that targets resting populations [129]. Larval source management targeting well-marked *Anopheles* breeding sites by help of local communities has been shown to confer malaria protection [130, 131]. A systematic review reported that with research using carefully controlled field studies or quasi-experimental designs, the potential of malaria prevention by introducing lavivorous fish in *Anopheles* larval sources could be evaluated, based on the observation in some areas that high fish stocks arrested densities of *Anopheles* larvae and pupae [132]. Zooprophylaxis that targets animal-biting species such as the *An. arabiensis* could be part of an effective strategy to reduce malaria transmission under specific ecological and geographical conditions [133] and may be enhanced by spraying animals with insecticides [134, 135]. Ivermectin, a common anthelminthic that is also known to confer a poisonous effect on *Anopheles* mosquitoes upon ingestion in a blood meal from treated humans or livestock has been explored for malaria prevention [136-138].

The opportunity to target *Anopheles* at multiple stages of life history with a variety of vector control technologies not only enhances malaria control effort but also limits selection pressure by the interventions [120]. The WHO has provided a road map for the use of an array of current and future products with potential to improve the impact of malaria vector control [1]. They

include new options for IRS and ITNs as well as other vector control tools as follows: (i.) IRS: - clothianidin (SumiShield), clothianidin plus deltamethrin (Fludora Fusion) and chlorfenapyr and other potential active ingredients. (ii.) ITNs: - pyrethroid plus piperonyl butoxide, pyrethroid plus repurposed active ingredient, ideally alpha-cypermethrin plus chlorfenapyr, pyrethroid plus the insect growth regulator, ideally alpha-cypermethrin plus pyriproxyfen, next generation ITNs with new active ingredients. (iii.) Targeted sugar baits: - designed to attract and kill mosquitoes and that may exploit a range of active compounds. (iv.) Others: - including larvicides, vector traps, eave tubes and baffles, spatial repellents and genetic control including gene drive and population suppression measures [1, 108].

Broadening range of possible targets by insecticidal compounds

A recommended strategy for preventing and mitigating insecticide resistance is to combine interventions employing insecticides with different modes of action to reduce selective pressure [108]. Presently, WHO-approved active compounds fall broadly into five insecticide classes and exploit three modes of action in general. They include: (1.) *Sodium channel modulators*: These are (i.) pyrethroids such as alpha-cypermethrin, deltamethrin, lambda-cyhalothrin, transfluthrin, etofenprox, bifenthrin and cyfluthrin, and (ii.) organochlorines, with the main compound being DDT. These compounds bind voltage-gated sodium channels on membranes of insect nerve cells resulting in a prolonged influx of sodium ions into cells that causes insect paralysis manifested by the characteristic knockdown effect of the insecticides [139]. Widespread use of DDT is however prohibited because of its high persistence in the environment and contamination [140]. (2.) *Acetylcholinesterase inhibitors:* This category comprises two insecticide classes namely, (i.) organophosphates, which include malathion, fenitrothion and pirimiphos-methyl, and (ii.) carbamates that include bendiocarb and propoxur. Acetylcholinesterase inhibition impedes breakdown of the neurotransmitter acetylcholine

resulting to its accumulation at post-synaptic junctions. Susceptible mosquitoes exhibit paralysis and knockdown similar to the response induced by pyrethroids and DDT. (3.) *Nicotinic acetylcholine receptor competitive modulators:* These comprise the latest addition to the WHO-approved insecticide classes, namely the neonicotinoids, with a main example being clothianidin. Neonicotinoids bind and interact with the nicotinic/nicotinergic acetlycholine receptors at the post-synaptic junctions of the insect nervous system through a competitive modulation process causing overstimulation of nerve cells, paralysis and possible mosquito death [108].

A major current challenge with the use of insecticides for malaria control is the high risk of selection pressure by use of compounds with similar modes of action as this often results in cross-resistance [141-143]. Accordingly, newer compounds that act independently to cause insect harm or alongside insecticides to enhance effects are adopted for vector control. They include: (1.) *Synergists:* These compounds act by inhibiting enzymes that break down the active ingredient, causing more molecules to interact with the active site, and thereby having a permanent insecticidal effect on the mosquito. A common synergist in malaria vector control is piperonyl-butoxide (PBO). PBO inhibits the P450 monooxygenase enzymes, which are responsible for detoxification of the pyrethroid before the neurotoxin reacts with its target site. Modern pyrethroid treatments are accompanied by a PBO component to enhance the effect of pyrethroids [144]. (2.) *Inhibitors of energy metabolism:* A common compound in malaria control is the pyrrole chlorfenapyr, which acts through competitive inhibition of oxidative phosphorylation, the energy generating process in the cells. Chlorfenapyr is a pro-insecticide whose activation occurs because of metabolism in the mosquito. Chlorfenapyr application is thought to be a mitigation strategy where metabolic resistance is prevalent [145]. (3.) *Insect growth regulators:* These compounds mimic the insect juvenile hormone hence impairing

normal growth in the mosquito. Pyriproxyfen is a well-known insect growth regulator that has been applied to control malaria vector populations at field scale in some malarious parts [146].

1.8 Malaria vector control challenges

Vector biodiversity in high burden areas

Anopheles populations thrive in the tropical climate alongside many other mosquitoes and insect species. There is often more than a single *Anopheles* species in most malaria endemic settings [59, 147]. In many cases, the local *Anopheles* populations defer bionomically with often one or two species being the greatest agents of malaria [147]. The challenge for vector control is usually to characterise those populations and identify the species that cause the biggest problem while remaining alert to possible shifts in species dominance and malaria transmission importance [147, 148]. Bart Knols, a medical entomologist, in a review of the book: *Mosquitoes of the World vol 1 and 2*, noted that, "When trapping mosquitoes out in the field, there's always that pile of "unidentifed" mosquitoes that remains. And only recently have we discovered that some of these "ignored" species may actually play a significant role in disease transmission, even though their abundance may be low" [149]. Such changes in malaria vectorial systems was reported in the South Pare region of Tanzania where following widespread implementation of IRS, the highly endophilic vector *An. funestus* decimated, leaving mainly *An. gambiae* s.l. populations that exhibited exophilic behaviours [150]. Further south in the country, in Ifakara, *An funestus* and *An. arabiensis* have gained dominance in transmission after widespread use of ITNs over the past decades that dramatically reduced *An. gambiae* s.s populations [151]. More recently, the invasive spread of *An. stephensi* across West and East Africa has become a key concern for the future of vector control and malaria transmission [152-154]. Originally from the South Asia and the Arabian Peninsula, the species

easily colonises new areas primarily because it can breed in habitats such as containers or cisterns with clean water and can adapt well in the environment such as hot weather [155].

Insecticide resistance and behavioural avoidance

Widespread use of ITNs and IRS may cause the following changes in local vector populations (i.) The pressure exerted by the interventions may select in favour of species with more flexible behaviours, this often results to a shift in species dominance [151]. (ii.) Endophilic species may change their behavioural patterns by for example resorting to biting and resting outdoors [156]. (iii.) Exposure to insecticides may cause insecticide resistance selection pressure, the greatest existential challenge of vector control [142, 143, 157-159]. Mosquitoes tolerate insecticides through a number of mechanisms. (1.) *Target site resistance:* Occurs when the site of action of an insecticide, which are mostly in the nervous system of the mosquito, is modified in exposed strains, such that the insecticide no longer binds effectively and the mosquito is no longer affected or is less affected and therefore withstands the insecticide. Resistance mutations, known as knockdown resistance or "kdr" mutations can affect acetycholinestrase, which is the molecular target of organophosphates and carbamates, or voltage-gated sodium channels, the target sites of pyrethroids and DDT. (2.) *Metabolic resistance:* Enzyme systems of insects metabolise foreign chemicals to detoxify them and mitigate harm. Metabolic resistance occurs when increased or modified activities of an enzyme system prevents the insecticide from reaching its intended site of action. The three main enzyme systems are esterases, mono-oxygenases and glutathione S-transferases. (3.) *Behavioural resistance:* This involves modifications in insect behaviour that helps it to avoid the lethal effects of insecticides. Several publications have suggested the existence of behavioural resistance and described changes in vectors' feeding or resting behaviour to minimize contact with insecticides [156, 160, 161]. (4.) *Cuticular resistance:* This refers to reduced uptake of insecticide due to modifications in

the insect cuticle that prevents or slows uptake of insecticides. Although rarely reported, a study in South Africa found an association between thicker cuticles and pyrethroid resistance in a local *An. funestus* population, and that overall, females had thicker cuticles than males suggesting resistance mechanism by cuticle thickening [162]

Ineffective intervention use

Although ITNs and IRS are proven to offer effective protection against malaria transmission, their use is often met by several challenges that undermine their effectiveness. These may include use of poor quality ITNs due to manufacturing defects [163]. Defects in ITN manufacturing may influence bio-efficacy, physical integrity or durability that are important for optimal ITN effectiveness. Closely related to defective ITNs is also a problem of counterfeit/illegitimate ITNs [164]. For ITNs to advance substantial effects on the disease burden a large number of people at risk are required to use them [165]. Accordingly, poor access to ITNs in the household can be a significant limitation to programme effectiveness as it does not only directly disadvantage non-users but limits attainment of the intended communal effect requiring high coverage [166]. Poor access could be because of attrition or insufficient nets received from distributions and losses of ITNs from the households or due to repurposing of ITNs, although rarely done [167, 168]. Finally, nets only protect optimally if they are used well, thus any hindrance from sleeping under ITNs all of the times when in bed for the night impedes their effectiveness [169, 170].

High vector control research and operational costs

Although the individual malaria vector control commodities such ITNs have been shown to be highly cost-effective, malaria vector control operations are generally not inexpensive [24]. The full scope of research infrastructure and personnel required in stimulating effective vector control programmes globally and locally, ranging from costs towards basic research and

development of products, to experimental and field work, expenditures towards technical expertise and training of field personnel, and logistical costs of deploying interventions often demand huge budgets.

Malaria is a top priority infectious disease in terms of health funding by governments across endemic countries [24, 25]. Although the World Health organisation (WHO) predicts that the current global malaria control expenditure will need to double in order to stimulate the required progress, substantial financial resources are presently committed towards malaria control and elimination [25]. Between USD 3.5 billion and USD 5.6 billion malaria control funding was required per year between 2006 and 2015 [171]. The global malaria expenditure totalled USD 3.5 billion in 2021, put in perspective, the sum approximates individual gross domestic products (GDPs) of the Republics of Eritrea, Malawi and Burundi [1]. The Global Fund, the largest malaria funding enterprise as of June 2022 had invested an estimated total USD 16.4 billion towards malaria control initiatives across the malarious world [172]. Significant malaria funding has also been provided by international, governmental and private partners. According to the latest report of the US President's Malaria Initiative (PMI), the largest governmental anti-malaria agency, each year an average sum of USD 9 billion are spent in support of 24 partner countries in Africa and three programmes in the Greater Mekong sub-region in Southeast Asia, that represent about 80% of the global malaria burden [173]. Based on an average LLIN price of USD 2.00 per net, approximately USD 400-500 million is spent annually on procuring LLINs, which excludes the costs of shipping and secondary distribution with approximately 56% of the expenditure channelled via GF and about 20% via PMI [115]. In a move aimed at easing the funding burden to foster capacity development and African leadership to end malaria and neglected tropical diseases, the Bill and Melinda Gates Foundation in mid-2022 announced a USD 140 million US dollar commitment [174].

1.9 Lessons from key past vector control efforts

The current knowledge, planning and practise of malaria vector control continues to derive from lessons of past successes and failures. Two key examples are as follows:

The Global Malaria Eradication Programme (1955-69) and the Garki Project (1969-76)

GMEP was the first globally coordinated and implemented anti-malaria agenda [106]. The programme was initiated towards the mid-1900s following the discovery of DDT in 1940. DDT provided hope as an insecticide that could dramatically hold back malaria transmission due to its high efficacy against mosquitoes and a long insecticidal residual effect [175]. *Anopheles* control at their common resting sites inside human shelters by spraying inner walls of structures with primarily pyrethrum extracts was already a common malaria control practice as early as the 1930s [176]. However, the pyrethrum-based insecticides, although highly efficacious against *Anopheles* mosquitoes were less effective for house spraying due to the typical short residual effect of the insecticides [177]. Applications required weekly to bi-weekly repeats to sustain sufficient *Anopheles* mortality and restrain transmission. DDT [105], a highly persistent insecticide requiring only bi-yearly to yearly rounds of spraying revolutionised malaria vector control [106]. Promising results of DDT use first by the United States army during World War II and later by national malaria control programmes stimulated a conversation by the scientific community to implement a global vector control approach taking advantage of IRS by DDT. In 1955, the WHO embarked on the ambitious GMEP, but nearly a decade and half later, GMEP aborted. At the time of GMEP collapse malaria had been reduced dramatically and eliminated in many parts in North America, Europe and northern Africa [106].

GMEP collapsed and held-back on its mission after realising that eradication as had been intended would not be achievable by the applied approach since malaria transmission persisted across many areas. Some key GMEP flaws were as follows: (i.) GMEP relied on a single

strategy namely IRS with DDT. Despite the fact that DDT had offered great promise in select countries before GMEP and in the programme's pilot studies, resistance to DDT had already been reported in Greece as early as 1950. Environmental management involving destruction of mosquito breeding marshes and bite prevention, longstanding key strategies were abandoned. (ii.) The preparedness necessary to undertake the huge enterprise was lacking, operations were mainly left to malariologists who had only been field scientists guiding governments and local authorities and had no appropriate operational expertise for the programme. (iii.) The feasibility of the programme in vast areas that had poor communications and adverse environments and that lacked public health systems would have been impossible, but that was not appreciated. (iv.) GMEP was operationalised on a rigid framework that disregarded scientific and research criticism and advocated strict discipline to a set of procedures, which prevented recognition of limitations of the campaign. (v.) Countries following the strict directives of the campaign neglected their local strategies and minimised the role of community engagement hence were incapable of adjusting to changes in their epidemiological situations. (vi.) Acute focus on the campaign goals overlooked emerging problems including reports of chloroquine resistance as all other strategies including use of antimalarials had been abandoned. Nájera et al. [106] summarise lessons learned from these key GMEP mistakes as follows: (i.) No single strategy can be applicable everywhere. (ii.) A long-term commitment with a flexible strategy is required (iii.) Malaria control needs to be integrated with the health system. (iv.) Community engagement is key to malaria control success. (v.) An agile surveillance system with a capacity to track changes in epidemiological situations is needed.

SSA missed out largely on the GMEP agenda mainly because it was considered that the malaria transmission contexts in the region were not well known and that countries did not have well-established health systems. However, immediately after GMEP collapse, with the lessons learned, a model 'eradication' programme for SSA was initiated in the district of Garki in

Nigeria with two primary objectives: (i.) to determine the impact of indoor residual spraying and mass drug administration and (ii.) to construct and test a mathematical model of malaria transmission [178]. Although Garki was implemented with much more scientific caution than GMEP, its main goals were also not realised. The IRS programme in particular did not achieve much. Failure of the programme was largely attributed to little effect of IRS on the primary local vector species *An. arabiensis* that predominantly bites and rests outdoors [179]. It was concluded that a good understanding of the local vector species composition and bionomics, including biting and resting behaviours was crucial for the success of malaria vector control programmes [180].

The GMEP and Garki projects offer important lessons both for policy and technical levels of malaria control, and could be summarised as follows: (i.) Strategy should not be based on a rigid central campaign with a fixed timeframe, but should ideally be based on continuous and flexible evidence-driven programmes that apply multilateral and integrated vector control interventional approaches. (ii.) Malaria vector control needs to be strengthened, well funded, and elevated as a core public health service and integrated across other sectors such as education, water and sanitation and agriculture. (iii.) Community engagement in various intervention initiatives needs to be promoted. (iv.) A localised understanding of malaria vector populations, including species compositions and their bionomics as it relates to human exposure is vital to initiate appropriate responses. (v.) An agile surveillance system with appropriate methods of monitoring local malaria vector populations to track changing patterns and identify emerging problems is needed to enhance effective vector control responses.

1.10 Narrowing the gap in effective vector control

The *Anopheles* problem is nowhere near solving, going by the current global estimates of malaria burden [1]. The WHO's malaria control strategy [75] outlines steps that can be

followed to narrow the gap in effective malaria protection by use of preventive approaches particularly vector control interventions [75]. They include:

High and rational coverage by core malaria vector control measures

The WHO recommends that a high number of people in the population need to have access and use of vector control interventions in order to stimulate sufficient intervention effects capable of interrupting malaria transmission [75]. It is estimated that for the ITN programme effectiveness, greater than 80% of the population at risk of endemic malaria transmission need to use ITNs of good quality. Locally appropriate campaigns complemented by appropriate catch-up distribution channels can help maintain a high access to ITNs in the households accompanied by health education to support a proper ITN use culture in the communities including sleeping under the nets at all times when in sleeping spaces during the active mosquito biting periods [101, 181, 182]. Use of IRS in addition to ITNs should be informed from malaria transmission intensity and behaviours of local vector populations including biting and resting with spraying targeting >80% of all structures in the endemic area [75, 183].

Appropriate, settings-specific choice of supplementary tools

However little the extent of residual malaria risk, appropriate personal protection measures during active mosquito biting times when it is impractical to use ITNs or where IRS may have limited benefits are needed for interrupting malaria transmission. The choice of specific supplementary measures depends on an understanding of who gets bitten where and when.

Locally adapted approaches for insecticide-resistance management

A series of guidelines are provided by the WHO for use of malaria vector control insecticides in a manner that mitigates insecticide resistance [108]. Use of pyrethroids-only ITNs is advocated in areas with no pyrethroid resistance, as the first-line malaria prevention strategy.

In areas of pyrethroid-resistance, new generation ITNs combining pyrethroids with the pro-insecticide chlorfenapyr, the synergist piperonyl butoxide or the insect growth regulator pyriproxyfen are recommended based on evidence from studies [184]. Where IRS is implemented in addition to ITNs, non-pyrethroid insecticides should be used. Deployment of IRS should ideally follow mosaics, combinations, mixtures or rotations as locally appropriate to minimise selective pressure and risk of insecticide resistance [185].

Improved study designs and field methods for better quality data

The importance of improving the quality of data for use in guiding effective malaria vector control work cannot be overemphasised and is reiterated by WHO [1]. In the WHO Global Vector Control Response [183], critical review and improvements of both experimental and field methods and strategies of generating data are emphasised. The guidelines further direct that assessments of vector populations should use up-to-date methods and techniques to ensure that results are informative for guiding and assessing vector control. Of particular need are robust indicators for vector-borne disease risk, especially in low transmission settings, and methods for assessing vector behaviour such as human outdoor biting. Improvements advised also include search for opportunities to use new technologies, such as novel adult mosquito sampling tools, and that experiences from other countries with similar vector ecologies or transmission conditions may be adapted.

Of importance also is the need to strengthen the evidence base showing impact of vector control on infection and human disease beyond the core malaria interventions of ITNs and IRS. This should as well include employing improved methods to assess the actual effects of ITNs under the contexts of increasing *Anopheles* outdoor biting. There is urgent need to understand the efficacy of current interventions, such as novel active ingredients developed for use against pyrethroid-resistant *Anopheles* populations. Here, applied research is required to measure the

field suitability and performance of the new insecticides, such as through cluster-randomized community trials (cluster RCTs) with entomological and clinical outcomes where possible. Interventions can also be recommended based on safety, quality and entomological efficacy data prior to establishing their epidemiological impacts.

Cluster RCTs are effective when conducted on large scales, which is often not the case due to resource constraints in low-income settings. As a result, outcomes of cluster RCTs conducted to evaluate new vector control interventions often provide results with limited certainty, which makes it hard to draw conclusions that can support vector control planning, as has often been found in systematic reviews of trials [93, 186]. Much larger data sizes can be reached with much less logistical difficulty by employing experimental huts (EHTs). EHT study designs comprise reproducible assays for capturing the complex entomological efficacy of ITNs and IRS on blood-feeding mosquitoes.

A meta-analysis revealed that mosquito data collected in EHTs could be used to parameterize mechanistic models for *P. falciparum* malaria and reliably predict the epidemiological efficacy of quick acting, neuro-acting ITNs and IRS. The findings suggest that for certain types of ITNs and IRS using entomological endpoints assessed in EHTs instead of clinical endpoints from cluster RCTs could support policy and expedite the widespread use of novel technologies [187].

2. Goal and objectives

2.1 Goal

The goal of this thesis was to assess *Anopheles* human biting, human exposure to infective *Anopheles* bites and protection by high coverage with insecticide treated nets and with indoor residual spraying in a rural area where the primary malaria vectors comprise of *Anopheles*

funestus and *An. arabiensis* that bite both indoors and outdoors and show high resistance to pyrethroids.

2.2 Objectives

Objective 1: To measure and compare *Anopheles* human biting from mosquito catches by the human landing catches method, CDC light trap and human decoy trap

Objective 2: To assess timing of bites by malaria-infected *Anopheles* mosquitoes and the use of insecticide treated nets during the night in a typical rural African community

Objective 3: To discuss the importance of linking human and mosquito behaviours for evaluating effectiveness of insecticide treated nets

Objective 4: To evaluate efficacy of clothianidin for indoor residual spraying against resistant *Anopheles* mosquitoes in a typical rural African community

3. Study site

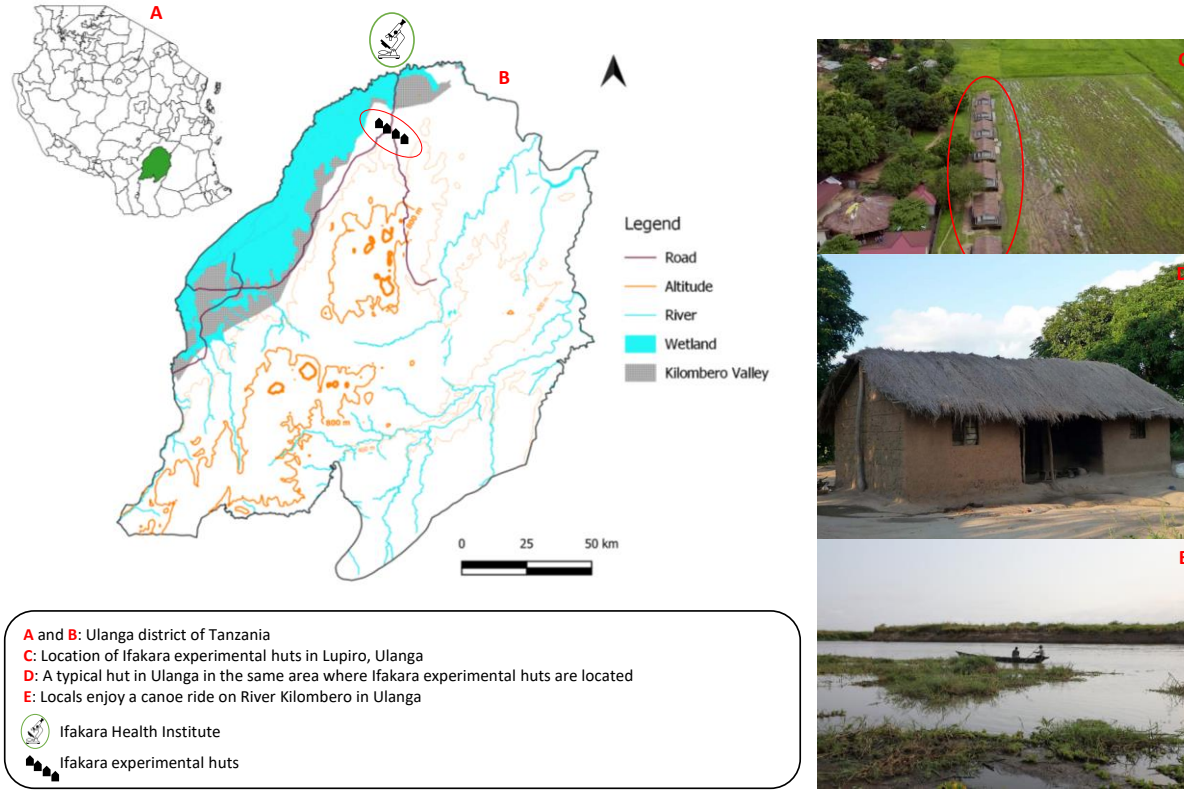

Figure 3. Study site

4. The Centres for disease control light trap (CDC LT) and the human decoy trap (HDT) compared to the human landing catch (HLC) for measuring *Anopheles* biting in rural Tanzania

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4.1 Abstract

Background

The intensity of vector mosquito biting is an important measure for malaria epidemiology and control. The human landing catch (HLC) is an effective entomological surveillance tool, but is labour-intensive, expensive and raises safety issues. The Centres for Disease Control light trap (CDC LT) and the human decoy trap (HDT) are less costly and exposure-free alternatives. This study compared the CDC LT and HDT against the HLC for measuring *Anopheles* (*An.*) biting in rural Tanzania and assessed their suitability as HLC proxies.

Methods

Indoor mosquito surveys using HLC and CDC LT and outdoor surveys using HLC and HDT were conducted in 2017 and in 2019 in Ulanga, Tanzania in 19 villages, with one trap per house per night. Species composition and the numbers of mosquitoes caught by different trap types were compared. Aggregating the data by village and month, the Bland-Altman approach was used to assess agreement. Mosquito sporozoite rates were also assessed.

Results

Overall, 66,807 *Anopheles funestus* and 14,606 *An. arabiensis* adult females were caught from 6,013 CDC LT, 339 indoor HLC, 136 HDT and 195 outdoor HLC collections. Overall, the CDC LT caught fewer malaria vectors than indoor HLC: *An. arabiensis* (Adjusted rate ratio (Adj.RR) = 0.35 (95% confidence interval (CI): 0.27 - 0.46)) and *An. funestus* (Adj.RR = 0.63 (95% CI: 0.51 - 0.79))**.** HDT caught fewer malaria vectors than outdoor HLC: *An. arabiensis* (Adj.RR = 0.04 (95% CI: 0.01 - 0.14)) and *An. funestus* (Adj.RR = 0.10 (95% CI: 0.07 - 0.15)). The bias and variability of the ratios of geometric mean mosquitoes caught by CDC LT and HDT relative to HLC collections for the same village-month were dependent on mosquito densities. The relative efficacies of both CDC LT and HDT declined with mosquito abundance.

The variability in the ratios was substantial for low HLC counts and decreased as mosquito abundance increased. Only CDC LT caught infected *An. arabiensis* and the HDT caught no infected mosquitoes.

Conclusions

If caution is taken in appreciation of its limitations, the CDC LT is suitable for use in routine entomological surveys and may be preferable for measuring sporozoite rates. Use of HLC is still essential to ratify parameters such as the EIR. The present design of the HDT is not amenable for use to conduct large-scale entomological surveys.

4.2 Background

Measuring *Anopheles* biting is a core part of the monitoring and surveillance of malaria vectors. The *Anopheles* females, responsible for the transmission of malaria, bite humans to obtain a blood meal needed for egg production. The proportion of biting mosquitoes that are infected is essential to quantify the entomological inoculation rate (EIR), the most reliable vector-based index for estimating infection transmission intensity and the impact of vector control interventions. *Anopheles* biting is assessed by collecting host-seeking mosquitoes around areas occupied by humans over regular time intervals throughout the night [169, 188-193].

The human landing catch (HLC) is considered the gold-standard method to assess human exposure to *Anopheles* biting [194, 195]. Individuals recruited to perform HLC (catchers), collect mosquitoes attracted to and alighting on their lower limbs using an aspiration tube before the mosquitoes attempt to bite, ideally over hourly intervals all night (Figure 4). The number of mosquitoes collected by the HLC is presumed to represent the actual intensities and patterns of malaria vector biting. The biting rates and EIRs assessed by the HLC are the most reliable for malaria surveillance and are used as a reference for standardizing other methods [193-197].

Figure 4. Illustrations of mosquito traps.

Panel **A**: The human landing catch (HLC) technique showing a catcher transferring a trapped mosquito into a collection container. Panel **B**: The standard CDC LT (Model 512; John W. Hock Company, Gainesville, FL). Panel **C**: A study field assistant setting up a CDC LT inside a house. Panel **D**: The human decoy trap (HDT). A study field assistant preparing the tent to be occupied by a human.

HLC surveys, however, have important limitations that restrict use. Although it has been demonstrated that observing proper HLC protocol minimises the risk of malaria infection

among catchers [198, 199], use of human baits to catch mosquitoes that have the potential to transmit malaria creates ethical and safety concerns, particularly with the emergence of antimalarial drug-resistance [198, 200], or in areas with active arbovirus circulation. HLCs are also labour-intensive, cumbersome and incur considerable costs to run on a large scale [201]. Variations in the alertness and skill of catchers requires careful supervision, and differences in attractiveness to mosquitoes make HLC surveys hard to standardize [194, 202, 203]. As such, the HLC has been found unsuitable for the extensive and continuous operational exercise of malaria vector monitoring and surveillance for disease control [204]. Accordingly, the World Health Organisation (WHO) encourages research and use of alternative mosquito traps with only sparing use of HLC for purposes such as calibrating new tools [205, 206].

Several attempts have been made to find options that measure biting rates but do not rely heavily on human effort or involve exposure to infection [194]. The target profile for anopheline collection methods to be used as HLC surrogates comprise traps that actively lure host-seeking females by use of host-based cues, usually a combination of olfactory, visual and thermal cues and their selection depends on the effectiveness to replicate efficiency of human attraction [194, 195, 207]. The number of mosquitoes caught by a trap should be comparable via a reliable algorithm to those caught by the HLC [208]. Entomological monitoring for disease control further requires traps that are easily scalable.

Developed initially for sampling agricultural pests, light traps have found common use for collecting malaria vectors after Odetoyinbo first demonstrated their efficacy against host-seeking anophelines [209]. The common battery-powered CDC LT, shown in Figure 4, is usually used alongside untreated bed nets to collect mosquitoes lured by odour cues from individuals sleeping nearby [210-212]. According to Garrett-Jones and Magayuka, the CDC LT-untreated bed net combination enhances the use of the trap for estimating *Anopheles* biting rates [212]. Compared to the HLC, CDC LTs are easy to use, have considerably lower costs to

operate, are easily scalable and reduce human reliance. Mechanical malfunctioning and battery problems, highlighted as the main limitations of these traps usually occur on a minimal scale and faulty traps are often conveniently excluded from mosquito surveys [210]. CDC LTs are also used for outdoor mosquito catches, but they tend to perform poorer compared to their use indoors [213]. Although generally regarded as a reliable mosquito trap [210, 214] there is no clear consensus on the CDC LT performance relative to the HLC and their comparative efficacy to estimate populations of malaria vectors appears to vary based on the local settings [203, 208, 215-219].

The host/human decoy trap (HDT) (Figure 4), was first trialled against *Anopheles* mosquitoes in an attempt to cover a malaria vector monitoring gap for outdoor biting populations in Burkina Faso [220]. The trap optimises mosquito attraction by use of a combination of odour and visual stimuli and a thermal signature in the range equivalent to the human body temperature. Host odour emanating from a protected human in a close tent is blown down a plastic pipe and delivered around a visually conspicuous adhesive trap kept warm at 35±5°C by a heating mechanism, usually hot water (Figure 4). The HDT is a promising entomological surveillance tool based on several studies that demonstrate its capacity to catch a wide range of exophagic mosquito species [220-224].

The present study focused on human biting rates of malaria vectors. The CDC-LT and the HDT were compared to the HLC with an overall aim of determining if the traps could replace the HLC for measuring human biting rates in Ulanga, Tanzania. The current study was interested in whether this calibration was accurate at the population level rather than the individual level as has been done in many other studies (Table 1).

Table 1. Some past studies of the efficacy relative to HLC of the CDC LT and HDT against *Anopheles* species

No.	Area of study	Dominant anophelines	Relative efficacy: Ratio to HLC (95% confidence intervals)		Was trap efficacy dependent on mosquito density?	Reference
	A. CDC LT					
	i. Mosquito species					
1.	Ulanga, Tanzania	*An. arabiensis* *An. funestus*	0.35 (0.27-0.46) 0.63 (0.51-0.79)		Yes Yes	**This study**
2.	Ulanga, Tanzania	*98% An. gambiae* s.l *2% An. funestus*	0.33 (0.24-0.46) 0.82 (0.61-1.10)		NA NA	Okumu *et al*. 2008 [52]
3.	Kenya, Zambia, Burkina Faso, Ghana, Tanzania	*An. gambiae* s.l *An. funestus*	1.06 (0.68-1.64) 1.37 (0.70-2.68)		Yes Yes	Briët *et al.* 2015 [17]**
4.	Lwanda, Kenya	*74% An. gambiae* s.l *26% An. funestus*	1.86 (1.73-2.00) 1.91 (1.66-2.19)		No No	Mathenge *et al*. 2004 [30]*
5.	Ahero, Kenya	*An. arabiensis* *An. funestus*	0.56 (0.49-0.66) 1.19 (1.03-1.37)		Yes Yes	Mathenge *et al*. 2005 [31]
6.	Rarieda, Kenya	*An. gambiae* s.l *An. funestus*	1.18 (0.55-2.54) 0.69 (0.49-0.98)		NA NA	Wong *et al*. 2013 [22]
	ii. ITNs vs no ITNs					
			With ITNs	**Without ITNs**		
7.	Bo, Sierra Leone	*An. gambiae* s.l	0.88 (0.72-1.05)	0.78 (0.60-1.01)	No (without ITNs) Yes (with ITNs)	Magbity *et al*. 2002 [28]‡
	iii. Indoors vs outdoors					
			Indoors	**Outdoors**		
8.	Wosera, Papua New Guinea	*An. koliensis* *An. punctulatus* *An. karwari* *An. farauti* s.l *An. longirostris* *An. bancroftii*	0.28 (0.27-0.29) 0.10 (0.09-0.11) 0.12 (0.11-0.13) 0.07 (0.06-0.09) 0.12 (0.08-0.15) 0.20 (0.15-0.27)	0.27 (0.26-0.28) 0.09 (0.08-0.09) 0.12 (0.11-0.13) 0.06 (0.05-0.08) 0.07 (0.05-1.05) 0.15 (0.11-0.20)	Yes Yes Yes Yes Yes Yes	Hii *et al*. 2000 [51]
9.	Bioko Island, Equatorial Guinea	*An. gambiae* s.s & *An. melas*	0.12 (0.11-0.14) (Mongola area) 0.36 (0.32-0.40) (Arena Blanca area) 0.13 (0.10-0.16) (Riaba area)	0.009 (0.01-0.012) (Mongola area) 0.10 (0.09-0.12) (Arena Blanca area) 0.07 (0.05-0.09) (Riaba area)	Yes (indoors) No (outdoors) Yes Yes	Overgaard *et al*. 2012 [29]†
	iv. Location					
			Kakola-Ombaka area	**Masogo area**		
10.	Nyando & Muhoroni, Kenya	*An. arabiensis* *An. funestus* *An. coustani*	1.98 (1.01-3.86) 0.88 (0.37-2.11) 3.03 (1.65-5.56)	1.83 (0.70-4.79) 0.45 (0.13-1.57) 2.88 (1.15-7.22)	NA NA NA	Abong'o *et al*. 2021 [35]
	B. HDT					
1.	Ulanga, Tanzania	*An. arabiensis* *An. funestus*	0.04 (0.01-0.14) 0.10 (0.07-0.15)		Yes Yes	**This study**
	i. Type of host bait					
			Cow-baited	**Human-baited**		

2.	Kisumu & Homa Bay, Kenya	*An. gambiae* s.s & *An. arabiesnsis* & *An. funestus* & *An. coustani*	7.08 (Kisian) 8.34 (Homa Bay)		0.17 (Kisian) 0.60 (Homa Bay)	NA NA	Abong'o *et al*. 2018 [38]†
ii. Location							
			Kakola-Ombaka area		**Masogo area**		
3.	Nyando & Muhoroni, Kenya	*An. arabiensis* *An. funestus* *An. coustani* *An. pharoensis*	5.69 (2.98-10.86) 1.38 (0.60-3.18) 0 18(0.09-0.37) NA		1.32 (0.49-3.59) 0.66 (0.21-2.09) 2.88 (1.15-7.22) NA	NA NA NA NA	Abong'o *et al*. 2021 [35]
			Lakkang area		**Pucak area**		
4.	Chikwawa, Malawi	*An. gambiae* s.s & *An. Arabiensis* & *An. coustani* & *An. quadriannulatus* & *An. tenebrosus*	1.03 (0.80-1.30)		1.52 0.83-3.17)	NA	Zembere *et al*. 2021 [37]†
iii. Season							
			Rainy season	**Early dry season**	**Late dry season**		
5.	Vallée de Kou, Burkina Faso	*An. gambiae* *An. pharoensis* *An. coustani*	9.6 (9.4-9.7) 10.5 (10.4-10.7) NA	2.2 (2.0-2.4) 2.8 (2.5-3.0) 18.6 (18.2-19.1)	1.7 (1.3-2.0) 1.7 (1.3-2.1) NA	NA NA NA	Hawkes *et al*. 2017 [34]

NA = not assessed because of data scarcity
† ratio estimated for pooled mosquito species
‡ three CDC LTs were compared to two HLC catchers

4.3 Methods

Study area

The study area was in Ulanga District, south-eastern Tanzania (Figure 5). Ulanga is located in the wider Kilombero River valley. The region is characterised by a hot-humid climate, seasonal floodplains and irrigated rice paddies. The main malaria vectors are *An. funestus* and *An. arabiensis* [188, 225-227].

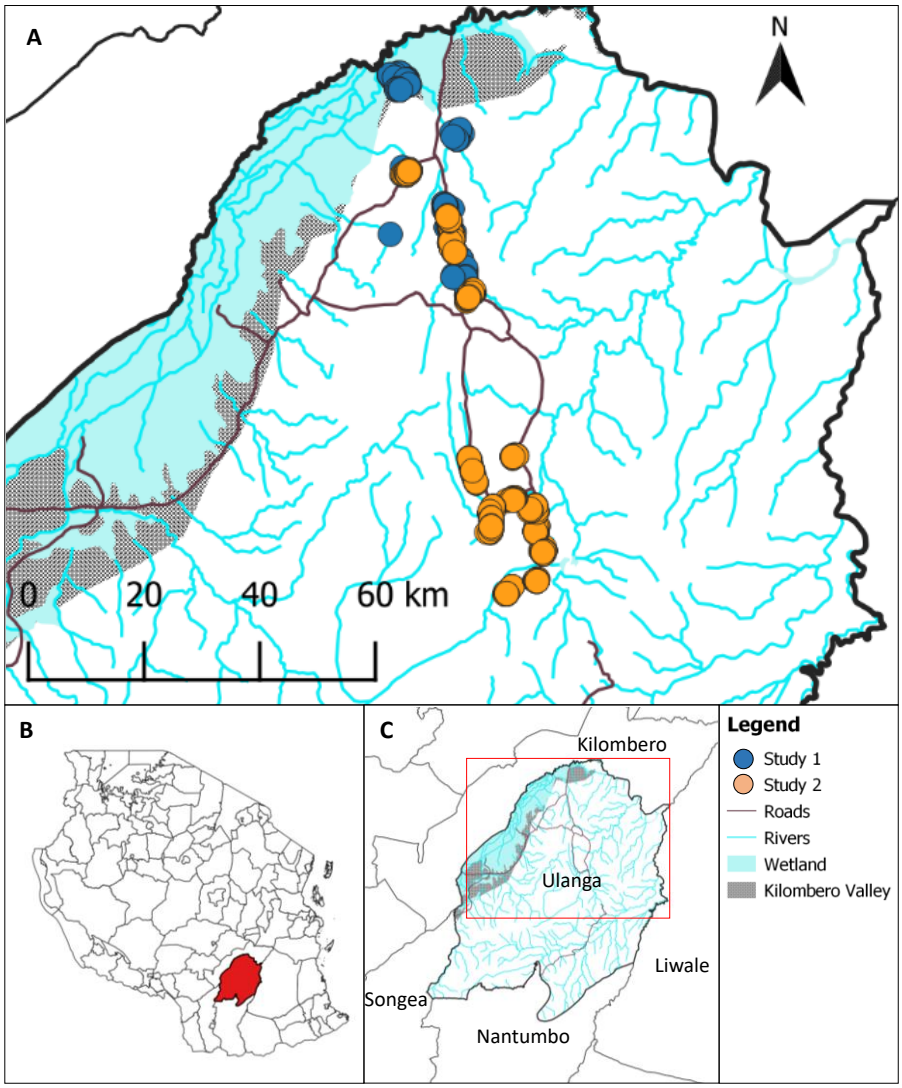

Figure 5. Map of the study area.

Panel **A** shows house locations where mosquito surveys were conducted. Overlapping dots represent closely located households. Panels **B** and **C** show the locations of Ulanga District in Tanzania and of the study area in Ulanga District, respectively.

Study design

Two phase III community randomized studies to evaluate the effectiveness of two new indoor residual spraying (IRS) products were conducted in 2017 (Study 1) and in 2019 (Study 2). Detailed descriptions of the IRS trials are presented in two papers (in preparation). The mosquito surveys were performed by HLC, the CDC LT and the HDT in separate houses. Sampling was partially randomised with population clusters (villages) selected close to rice paddies where high mosquito densities were presumed to occur and study houses randomly selected within the villages. Overall, 19 villages were surveyed; Study 1 covered ten villages while Study 2 covered 14 villages that partly overlapped five villages from Study 1. The villages were paired into intervention and control arms and were separated by at least 2km to limit mosquito migration between treatment arms. House surveys were conducted to collect data on household characteristics such as the number of occupants, number of sleeping spaces, presence of pets and livestock, materials used on house walls, roof, ceiling, floor, and condition of eaves, window and door screening. The global positioning system (GPS) coordinates for house locations were recorded for all surveyed households.

Human landing catch (HLC) collections

The HLC surveys followed the WHO guidelines [228]. Two catchers collected mosquitoes indoors and outdoors, alternating positions every hour. Collections were performed for 45 minutes followed by 15 minutes break. The catchers received doxycycline for malaria prophylaxis and were tested weekly for malaria infection using Bioline Malaria Ag Pf/Pan rapid diagnostic tests. Mosquitoes were collected from 18:00PM to 06:00AM in three randomly selected houses per village. The surveys were repeated for six nights per month for five months in Study 1 and for eight months in Study 2.

CDC LT collections

The standard miniature CDC LT (Model 512; John W. Hock Company, Gainesville, FL.) was used for the surveys (Figure 4). Traps were set indoors at sleeping spaces protected by ITNs, at the foot end of the bed, with the light source positioned at approximately 0.7m from the ground as described by Mboera et al [229]. The traps were operated from 18:00 PM to 06:00 AM in three randomly selected houses per village in Study 1 and in four randomly selected houses per village in Study 2. The traps were used for six nights per month for five months in Study 1 and for 20 nights per month for 8 months in Study 2.

Human decoy trap (HDT) collections

The HDT used in this study was a modification of the standard Biogents, Regensburg, Germany, developed as described by Hawkes and colleagues [230] and is shown in Figure 4. HDT surveys were conducted as described by Hawkes and colleagues [220] and in accordance with the WHO general guidelines [228]. The traps were operated outdoors between 18:00PM to 06:00AM in 4 randomly selected houses per village and were repeated monthly for up to 3 months. The HDT surveys were only done in Study 2.

Sorting and molecular identification of mosquitoes

Field technicians sorted female adult mosquitoes morphologically to separate *Anopheles* mosquitoes. A sample of sibling *Anopheles gambiae* s.l. and *An. funestus* s.l. species were further sorted in the lab by polymerase chain reaction (PCR).

Sporozoite detection in mosquito salivary glands

Enzyme linked immunosorbent assays (ELISA) was used for detection of *Plasmodium falciparum* circumsporozoite protein (CSP) in the salivary glands of mosquitoes [231]. Detection of *P. falciparum* parasites were performed from heads and thoraxes for pooled

mosquito samples, separately for *An. arabiensis* and *An. funestus*. Sample pooling was done by house ID, date and hour of collection and by trap type. The optical density of post-ELISA lysate were measured at 405 – 414nm after 45 minutes using ELISA plate reader machine [231].

Data analysis

Violin plots were used to display the distribution of the number of mosquitoes caught per trap per night. Due to skewness, the counts were log transformed by first adding a value of 1 to the number of mosquitoes (n) per trap per night i.e. log (n+1). Nightly trap catches were summarised using Williams' means and medians with 90% central ranges. The relative proportions of *An. funestus and An. arabiensis* mosquito species caught by the traps were estimated using a logistic regression model with a random effect for house and date. The association between trap type and the number of mosquitoes caught was estimated by negative binomial regression with random effects for house and date and fixed effects for household size, livestock and pets reared, house screening, IRS treatment, ITNs use, seasonality, and whether the measurements were taken as part of Study 1 or 2 (Supplementary Table 1,Supplementary Table 2 Supplementary Table 3).

Agreement for individual catches could not be assessed since there were no paired observations for the same households and nights. Instead, collections were aggregated by village and month to calculate the geometric mean number of mosquitoes caught per house per night for each trap.

The Bland and Altman approach [232] was used to assess agreement between the trap types, providing estimates of the overall bias and the variability. The bias was measured by the ratio of the geometric mean for each trap type (HDT or CDC) compared to the geometric mean using HLC, calculated for the village-months. The ratios were logarithmically transformed (because the distribution of the ratios was skewed). The log ratios were then plotted against the HLC

density [233, 234]. The HLC density rather than the mean of two trap types were used because HLC was considered to be a gold standard. The estimates of the variability were presented as 95% limits of agreement, which represent the range in which 95% of the ratios were expected to lie. The mean bias and limits of agreement were estimated by the regression approach as described by Bland and Altman [235].

To investigate the effect of the trap on the mean ratio by density, a regression model was fitted with the log ratio as the outcome variable and HLC density as the explanatory variable. This way, an estimate of the effect of mosquito densities on the ratio of the geometric means of CDC LT (or HDT) to HLC could be obtained. The effect of mosquito densities on the variability and limits of agreement was estimated by regressing the absolute values of the residuals of the previous model on HLC catches. Village-months with 10 or less CDC LT and indoor HLC collection pairs were excluded from the agreement analysis due to stochasticity.

The prevalence of *P. falciparum* CSP ELISA positive mosquitoes was estimated for each trap type. Due to a very low sporozoite prevalence, no comparative analyses were made between the traps.

The statistical analyses were performed in Stata (16.1, StataCorp LLC, College Station, TX) and in R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

Ethical clearance

The studies received ethical clearance from the Medical Research Coordinating Committee of the Tanzanian National Institute of Medical Research. The reference numbers were as follows: Study 1: NIMR/HQ/R.8a/Vol.IX/1725 & 2270 and Study 2: NIMR/HQ/R.8a/Vol. IX/2894). Clearance by the Ifakara Health Institute Review Board (IHI-IRB) was issued under the following reference numbers: Study 1: IHI IRB 021/2016 & 015/2017 and Study 2: IHI/IRB/No: 031-2018).

4.4 Results

Altogether, there were 6,013 CDC LT, 339 indoor HLC, 136 HDT and 195 outdoor HLC collections. A greater number of *An. funestus* (66,807) than *An. arabiensis* (14,606) adult females were caught. The traps also caught a total 75,248 *Culex* spp mosquitoes, known vectors of other disease-causing pathogens and a common source of biting nuisance throughout the tropics. The number of mosquitoes collected per trap per night were generally low across all traps throughout the study, with a skewed distribution (Figure 6) and (Table 2). The skew was largely due to collections when no mosquitoes were caught by either of the traps but were included in the analysis since such observations are frequently encountered in natural populations. There was substantial variation in the number of mosquitoes caught per trap per night (Table 2).

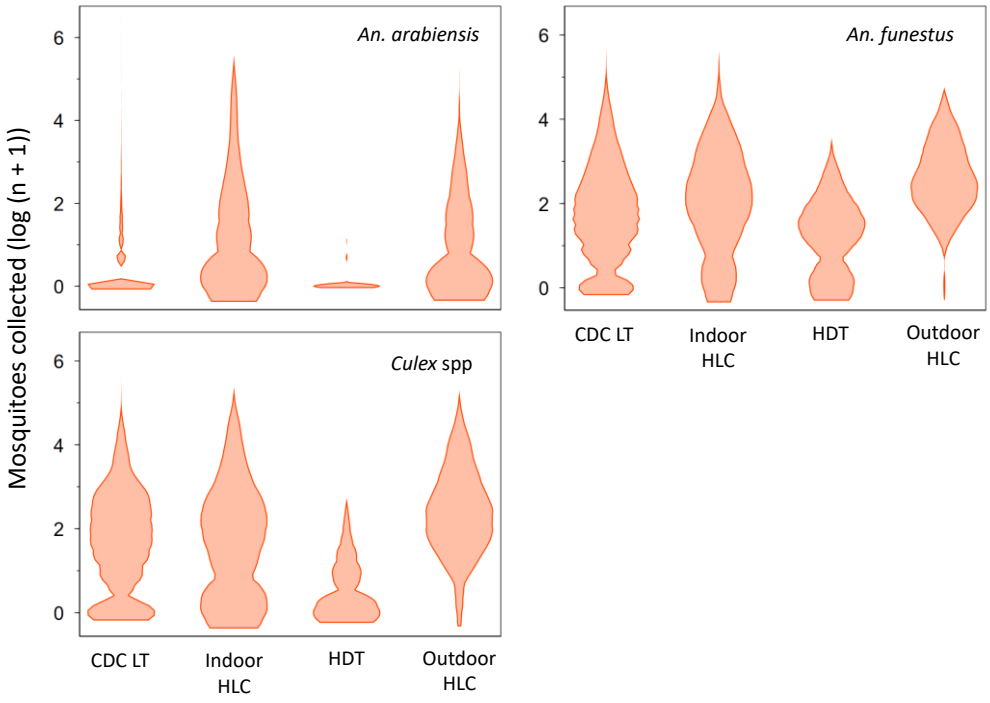

Figure 6. Density distribution of log nightly mosquito catches per trap.

The violin plots were plotted from log transformed mosquito numbers due to skewness. Because of zeros in the data, a value of 1 was added to the nightly numbers of mosquitoes prior to the logarithmic transformation.

Table 2. Number of trap collections and number of mosquitoes caught per trap per night

Trap type		*An. arabiensis*				*An. funestus*				*Culex* spp			
	Total collections	Total caught	Williams' mean (95%CI)	Median (90% central range)	Range	Total caught	Williams' mean (95%CI)	Median (90% central range)	Range	Total caught	Williams' mean (95%CI)	Median (90% central range)	Range
Indoor HLC	339	3380	2.39 (1.93-2.91)	1(0-61)	0-180	3934	4.22 (3.53-5.02)	5(0-58)	0-147	4803	6.51 (5.58-7.57)	7(0-46)	0-196
CDC LT	6013	10281	0.51 (0.48-0.54)	0(0-7)	0-658	59276	4.61 (4.45-4.78)	5(0-39)	0-240	66459	5.01 (4.84-5.19)	5(0-45)	0-250
Outdoor HLC	195	940	1.56 (1.18-2.00)	1(0-23)	0-139	3408	4.14 (3.48-4.91)	9(1-63)	0-143	3460	11.71 (10.15-13.48)	11(2-58)	0-86
HDT	136	5	0.02 (0.00-0.05)	0(0-0)	0-2	189	0.77 (0.56-0.99)	0(0-7)	0-11	526	2.33 (1.86-2.88)	3(0-12)	0-25

The Williams' means were computed by exponentiating the arithmetic means of the log transformed nightly catches per trap. A value of 1 was added to the nightly figures of caught mosquitoes prior to the logarithmic transformation i.e. log (n+1).

The proportions of anophelines caught that were *An. arabiensis* in the CDC LTs and HDTs were lower compared to indoor and outdoor HLC, respectively (Figure 7).

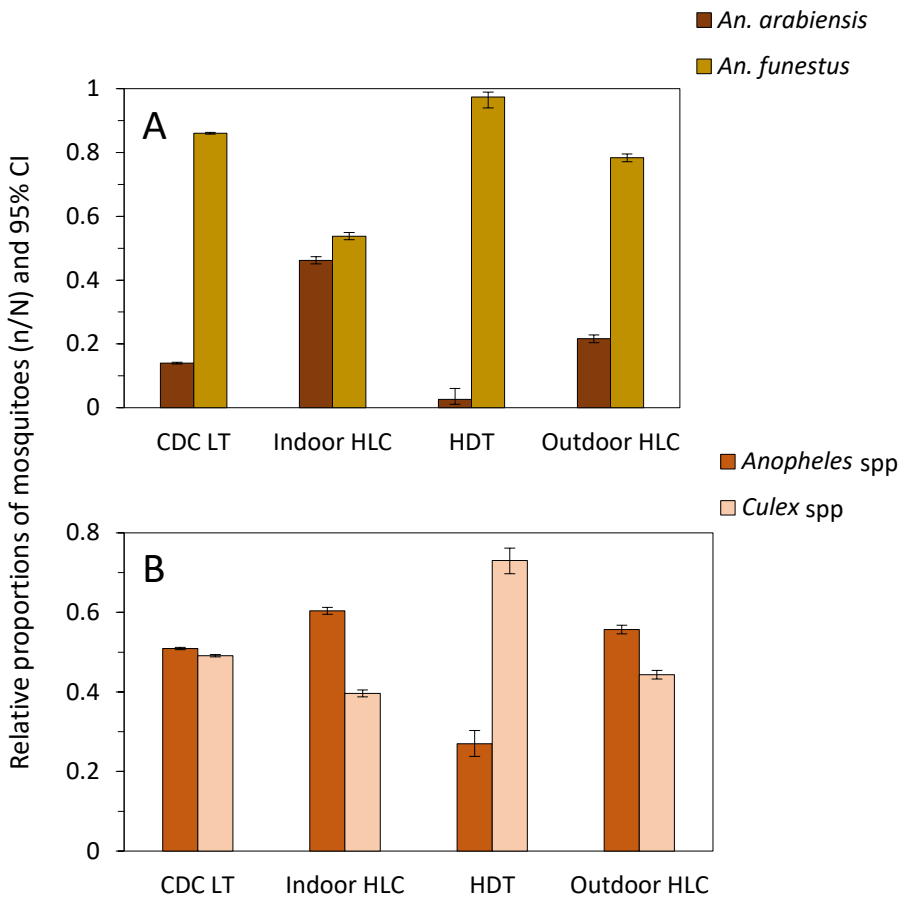

Figure 7. The proportions of *Anopheles* mosquitoes caught by traps.

The relative proportions of (A) *An. arabiensis* versus *An. funestus* and (B) Anophelines versus culicines were estimated from logistic regression models adjusted for random effects of house and date. (The error bars represent 95% confidence intervals (CI))

Overall, the CDC LTs caught approximately a third as many *An. arabiensis* (Adjusted rate ratio (Adj.RR) = 0.35 (95% confidence interval (CI) = 0.27-0.46)) and about two-thirds as many *An. funestus* (Adj.RR = 0.63 (95% CI = 0.51-0.79)) compared to indoor HLC (Table 3)**.** The HDT

caught much lower numbers of *An. arabiensis* (Adj.RR = 0.04 (95% CI: 0.01-0.14)) and *An. funestus* (Adj.RR = 0.10 (95% CI: 0.07-0.15)) compared to the outdoor HLC. The estimated rate ratios for CDC LT and HDT for *Culex* spp were 0.82 (95% CI: 0.67-1.01) and 0.20 (95% CI: 0.14-0.29), respectively.

Table 3. The estimated effect of the CDC LT and the human decoy trap (HDT) compared to the human landing catch (HLC)

Trap type	***An. arabiensis***		***An. funestus***		***Culex* spp**	
	Adj.RR† (95%CI)	p value	Adj.RR† (95%CI)	p value	Adj.RR† (95%CI)	p value
Indoor HLC	1*		1*		1*	
CDC LT	0.35 (0.27-0.46)	< 0.001	0.63 (0.51-0.79)	< 0.001	0.82 (0.67-1.01)	0.061
Outdoor HLC	1*		1*		1*	
HDT	0.04 (0.01-0.14)	< 0.001	0.10 (0.07-0.15)	< 0.001	0.20 (0.14-0.29)	< 0.001

†The adjusted mosquito sampling rate ratios (Adj. RR) and 95% confidence intervals (95%CI) were estimated from negative binomial regression models. The models included random effects for day and house, and fixed effects for the **household size, livestock and pets reared, house screening, IRS treatment, ITNs use, seasonality, and whether the trap surveys were conducted in Study 1 or 2**.
1* reference method.

Aggregating the trap collections per village and per month gave a total of 116 CDC LT and indoor HLC pairs with a median of 66 (90% central range (CR): 6-89) collections per village-month and 40 HDT and outdoor HLC pairs with a median of 6 (90% CR: 4-9).

Geometric mean mosquito catches per village-month by the CDC LT and indoor HLC and by the HDT and outdoor HLC appeared to be positively associated (Figure 8). The mean ratios of geometric means of HDT or CDC LT to HLC and limits of agreement were dependent on mosquito density for all species (Figure 9). The mean ratios decreased significantly with higher HLC catches, indicating that trap efficiency was lower at higher mosquito densities. The limits of agreement for the village-months were wide across most of the range of HLC densities in this study but decreased for higher densities.

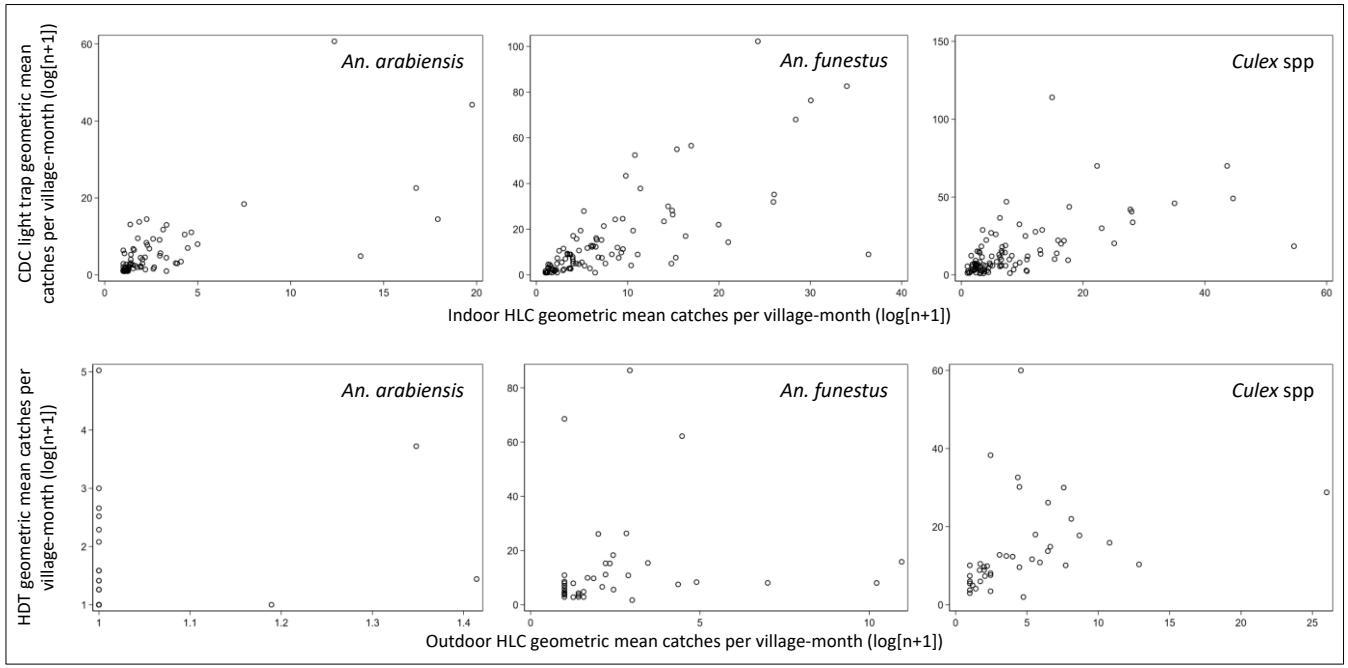

Figure 8. Mosquito catches per village-month by the CDC LT and indoor HLC (upper panels) and by HDT and outdoor HLC (lower panels).

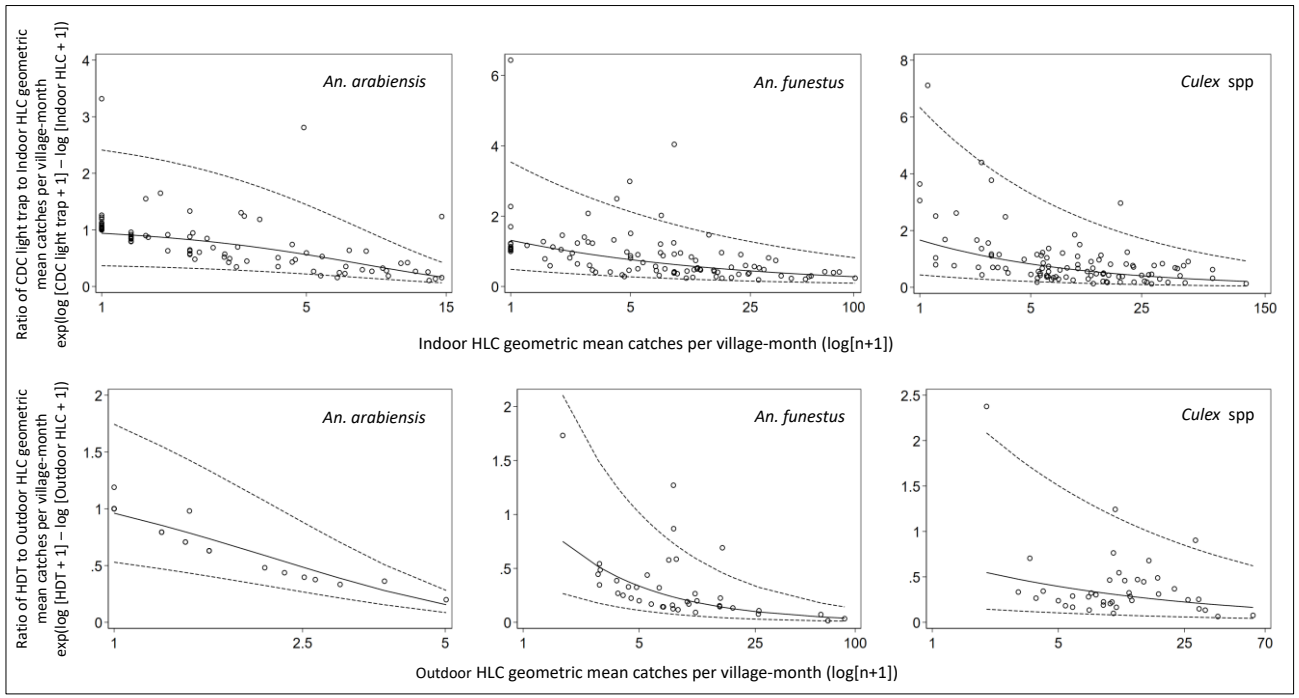

Figure 9. Bland-Altman-based plots showing agreement between CDC LT and indoor HLC (upper panels) and between HDT and outdoor HLC (lower panels).

The solid lines (—) represent the mean ratios of geometric mean catches for the village-month for CDC LT or HDT compared to HLC (the overall bias). The regression equations used to estimate the overall biases are the translation algorithms that account for the density-dependence of the CDC LT or HDT effects relative to the HLC. The dotted lines (----) represent the 95% limits of agreement, in which 95% of the ratios were expected to lie.

Plasmodium falciparum infection rates for *An. arabiensis* and *An. funestus* caught by the different traps were low (Table 4). Only CDC LTs caught any infected *An. arabiensis* mosquitoes and estimated a higher prevalence of infected *An. funestus* compared to indoor HLC. HDT did not catch any infected mosquitoes.

Table 4. *Plasmodium falciparum* infection rates for *An. arabiensis* and *An. funestus* collected by different traps

	Malaria vectors positive by ELISA test								
	An. arabiensis			*An. funestus*			Total		
Trap type	positive	tested	% positive	positive	tested	% positive	positive	tested	% positive
Indoor HLC	0	286	0	12	998	1.20	12	1,284	0.93
CDC LT	10	1,461	0.68	255	5,701	4.47	265	7,162	3.70
Outdoor HLC	0	335	0	10	966	1.04	10	1,301	0.77
HDT	0	3	0	0	39	0	0	42	0

% positive represents the number of mosquitoes with a positive *P. falciparum* circumsporozoite protein (CSP) ELISA test divided by the total number of mosquitoes tested.
ELISA = Enzyme-linked immunosorbent assay.

4.5 Discussion

Monitoring malaria vectors requires accurate, safe and reliable mosquito traps that can be deployed at scale. Despite being the most accurate man-biting mosquito trap, the use of the HLC for the continuous exercise of monitoring malaria vectors is discouraged due to safety concerns. The primary goal of this study was to measure the efficacy relative to HLC of the CDC LT and the HDT to estimate the numbers of different species of host-seeking female *Anopheles* mosquitoes in Ulanga, Tanzania and to determine the suitability of the methods to replace the HLC for routine malaria entomological monitoring in the region.

Controlling for other effects influencing mosquito densities, the CDC LT caught roughly a third as many *An. arabiensis* and about two-thirds as many *An. funestus* as the HLC overall, while the HDT barely caught a tenth of these species compared to the HLC. Although these mean estimates highlight the relative capacities of the traps in general, they have limited relevance in comparing the methods under diverse field settings where mosquito densities are likely to change even across fine spatial and temporal scales [208]. Instead, agreement analysis has been proposed by statisticians, whereby traps are compared on the basis of the overall bias and the variability of a series of matched mosquito collections spanning different location and time points [235, 236]. In the present study, compared to the HLC in matched village-month collections, the CDC LT and the HDT underestimated *An. arabiensis* and *An. funestus* biting and their performance was poorer at high mosquito densities. Mathenge and colleagues [217] explained that this trend may be due to reduced attentiveness of catchers performing the tedious HLC exercise at low mosquito densities. The limits of agreement representing the ratios of geometric mean catches per village-month to the HLC were quite wide, although this declined with increasing abundance of mosquitoes. High variability in the observed ratios presented a challenge to translate *Anopheles* biting rates via consistent algorithms between the CDC LT and indoor HLC and between the HDT and outdoor HLC thereby rendering the use of the

methods as HLC proxies for estimating *Anopheles* biting at the village-month level difficult. However, the variability would be expected to reduce if the comparative estimates are aggregated at periods longer than a month and for areas larger than the villages of this study. In a trial for instance, where absolute numbers of mosquitoes are required to evaluate the effects of treatment arms, the regression equations used in the agreement analysis (Figure 9) could be employed as the conversion algorithms to account for the density-dependent bias of the traps.

Although the sporozoite rates data collected was not sufficient to conduct meaningful statistical comparisons between the CDC LT and the indoor HLC, the proportion of mosquitoes that were infected was higher in the CDC LT than in the indoor HLC samples, a finding similar to that of Mbogo and colleagues in Kilifi [219]. If indeed the CDC LT has higher sensitivity for measuring infection rates of mosquitoes, stemming from the biological premise that older mosquitoes are more likely to be infected, and that the CDC LT has a tendency to catch older mosquitoes [237], then the method is preferable for evaluating the impact of vector control programmes.

Past studies of the CDC LT and the HDT (Table 2), suggest that the performance of these traps may also differ depending on a number of factors. For instance, the CDC LT under- [238] or out-performed [203, 216] the HLC independent of the mosquito species, but in some circumstances its performance differed based on the caught populations [208, 217]. Other observed sources of variation included location [221], dissimilarities indoors and outdoors [215, 237] and the presence or absence of ITNs [214]. Overgaard and colleagues observed that the results of CDC LT efficacy also varied by the different methodological approaches of their study [215]. The choice of host decoy [224], location [221, 223] and seasonality [220] were among the factors observed to influence the HDT performance.

Taken together, if used cautiously with case-by-case appreciation of its limitations, the CDC LT is a suitable and necessary entomological surveillance tool particularly in light of the HLC ethical controversy. In any case, the traps are more objective since they are less prone to human sources of error, they are more acceptable within households than catchers visiting at night, and are convenient to deploy on largescale [210, 220, 224]. However, for measuring the EIR, concurrent use of the HLC on a limited scale is still crucial to calibrate the CDC LT estimates.

The HDT's poor performance in largescale surveys has been ascribed mostly to operational challenges due to its design [221]. The field personnel involved in the surveys of this study mentioned logistical difficulties of transporting and setting up the traps from location to location. The CDC LTs adapted for outdoor surveys [221, 239] and the furvela tent trap (FTT) [221] are some of the possible alternatives for outdoor biting surveys and where necessary, restrained use of the HLC.

4.6 Conclusion

Although the CDC LT caught fewer mosquitoes than the indoor HLC in this study, the traps have shown similar or better efficiency to the HLC elsewhere. The tendency of the traps to under- or oversample host-seeking anophelines can be resolved by regression methods with reference to the HLC, as long as the limits of agreement are reasonably narrow. Therefore, in light of the ethical problems presented by HLC use, the CDC LT could be considered for routine surveys with the HLC only used to ratify parameters such as the EIR. The present design of the HDT is not amenable for use to conduct largescale entomological surveys.

Authors' contributions

Conceived and designed the study: CM and SM. Implemented the study: CM, AS, DK, FT, NM, OO, GL (Deceased), HN, IM, JB, JM and SM. Conducted or contributed to the analysis:

IHN, SM, AR and MH. Drafted or edited the manuscript: IHN, CM, AS, AR, DK, FT, OO, JB, SM, and MH. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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Additional files

Supplementary Table 1. Household and study area factors

Baseline factors	Study 1		Study 2			
	Indoor surveys				Outdoor surveys	
	CDC LT	Indoor HLC	CDC LT	Indoor HLC	Outdoor HLC	HDT
Number of households	214	68	543	39	39	71
Household size						
≤ 5 members	154 (72%)	47 (69%)	463 (85%)	34 (87%)	34 (87%)	61 (86%)
> 5 members	60 (28%)	21 (31%)	80 (15%)	5 (13%)	5 (13%)	10 (14%)
Total number of households (N)	214 (100%)	68 (100%)	543 (100%)	39 (100%)	39 (100%)	71 (100%)
Seasonality of collections						
long rains (Jan-Jun)	439 (81%)	141 (98%)	3280 (60%)	119 (61%)	119 (61%)	25 (18%)
dry season (Jul-Dec)	100 (19%)	3 (2%)	2194 (40%)	76 (39%)	76 (39%)	111 (82%)
Total collections (N)	539 (100%)	144 (100%)	5474 (100%)	195 (100%)	195 (100%)	136 (100%)
IRS treatment						
Intervention arm	307 (57%)	71 (49%)	2544 (46%)	97 (50%)	97 (50%)	64 (47%)
Control arm	232 (43%)	73 (51%)	2930 (54%)	98 (50%)	98 (50%)	72 (53%)
Total collections (N)	539 (100%)	144 (100%)	5474 (100%)	195 (100%)	193 (100%)	136 (100%)
Persons per ITN in households						
1 ITN/≤2 persons	121 (57%)	35 (51%)	360 (66%)	29 (74%)	29 (74%)	45 (63%)
1 ITN/>2 persons	93 (43%)	33 (49%)	183 (34%)	10 (26%)	10 (26%)	26 (37%)
Total number of households (N)	214 (100%)	68 (100%)	543 (100%)	39 (100%)	39 (100%)	71 (100%)
House screening*						
no mosquito proofing	196 (36%)	70 (48.6%)	2863 (52%)	111 (57%)	111 (57%)	69 (51%)
mosquito proofing	343 (64%)	74 (51.4%)	2611 (48%)	84 (43%)	84 (43%)	67 (49%)
Total collections (N)	539 (100%)	144 (100%)	5474 (100%)	195 (100%)	195 (100%)	136 (100%)
Livestock and pets						
No animals	70 (33%)	21 (31%)	12 (2%)	1 (3%)	1 (3)	2 (3%)
Poultry, cats and dogs only	109 (51%)	34 (50%)	246 (45%)	15 (38%)	15 (38%)	33 (38%)
At least goat, donkey or cow	35 (16%)	13 (19%)	20 (4%)	2 (5%)	2 (5%)	2 (5%)
Not recorded	0 (0%)	0 (0%)	265 (49%)	21 (54%)	21 (54%)	34 (54%)
Total number of households (N)	214 (100%)	68 (100%)	543 (100%)	39 (100%)	39 (100%)	71 (100%)

These factors were added as covariates in the multivariable negative binomial GLMMs to account for associated variability of mosquito densities and resultant influence upon the efficacy of traps.
*House screening was coded on the basis of window screens and eaves condition as follows: 1. not mosquito proofed = open eaves + no window screening, 2. partially mosquito proofed = closed eaves or screened windows, 3. mosquito proofed = screened windows + closed eaves

Supplementary Table 2. Estimated rate ratios and 95% CI of indoor biting mosquito densities for trap type and other factors

Covariates		*An. arabiensis*		*An. funestus*		*Culex* spp	
		Adj.RR (95%CI)	*p value*	Adj.RR (95%CI)	*p value*	Adj.RR (95%CI)	*P value*
Trap type	1 = Human landing catch	1*		1*		1*	
	2 = CDC LT	0.35 (0.27-0.46)	*< 0.001*	0.63 (0.51-0.79)	*< 0.001*	0.82 (0.67-1.01)	*0.061*
Household size							
	1 = ≤ 5 members	1*	*0.220*	1*	*0.439*	1*	*0.481*
	2 = > 5 members	0.86 (0.68-1.09)		0.93 (0.78-1.12)		1.08 (0.88-1.33)	
Study (1&2) category							
	1 = Study 1 (2017)	1*	*0.065*	1*	*< 0.001*	1*	*0.082*
	2 = Study 2 (2019)	0.63 (0.38-1.02)		7.85 (5.34-11.52)		1.33 (0.96-1.82)	
Seasons							
	1 = long rains	1*	*< 0.001*	1*	*0.002*	1*	*0.006*
	2 = dry season	0.07 (0.04-0.10)		0.64 (0.49-0.84)		0.82 (0.72-0.94)	
Indoor residual spraying							
	0 = positive control	1*	*< 0.001*	1*	*< 0.001*	1*	*< 0.001*
	1 = IRS product 1	1.13 (0.74-1.71)		1.80 (1.27-2.57)		0.66 (0.48-0.92)	
	2 = IRS product 2	0.52 (0.43-0.62)		2.67 (2.33-3.07)		1.66 (1.41-1.96)	
Insecticide treated nets							
	1 = 1 ITN/≤2 persons	1*	*0.940*	1*	*0.012*	1*	*0.630*
	2 = 1 ITN/>2 persons	0.99 (0.82-1.20)		0.84 (0.73-0.96)		1.96 (0.82-1.13)	
House screening							
	1 = not mosquito proofed	1*	*0.243*	1*	*0.604*	1*	*0.702*
	2 = mosquito proofed	0.90 (0.76-1.07)		0.97 (0.85-1.10)		0.97 (0.84-1.13)	
Livestock and pets							
	1 = none	1*	*0.472*	1*	*< 0.001*	1*	*0.126*
	2 = poultry, dogs, cats only	1.09 (0.78-1.54)		1.06 (0.80-1.40)		0.86 (0.64-1.16)	
	3 = At least goat, cow or donkey	0.82 (0.51-1.31)		0.59 (0.40-0.87)		1.92 (0.62-1.36)	
	4 = Not recorded	1.13 (0.78-1.63)		0.86 (0.64-1.15)		1.05 (0.76-1.46)	

1* refers to the reference method or category
The single p values for the categorical variables were estimated by the likelihood ratio test (lrtest)
CI = Confidence interval
Adj.RR = Adjusted rate ratios estimated from multivariable mixed effects regression models

Supplementary Table 3. Estimated rate ratios and 95% CI of outdoor biting mosquito densities for trap type and other factors

Covariates		*An. arabiensis*		*An. funestus*		*Culex* spp	
		Adj.RR (95%CI)	*p value*	Adj.RR (95%CI)	*p value*	Adj.RR (95%CI)	*P value*
Trap type	1 = Human landing catch	1*		1*		1*	
	3 = Host decoy trap	0.04 (0.01-0.14)	*< 0.001*	0.10 (0.07-0.15)	*< 0.001*	0.20 (0.14-0.29)	*< 0.001*
Household size							
	1 = ≤ 5 members	1*	*0.454*	1*	*0.068*	1*	*0.348*
	2 = > 5 members	1.52 (0.52-4.45)		1.68 (0.96-2.93)		1.27 (0.77-2.09)	
Season							
	1 = long rains	1*	*< 0.001*	1*	*< 0.001*	1*	*0.007*
	2 = dry season	0.05 (0.02-0.12)		0.57 (0.46-0.71)		0.73 (0.59-0.91)	
Indoor residual spraying							
	0 = positive control	1*	*0.032*	1*	*< 0.001*	1*	*0.172*
	2 = IRS product 2	0.47 (0.23-0.95)		2.50 (1.68-3.70)		1.27 (0.90-1.80)	
Insecticide treated nets							
	1 = 1 ITN/≤2 persons	1*	*0.668*	1*	*0.325*	1*	*0.861*
	2 = 1 ITN/>2 persons	1.20 (0.52-2.78)		0.80 (0.51-1.26)		0.97 (0.65-1.43)	
Livestock and pets							
	1 = none	1*	*0.439*	1*	*0.611*	1*	*0.399*
	2 = poultry, dogs, cats only	3.25 (0.40-26.52)		1.49 (0.46-4.81)		0.67 (0.25-1.81)	
	3 = At least goat, cow or donkey	7.61 (0.64-90.05)		2.01 (0.47-8.52)		0.33 (0.09-1.25)	
	4 = Not recorded	2.97 (0.39-22.59)		1.22 (0.38-3.88)		0.63 (0.24-1.70)	

1* refers to the reference method or category
The single p values for the categorical variables were estimated by the likelihood ratio test (lrtest)
CI = Confidence interval
Adj.RR = Adjusted rate ratios estimated from multivariable mixed effects regression models

5. A matter of timing: Biting by malaria-infected Anopheles mosquitoes and the use of interventions during the night in rural south-eastern Tanzania

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5.1 Abstract

Background: Knowing when and where infected *Anopheles* mosquitoes bite is required for calculating accurate measures of malaria risk, assessing the contribution of outdoor exposure, and for designing effective intervention strategies. This study combines human behaviour and entomological data to estimate human exposure to malaria-infected *Anopheles* mosquitoes throughout the night in rural Tanzanian villages.

Methods: This study involved secondary analyses of an entomological survey and a separate human behaviour survey carried out in the same area in south-eastern Tanzania. Mosquitoes were collected hourly from 6PM to 6AM indoors and outdoors by human landing catches (HLC) in 2019, and tested for *Plasmodium falciparum* sporozoite infections using ELISA. In nearby villages, trained members of selected households recorded the whereabouts and activities of individual household members from 6PM to 6AM in 2016 and 2017. Vector control use was high: at the time of the human behaviour survey 99% of individuals were reported to use insecticide treated nets and at the time of the entomological study a recent trial of indoor residual spraying had achieved 80% coverage. Human and mosquito data were analysed by hour from 6PM to 6AM to assess the risk of being bitten by infected mosquitoes outdoors, indoors in bed, and indoors but not in bed, and whether or not mosquito nets were used.

Results: Individuals were mainly outdoors before 9PM, and mainly indoors between 10PM and 5AM. The main malaria vectors caught were *Anopheles funestus* sensu stricto and *An.*

arabiensis. Biting rates were higher in the night compared to the evening or early morning. Due to the high use of ITNs, an estimated 84% (95% CI 80%, 88%) of all exposure in children below school age and 76% (71%, 81%) in older household members could potentially be averted by ITNs under current use patterns. Outdoor exposure accounted for an estimated 18% (13%, 22%) of infective bites in school-age children and 12% (8%, 16%) in older individuals.

Conclusion: At night, the majority of exposure to infected mosquitoes in this study setting occurs indoors. Maintaining high levels of access and use of ITNs remains an effective means to reduce malaria transmission in this area. Interventions against outdoor exposure would provide additional protection.

Keywords: *Anopheles* biting, *Plasmodium falciparum*, malaria risk, insecticide treated nets

5.2 Background

Vector control interventions protect people by reducing or preventing human-vector contact [240]. Between 2000 and 2021 an estimated two billion cases and 12 million deaths were averted by malaria control programmes [1]. A substantial proportion of the cases averted have been attributed to the most widely used measures against malaria-transmitting *Anopheles* mosquitoes, insecticide-treated nets (ITNs) and indoor residual spraying (IRS) of insecticides [241, 242]. These tools remain a critical part of the global malaria control and elimination agenda [1, 75]. However, the gains made by vector control are being undermined by multiple factors, among them, insecticide resistance [243-245], sub-optimal bioefficacy and sub-optimal durability of nets [246, 247], inefficient distribution of nets to households, unequal allocation of nets to household members and the poor use of nets [248, 249]. In many settings, the effectiveness of indoor interventions is also attenuated by mosquito behavioural adaptations, such as biting alternative hosts, exiting houses immediately after feeding to rest outdoors and biting humans outdoors [250-255].

Although malaria vectors still bite predominantly indoors at night [61], a systematic review of human-vector interactions across sub-Saharan Africa (SSA) estimated that the proportion of bites occurring outdoors had risen by 10% between 2003 and 2018 [156]. This increase in the proportion of outdoor biting has been predicted to result in an additional 10.6 million malaria cases a year in SSA assuming universal coverage with ITNs and IRS is achieved [156]. These findings highlight the need to characterise the risk of malaria transmission in the context of the increasing use of interventions.

A standard measure of malaria transmission is the entomological inoculation rate (EIR), the mean number of infective bites per person per unit of time. The EIR is estimated by multiplying the biting rate, estimated from the number of host-seeking mosquitoes caught, by the estimated proportion of mosquitoes that are infected with sporozoites [256]. The EIR is useful for quantifying the risk of infective bites. However, it does not capture the actual risk experienced by the community since it does not include the use of personal protective interventions. The component metrics for EIR are typically obtained over the entire active mosquito-biting period, usually all night, and do not take changes in biting rates, the proportion of infected-bites and human behaviour throughout the night into account.

Estimating the risk for individual hours throughout the night may allow a more accurate estimation of the community's actual risk of malaria. The locations of humans (whether inside or outside dwellings and whether under a net) throughout the night are needed to properly characterise the actual risk of malaria infections [257, 258]. The relevance of *Anopheles* bites measured by catches of host-seeking mosquitoes depends on the availability of unprotected humans at the respective times and locations [257, 259, 260]. Some studies have also indicated that mosquitoes with *Plasmodium* sporozoites or those that were parous and therefore more likely to be infected may bite at different times of the night (Table 1).

Malaria risk metrics that are more granular and that take human behaviour into account may improve the identification of gaps in the existing protection and assist the designing of more effective intervention responses. However, there are only a small number of studies that capture these metrics. A recent study from Burkina Faso combined entomological and human behaviour data and found that while the majority of infective bites would have occurred during times when people were using ITN, transmission outside these hours still occurred [261]. The present study aimed at understanding the patterns of human exposure to malaria-infected mosquitoes in rural Tanzanian villages where ITNs and IRS are used. We investigated the hourly numbers of host-seeking mosquitoes outdoors and indoors, the proportion of mosquitoes infected with sporozoites, and location of humans during the night to assess patterns of exposure to infected mosquitoes.

5.3 Methods

Study area

This study was conducted in Ulanga and Kilombero districts in south-eastern Tanzania (Figure 10). The area lies within the greater Kilombero valley with elevation averaging 270m above sea level. The climate is mostly hot and humid. The annual rainfall is 1200-1800mm, with a peak between October and November and a second peak between April and May, while temperatures range between 20ºC and 33ºC [262]. The communities practise rice farming in irrigated paddies, subsistence agriculture and small-scale fishing. Recent studies have shown that *An. gambiae sensu lato* in this area consists almost entirely of *An. arabiensis*, while *An. funestus* s.l. comprises more than 95% *An. funestus sensu stricto* [121, 226, 263-265]. Moderate to high levels of malaria transmission occur all year with seasonal peaks around the wet seasons. ITNs are the primary vector control intervention in the area [265-267], and a mass campaign shortly before the human behaviour survey resulted in self-reported use of ITNs of 99%. In addition, a community-wide IRS with pirimiphos-methyl (Actellic® 300, capsule

suspension) and a perlite-mineral insecticide (Imergard^{TM}, wettable powder) was also implemented between January and October in 2019 as part of an intervention trial [268]. At the time of the entomological survey, 80% of households had IRS

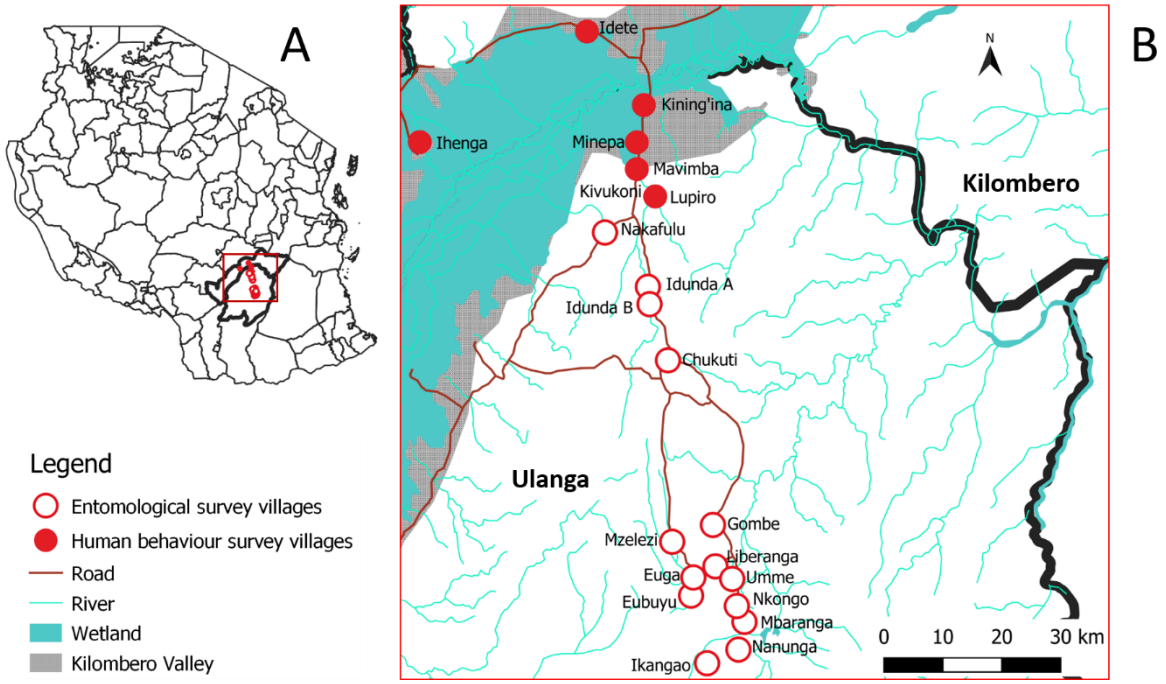

Figure 10. Map of study area

(**A**) Location of Kilombero and Ulanga Districts (bold line borders) in Tanzania. (**B**) Locations of the study villages in Ulanga and Kilombero Districts.

Study design

This study combines secondary analysis of data from two different studies: a human behaviour survey and an entomological survey.

Human behaviour survey: The human behaviour survey was conducted between August 2016 and June 2017 in rural communities comprising six villages (Kivukoni, Minepa,Lupiro, Idete, Ihenga and Kining'ina) in Ulanga and Kilombero districts and in urban and peri-urban settlements comprising three sites (Katindiuka, Ifakara Mjini and Viwanja Sitini) in Kilombero

district . The surveys were done around and inside houses from dusk to dawn, and have been described in detail elsewhere [188]. Ninety households from the villages (ten houses per village) were randomly selected from a house enumeration list extracted from the Ifakara Health and Demographic Surveillance System [269]. Consenting adult household members were recruited and provided with three-day training on how to observe and record nightly household activities among members of their households. The number of people doing different activities at different times and locations generally classified as outdoors, indoors but out of bed and in bed with or without ITN use were recorded every half hour from 6PM to 6AM. The observations were done for three days every month, for three months in the rainy season and another three months in the dry season. For purposes of this current study, data from peri-urban and urban settlements as captured in the original study [188] have been excluded to match the entomological surveys which were all done in rural communities.

Entomological survey: The entomological survey was conducted between August 2018 and September 2019 in fourteen villages in Ulanga district (Nakafulu, Idunda A, Idunda B, Chikuti, Gombe, Liberanga, Umme, Nkongo, Mbaranga, Ikangao, Eubuyu, Euga, Mzelezi and Nanunga) (Figure 10). The villages had been deliberately selected as having substantial mosquito populations for the purpose of the IRS evaluation. Host-seeking mosquitoes were collected by human landing catches (HLC) between 6PM to 6AM by a pair of volunteers alternating positions indoors and outdoors every hour [270]. The collections were conducted in three houses selected from each of fourteen villages and were repeated for six nights per month over eight months. Similar to the human behaviour survey, the entomological survey included the wet and dry seasons. The entomological survey has been described in detail elsewhere [271]. Female *Anopheles* mosquitoes were identified morphologically. Polymerase chain reaction (PCR) assays were conducted to identify the sibling species of the *An. gambiae* and *An. funestus* complexes [272, 273]. *Plasmodium* circumsporozoite protein (CSP) tests were

done by enzyme-linked immunosorbent assays (ELISA) to detect mosquitoes infected with malaria parasites [274].

Data analysis

We adapted the notation and formulae from initial work by Monroe *et al* for measuring human exposure to bites occurring in different locations and whether protected or unprotected [169].

***Total and infected mosquito biting rates*:** The biting rates, the mean number of mosquito bites per person, were estimated using the HLC collections. For each hour, t, and species, m, the outdoor, $B_{O,t,m}$, and indoor, $B_{I,t,m}$, biting rates were estimated using Poisson regression with crossed random effects to account for repeated observations by household and date and a fixed effect for hour. This takes into account the unbalanced sampling by household, date and hour. Separate models were run for each species and location.

The proportions of mosquitoes infected with sporozoites, $p_{l,t,m}$, were estimated with exact binomial confidence intervals for each location, l, hour, t, and species, m. Due to low numbers of infected mosquitoes, clustering was not accounted for. We also aggregated the results for the proportions of infected mosquitoes for some hours when calculating the rates of infective bites: for indoors, the categories were 6PM-11PM, 11PM-12AM, 12AM-1AM, 1AM-6AM and for outdoors, all hours were pooled together.

The mean number of infective bites per person outdoors, and indoors, were estimated per hour by multiplying the hourly biting rates by the proportion of bites from the infected mosquitoes.

A sum of the hourly *An. arabiensis* and *An. funestus* infective bites was then obtained to estimate the total hourly incidence of infective bites. We estimated the 95% confidence intervals for the infective biting rates taking the uncertainty for both the overall biting rates and the proportion of mosquitoes infected into account. For each hour, species and location, we

randomly sampled 1000 draws from the distribution for the number of bites per person per hour (we used a normal distribution parameterized with our estimated mean biting rate and standard error (SE)) and from the distribution of the proportion of mosquitoes infected (we used a normal distribution with our estimated mean proportion infected and SE). We calculated the number of infective bites for each of the 1000 samples. The 2.5 and 97.5 percentiles of the 1000 samples yielded the 95% confidence interval. We assume that the covariance between biting rates and the proportion of mosquitoes infected is zero.

We also aggregated the outdoor and indoor hourly infective bites over three time intervals. These intervals represent times when mostly people are outdoors or indoors prior to bed time (6AM to 10PM), during bedtime (10PM to 5AM) and after bedtime (later than 5AM) where different interventions would need to be used.

***Human exposure to infected mosquito bites in the absence of ITNs*:** For each hour of the night, *t*, between 6PM and 6AM, the proportion of recorded times that people were outdoors, O_t, indoors in bed (during sleep), S_t, with or without ITNs, and indoors out of bed (awake),A_t, were estimated. The human behaviour survey recorded the location of participants indoors as 'in bed' or 'out of bed' while the Monroe *et al* notation classifies participants as 'sleeping' or 'awake'. For purposes of using this notation, we assume that these are synonymous. The mean number of infective bites indoors awake per person per night, n_A, was estimated by the sum for all the hours and species of the time at risk multiplied by the incidence of infective bites, so that $n_A = \sum_m \sum_t B_{I,t,m}\, p_{I,t,m}\, A_t$. Similarly, the mean number of infective bites indoors while in bed sleeping per person per night assuming no net use, was given by $n_{S,u} = \sum_m \sum_t B_{I,t,m}\, p_{I,t,m}\, S_t$, and the mean number of infective bites per person per night outdoors by $n_O = \sum_m \sum_t B_{O,t,m,}\, p_{O,t,m}\, O_t$. The proportion of infective bites estimated to occur indoors and in bed sleeping assuming no net use was given by $\pi_{S,u} = \frac{n_{S,u}}{n_A + n_{S,u} + n_O}$.

***Proportion of infective bites occurring when people are using ITNs*:** For each hour of the night, *t*, the proportion of time spent in bed and protected by ITNs, $S_{p,t}$ was estimated. The proportion of infective bites occurring when people were using ITNs, P_S^*, was estimated by the sum over the hours *t* of infective bites occurring during sleep multiplied by the proportion of time in hour *t* that ITNs were used while sleeping divided by the number of all infective bites. This would represent the proportion of infective bites averted by ITNs if ITNs prevent 100% of bites while in use. Normally, ITNs do not block every single mosquito bite while in use [158, 275, 276], and so P_S^* would represent the maximum protection for ITN users in this study setting. This potential maximum protection from ITNs was estimated separately for children below school age and the rest of the household members.

Estimated number of infective bites per person per year

We summed the estimated infective bites over the night (taking the mean of indoor and outdoor bites) to give an estimate of the EIR without incorporating human behaviour. We calculated a similar measure taking into account human location and ITN use to estimate the actual transmission risk experienced by the community.

Data analysis was carried out using Stata (16.1, StataCorp LLC, College Station, TX).

Table 5. Quantities used to estimate behaviour-adjusted exposure to Anopheles infective bites

Quantity	Description
l	Location (Indoors or Outdoors)
t	Hour
m	Species (*An. arabiensis* or *An. funestus*)
$B_{O,t,m}$	Number of outdoor bites in hour *t* by species *m*
$B_{I,t,m}$	Number of indoor bites in hour *t* by species *m*
$p_{l,t,m}$	Proportion of bites that came from infective mosquitoes
O_t	Proportion of time spent by humans outdoors in hour *t*
S_t	Proportion of time spent by humans indoors asleep in hour *t*
A_t	Proportion of time spent by humans indoors awake in hour *t*
n_A	Number of infective bites per person indoors awake per night
n_O	Number of infective bites per person outdoors per night
$n_{S,u}$	Number of infective bites per person indoors asleep per night assuming no net use
$\pi_{S,u}$	Proportion of infective bites that occur indoors asleep assuming no net use
$S_{p,t}$	Proportion of time spent by humans indoors asleep using a bed net in hour *t*
P_S^*	Proportion of infective bites occurring during sleep when protected by a net

Ethics approval and consent to participate

Ethical clearance was obtained from Ifakara Health Institute Review Board, (Entomological surveys: IHI IRB 021/2016 & 015/2017, Human behaviour surveys: IHI/IRB/No: IHI/IRB/No: 35–2015) and the Medical Research Coordinating Committee of the Tanzanian National Institute of Medical Research (Entomological surveys: NIMR/HQ/R.8a/Vol.IX/1725 & 2270, Human behaviour surveys: NIMR/HQ/R.8a/Vol.IX/2162). Written informed consent was obtained from all household heads and volunteers prior to their participation in the surveys. Permission to publish this work was granted by Tanzanian National Institute of Medical Research: NIMR/HQ/P.12 VOL.XXXV/164.

5.4 Results

Indoor and outdoor biting rates

There were HLC collections in 46 households from 14 villages over 62 dates. A total of 8,276 *An. funestus* s.s. and 1,927 *An. arabiensis* mosquitoes were caught. The biting rates were higher for *An. funestus* compared to *An. arabiensis*, but the split between indoor and outdoor biting was similar for the two species (Table 6 and Figure 11).

Table 6. Mosquito biting rates estimated from HLC

	Indoors			Outdoors		
	Number of HLC	Estimated mean number of bites/person/hour (95% CIs)		Number of HLC	Estimated mean number of bites/person/hour (95% CIs)	
		An. arabiensis	*An. funestus*		*An. arabiensis*	*An. funestus*
6-7PM	171	0.10 (0.06, 0.18)	0.51 (0.36, 0.70)	162	0.10 (0.05, 0.19)	0.38 (0.27, 0.54)
7-8PM	208	0.11 (0.06, 0.19)	0.98 (0.72, 1.32)	207	0.12 (0.06, 0.23)	0.86 (0.64, 1.16)
8-9PM	212	0.10 (0.06, 0.17)	0.96 (0.71, 1.30)	215	0.10 (0.05, 0.20)	1.18 (0.88, 1.58)
9-10PM	213	0.11 (0.06, 0.19)	1.05 (0.78, 1.43)	215	0.12 (0.06, 0.22)	1.23 (0.91, 1.65)
10-11PM	222	0.12 (0.07, 0.20)	1.08 (0.79, 1.46)	214	0.12(0.06, 0.23)	1.16 (0.87, 1.57)
11-12AM	207	0.12 (0.07, 0.21)	1.07 (0.79, 1.45)	218	0.10 (0.05, 0.20)	1.05 (0.78, 1.41)
12-1AM	205	0.12 (0.07, 0.21)	0.95 (0.70, 1.29)	202	0.08 (0.04, 0.16)	1.12 (0.83, 1.50)
1-2AM	204	0.09 (0.05, 0.17)	1.02 (0.76, 1.39)	205	0.11 (0.06, 0.22)	1.13 (0.84, 1.52)
2-3AM	197	0.13 (0.07, 0.22)	1.03 (0.76, 1.41)	206	0.11 (0.05, 0.21)	1.90 (0.81, 1.47)
3-4AM	208	0.08 (0.04, 0.14)	1.09 (0.81, 1.48	211	0.11 (0.06, 0.22)	1.12 (0.83, 1.50)
4-5AM	197	0.07 (0.04, 0.12)	1.08 (0.80, 1.47)	195	0.07 (0.03, 0.13)	1.21 (0.90, 1.62)
5-6AM	160	0.06 (0.03, 0.11)	0.90 (0.66, 1.23)	159	0.04 (0.02, 0.09)	1.03 (0.76, 1.40)

The mean number of bites per person per hour was estimated using Poisson regression with crossed random effects for house and date and a fixed effect for hour. Separate analyses were run for indoor and outdoor locations, and by species and total.

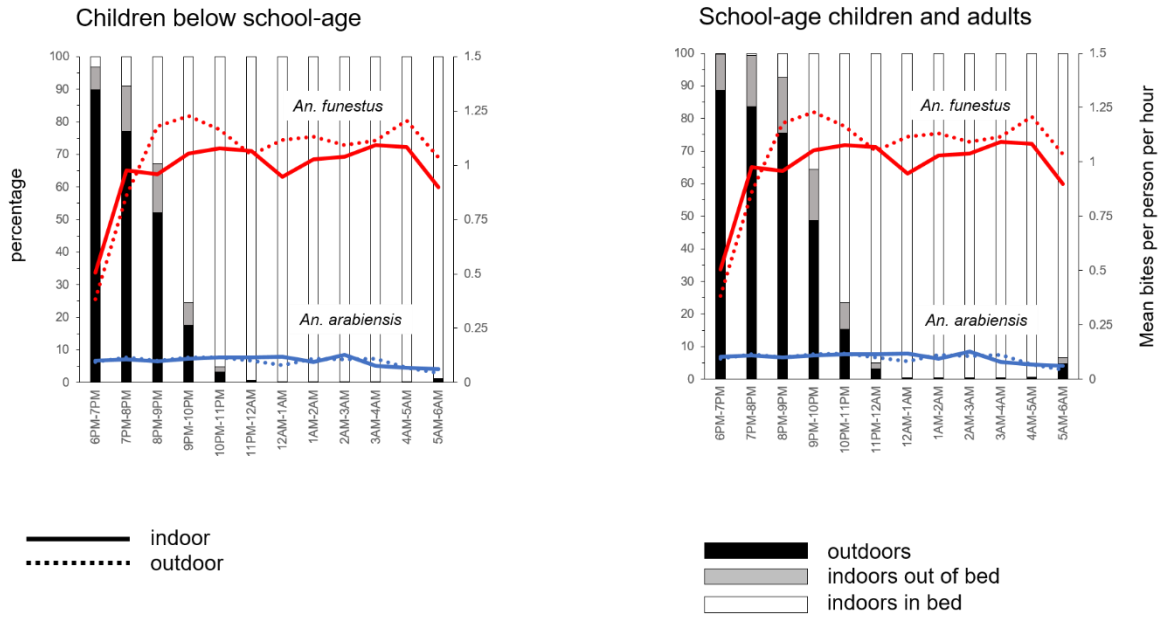

Figure 11. Locations of household members and *Anopheles* bites at night.

Proportion of mosquitoes that were infected with *Plasmodium falciparum*

The proportion of mosquitoes infected with *P. falciparum* sporozoites tended to be higher in mosquitoes collected indoors than in those collected outdoors (Table 7). For *An. funestus* s.s. the proportion infected indoors was 0.005 (326/72219) and outdoors was 0.003 (13/4025). For *An. arabiensis*, the proportion infected was 0.002 (17/7442) indoors and 0 (0/1044) outdoors. There were small numbers of infected mosquitoes and no clear patterns with time. The uncertainty, represented by the width of the CI, was greatest where few mosquitoes were available for testing due to low biting rates. For this reason, for the estimates of behaviour-adjusted exposure the proportion of mosquitoes infected were aggregated over multiple hours.

Table 7. Proportion of mosquitoes infected

	An. arabiensis				*An. funestus*			
	Number tested	Number positive	Estimated proportion infected (95% CI[1])		Number tested	Number positive	Estimated proportion infected (95% CI[1])	
Indoors								
6-7PM	81	0	0	(0, 0.04)	365	2	0.005	(0.001, 0.02)
7-8PM	67	0	0	(0, 0.05)	508	0	0	(0, 0.007)
8-9PM	70	0	0	(0, 0.05)	496	1	0.002	(0.0001, 0.01)
9-10PM	82	0	0	(0, 0.04)	413	1	0.002	(0, 0.01)
10-11PM	1630	4	0.002	(0.001, 0.006)	17731	75	0.004	(0.003, 0.005)
11-12AM	2006	4	0.002	(0.001, 0.006)	17424	82	0.005	(0.004, 0.006)
12-1AM	1606	4	0.002	(0.001, 0.006)	16780	77	0.005	(0.004, 0.006)
1-2AM	1711	5	0.003	(0.001, 0.007)	17065	80	0.005	(0.004, 0.006)
2-3AM	77	0	0	(0, 0.05)	375	4	0.011	(0.003, 0.027)
3-4AM	48	0	0	(0, 0.07)	405	2	0.005	(0.001, 0.018)
4-5AM	39	0	0	(0, 0.09)	393	1	0.003	(0.0001, 0.014)
5-6AM	25	0	0	(0, 0.14)	264	1	0.004	(0.0001, 0.021)
Outdoors								
6-7PM	82	0	0	(0, 0.04)	121	0	0	(0, 0.030)
7-8PM	102	0	0	(0, 0.04)	272	1	0.004	(0.0001, 0.020)
8-9PM	94	0	0	(0, 0.04)	375	0	0	(0, 0.010)
9-10PM	110	0	0	(0, 0.03)	402	1	0.002	(0.0001, 0.014)
10-11PM	108	0	0	(0, 0.05)	396	2	0.005	(0.0001, 0.018)
11-12AM	98	0	0	(0, 0.04)	337	2	0.006	(0.0001, 0.021)
12-1AM	77	0	0	(0, 0.04)	353	1	0.003	(0.0001, 0.016)
1-2AM	98	0	0	(0, 0.04)	371	3	0.008	(0.002, 0.023)
2-3AM	97	0	0	(0, 0.04)	346	0	0	(0, 0.011)
3-4AM	91	0	0	(0, 0.04)	380	3	0.008	(0.002, 0.023)
4-5AM	53	0	0	(0, 0.07)	384	0	0	(0, 0.010)
5-6AM	34	0	0	(0, 0.10)	288	0	0	(0, 0.013)

[1]Exact binomial 95% confidence intervals

Rate of infective bites

The estimated rate of infective bites varied by time of the night, species and location (Table 8). The estimated infective biting rates were slightly higher indoors compared to outdoors, and were lowest in the early evening and late morning.

The percentage of infective bites occurring between 10PM and 5AM (representing 58% of the 12 hour period of HLC) was estimated to be 65% (60%, 70%) indoors and 63% (54%, 71%) outdoors.

Table 8. Estimated rates of infective bites

	Infective bites per 100 person-hours (95% CI)					
	Indoors			Outdoors		
	An. arabiensis	*An. funestus*	total	*An. arabiensis*	*An. funestus*	total
6-7PM	0.02 (0.01, 0.04)	0.21 (0.14, 0.30)	0.23 (0.16, 0.32)	0	0.12 (0.05, 0.21)	0.12 (0.05, 0.21)
7-8PM	0.02 (0.01, 0.04)	0.40 (0.03, 0.57)	0.42 (0.29, 0.60)	0	0.28 (0.12, 0.48)	0.28 (0.12, 0.48)
8-9PM	0.02 (0.01, 0.04)	0.39 (0.25, 0.56)	0.41 (0.27, 0.58)	0	0.39 (0.17, 0.66)	0.39 (0.17, 0.66)
9-10PM	0.02 (0.01, 0.04)	0.44 (0.30, 0.63)	0.46 (0.32, 0.65)	0	0.40 (0.16, 0.66)	0.40 (0.16, 0.66)
10-11PM	0.02 (0.01, 0.05	0.44 (0.29, 0.66)	0.47 (0.31, 0.69)	0	0.38 (0.17, 0.66)	0.38 (0.17, 0.65)
11-12AM	0.02 (0.01, 0.05)	0.51 (0.33, 0.73)	0.53 (0.36, 0.76)	0	0.34 (0.14, 0.59)	0.34 (0.15, 0.59)
12-1AM	0.03 (0.01, 0.06)	0.44 (0.30, 0.62)	0.47 (0.33, 0.65)	0	0.37 (0.16, 0.62)	0.37 (0.16, 0.62)
1-2AM	0.03 (0.01, 0.05)	0.49 (0.33, 0.70)	0.52 (0.35, 0.73)	0	0.37 (0.16, 0.63)	0.37 (0.16, 0.63)
2-3AM	0.03 (0.02, 0.06)	0.50 (0.33, 0.70)	0.53 (0.36, 0.75)	0	0.36 (0.15, 0.60)	0.36 (0.15, 0.60)
3-4AM	0.02 (0.01, 0.04)	0.52 (0.36, 0.74)	0.54 (0.38, 0.77)	0	0.36 (0.16, 0.62)	0.36 (0.16, 0.62)
4-5AM	0.02 (0.01, 0.03)	0.51 (0.34, 0.72)	0.53 (0.36, 0.74)	0	0.39 (0.16, 0.66)	0.39 (0.16, 0.66)
5-6AM	0.02 (0.01, 0.03)	0.43 (0.29, 0.61)	0.45 (0.31, 0.63)	0	0.34 (0.15, 0.57)	0.34 (0.15, 0.57)

The rate of infected bites was estimated by combining the biting rates and the sporozoite rates. We aggregated the proportion of mosquitoes infected from 6-11PM, 11PM-12AM, 12-1AM, 1-6AM indoors and all hours outdoors due to small numbers.

Observations of human behaviours and activities indoors and outdoors

Sixty households had records spread over three months, with a median of 8 nights per household with range 2 to 18.

There was a total of 171,139 observations of the locations of individuals made at half-hourly intervals. Overall, the majority of the observations of participants in the early evenings between 6PM and 9PM were outdoors (Table 9). Between 9PM and 10PM, the proportion of time spent outdoors dropped and by midnight, nearly all observations recorded were of individuals indoors. The proportion of recorded locations of individuals that were indoors in bed rose steadily each hour from 9PM to midnight. Nearly everyone who was recorded at 6AM was still indoors in bed. Time spent in bed tended to be longer for children below school age than for older household members (Figure 12).

Table 9. Proportion of people observed by location

	Children below school-age			School-age children and adults		
	Outdoors %(95% CIs)	Indoors awake % (95% CIs)	Indoors sleeping % (95% CIs)	Outdoors %(95% CIs)	Indoors awake % (95% CIs)	Indoors sleeping % (95% CIs)
6-7PM	89.8 (84.0, 93.6)	7.0 (3.7, 13.1)	3.2 (1.7, 6.0)	88.5 (84.6, 91.5)	11.2 (8.2, 15.1)	0.3 (0.1, 0.6)
7-8PM	77.2 (70.5, 82.7)	13.8 (8.7, 21.2)	9.0 (6.1, 13.1)	83.6 (79.1, 87.3)	15.6 (12.0, 20.1)	0.7 (0.4, 1.2)
8-9PM	52.1 (45.5, 58.7)	15.0 (8.6, 24.9)	32.9 (26.6, 39.8)	75.5 (69.9, 80.3)	17.1 (12.8, 22.6)	7.4 (5.4, 10.0)
9-10PM	17.6 (13.0, 23.4)	7.0 (3.0, 15.1)	75.4 (67.6, 81.9)	48.7 (42.76, 54.7)	15.7 (11.7, 20.6)	35.6 (29.6, 42.1)
10-11PM	3.2 (1.9, 5.4)	1.6 (0.8, 3.2)	95.2 (92.4, 97.0)	15.4 (11.5, 20.3)	8.0 (5.0, 12.5)	76.6 (70.6, 81.6)
11-12AM	0.4 (0.1, 1.0)	0.2 (0.1, 0.6)	99.4 (98.8, 99.7)	3.2 (1.7, 5.9)	1.9 (1.1, 3.2)	94.9 (91.9, 96.8)
12-1AM	0.1 (0.0, 0.5)	0	99.9 (99.5, 100)	0.3 (0.1, 0.9)	0.2 (0.0, 1.2)	99.5 (98.6, 99.8)
1-2AM	0.1 (0.0, 0.5)	0	99.9 (99.5, 100)	0.1 (0.0, 1.0)	0.2 (0.1, 0.7)	99.7 (99.0, 99.9)
2-3AM	0.1 (0.0, 0.5)	0	99.9 (99.5, 100)	0.2 (0.1, 0.8)	0.1 (0.0, 0.2)	99.7 (99.2, 99.9)
3-4AM	0.1 (0.0, 0.5)	0	99.9 (99.5, 100)	0.1 (0.0, 0.9)	0.0 (0.0, 0.2)	99.8 (99.2, 100)
4-5AM	0.1 (0.0, 0.4)	0	99.9 (99.6, 100)	0.3 (0.1, 0.9)	0.1 (0.0, 0.1)	99.6 (99.1, 99.8)
5-6AM	1.1 (0.4, 2.8)	0.2 (0.0, 1.3)	98.7 (96.6, 99.5)	4.8 (3.3, 7.0)	1.7 (1.0, 2.7)	93.5 (91.1, 95.3)

Percentages of time spent in different locations were estimated as the proportion of half hours spent in the locations per hour out of the total half hours spent by the population in the respective hour. Poisson regression was used with crossed random effects to account for repeated observations by household and date and a fixed effect for hour.

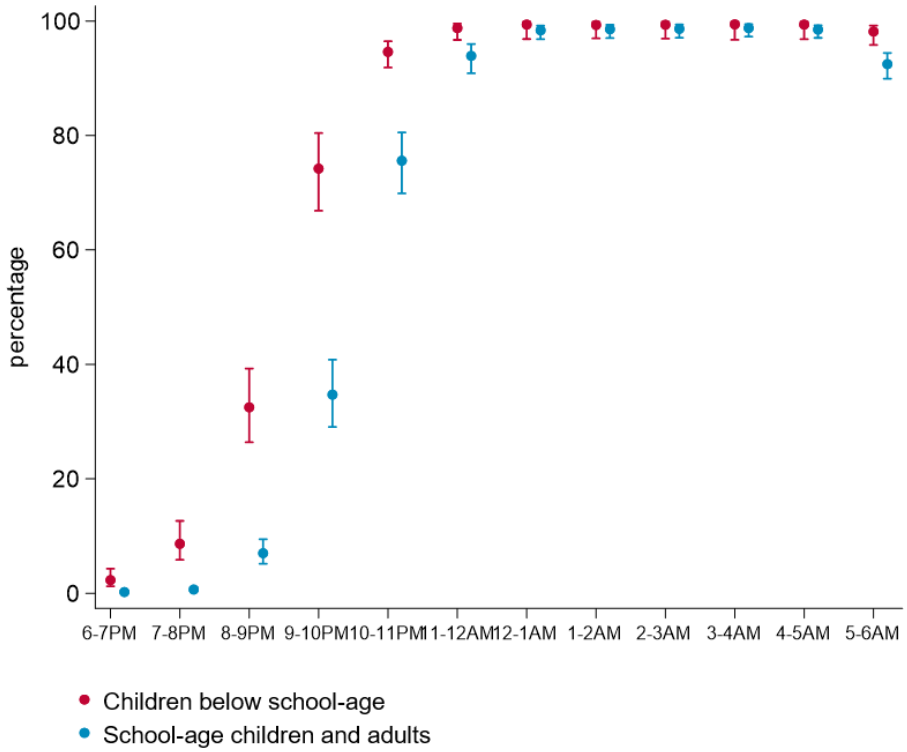

Figure 12. Hourly use of ITNs in the household.

Proportion of infective bites during times spent under ITNs

The proportion of time spent under ITNs out of the total time spent in bed, was high in both children below school age, 99.2% (95% CI 97.0%-99.8%) and older household members, 98.8% (95% CI 97.2%-99.5%). Nearly everyone who was recorded at midnight onwards was indoors in bed and under an ITN.

The proportion of infective bites between 6PM and 6AM that occurred during times when the individuals were sleeping under ITNs was estimated to be 84% (80%, 88%) for children below school age and 76% (71%, 81%) for older household members. The percentage of infective bites that would occur when people were outdoors was estimated to be 12% (8%, 16%) for children below school age children and 18% (13%,22%) for older participants (Figure 13).

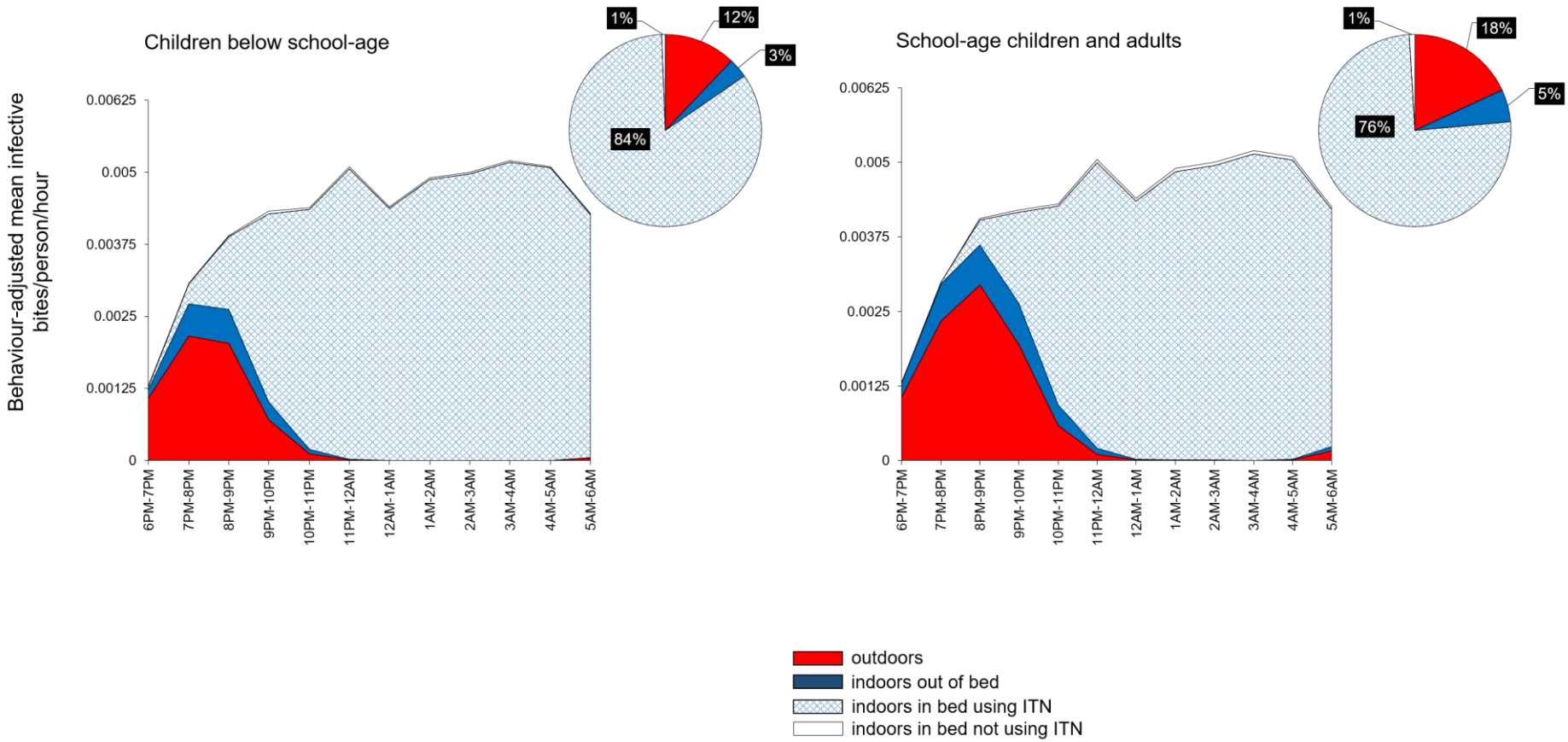

Figure 13. Human exposure to malaria and use of ITNs across the night

We summed the estimated infective bites for each hour to give an estimate of the EIR without incorporating human behaviour. For the months of the entomological collections, the standard EIR was estimated to be equivalent to 17.7 infective bites per person per year. Taking human location and ITN use into account, the mean rate of infective bites was estimated to be 4.6 per year for older participants and 3.0 for children below school-age.

5.5 Discussion

Residual malaria transmission has been raised as a potential challenge for malaria control programmes [68, 190, 277, 278] and can be in part due to outdoor biting as well as other factors [279]. In order to address gaps in malaria vector control, it is necessary to understand the behaviours of mosquito vectors and of human hosts that together can result in exposure to infective mosquito bites. In a setting with high ITN coverage and recent application of IRS, we investigated where and at what time during the night, *Plasmodium* sporozoite-positive local malaria vectors bite human hosts, and quantified the proportion of infective bites that would occur when household members are using ITNs in this setting.

While both species contribute to transmission in this area [151, 188, 226], *An funestus* s.s contributed higher numbers of mosquitoes caught and higher proportions of sporozoite-positive mosquitoes compared to *An. arabiensis* consistent with the recent finding of the relatively higher importance of *An. funestus* s.s for malaria transmission in the area [280]. *An. arabiensis* has previously been thought to be the main agent of outdoor malaria transmission owing to reports of predominant outdoor biting tendencies [252, 281, 282]. However, in our study, none of the *An. arabiensis* mosquitoes caught outdoors was infected, and other studies in the same area have also reported generally low proportions of infection [226, 280]. These findings may suggest a limited role for outdoor biting by *An. arabiensis* in malaria transmission in the area.

There was variation in biting rates and the proportion of mosquitoes infected. The biting rates were similar indoors and outdoors, but varied by time being lowest in the early evening and after 5AM. The proportions of mosquitoes infected tended to be higher for mosquitoes caught indoors compared to outdoors, and between 10PM and 3AM compared to other times during the night. Previous studies have reported variations in both biting rates and the proportions of sporozoite-positive or parous mosquitoes at different times of the night (Supplementary Table 4), although the differences are not consistent.

In our study, an estimated 65% (95% CI 60%, 70%) of the indoor infective bites occurred between 10PM and 5AM. A separate study [283] in the same region estimated that 8% of the infected *An gambiae* caught by light traps were between 7PM and 10PM, at hours of the night when people were unlikely to use a mosquito net. In a holoendemic setting in Burkina Faso, Perugini *et al* also found that the majority of infective bites were during the hours when people used ITNs [261]. The very high ITN use: 99.2% (95% CI 97.0%-99.8%) among children below school age and 98.8% (95% CI 97.2%,99.5%) among older household members in this study could protect these groups against 84% (95% CI 80%,88%) and 76% (71%,81%) of infective bites that they would be exposed to between 6PM and 6AM assuming 100% protection (Figure 14).

We assumed that 100% of bites are prevented while under a net. We recognize that this is unlikely to be true: it is likely that protection is reasonably high but wanes as the net ages. A large study of the effect of insecticide resistance on the risk of malaria demonstrated that pyrethroid ITNs are highly protective to users even in areas of pyrethroid insecticide resistance [284]. N'Guessan *et al* similarly showed that efficacy of ITNs may still remain high even when old and with holes [158]. We also assume that the biting rates in the entomological study would be the same for all age groups in the human behaviour study. There is evidence of different

biting rates by host size [285, 286], and carrying out activities other than HLC may affect mosquito landing. However, this would not affect comparisons within the same age group but would affect comparisons across age groups and absolute levels of risk.

A disadvantage to characterizing risk during the night is the need for sufficient data to characterize each segment of the night. There were only 356 infected mosquitoes in the study. This led to imprecision for some hours and locations for the estimated proportions of mosquitoes infected, and consequently we aggregated across some time-periods. We also do not capture day biting, which has been reported to contribute to transmission in a study in the Central African Republic [64]. Another limitation was that the two datasets for the entomological and human behaviour data were collected at different times; and from different villages, even though all were in the Kilombero valley. Villages where entomological surveys were done also tended to have higher altitudes (average: 420m (range: 311m, 1884m) above sea level) than villages where human surveys were done (average: 270m (range: 255m, 298m) above sea level) [287]. We needed to account for the variance structure introduced by the cluster sampling in our study, and different analysis methods may lead to different estimates. We used random effects for household and night for the entomological data because our data was unbalanced and our question focused on comparing the biting rates between the hours. We used robust standard errors for the human behaviour data. We investigated alternative methods as a sensitivity analysis: assuming zero for the proportion of mosquitoes infected in the hours with few mosquitoes caught and using robust variance estimates for the entomological data both lead to slightly higher estimates for the proportion of infective bites between 10PM and 5AM.

The high ITN use in our study area may have occurred for several reasons. It was self-reported, the human behaviour study occurred within two years of a mass distribution campaign and the

communities are within an area where several malaria transmission control studies have been done and the locals may be adequately sensitised about the benefits of nets. This may not apply to the village where the entomological surveys were carried out. In addition, the human behaviour survey only captures people who are at home. Those who have gone out may be less likely to use a net.

Evidence from our study reaffirms the need for an intervention, which protects people indoors when they are asleep, such as ITNs. Sustaining high levels of ITN use by ensuring sufficient availability within households and regular use of the nets at night by all household members remains key to reducing malaria transmission. Increased advocacy and community engagement to encourage the maintenance of ITNs [288] and increase their longevity [289, 290] can contribute to higher use where population access to ITNs is suboptimal [291]. The overall effects of widespread ITN use extend beyond the direct protection offered to users, by diminishing the overall mosquito population [170, 292-295]. In this study, the protection indoors would also be provided by IRS, which was implemented in all the study houses. Further indoor interventions and personal protection measures such as repellents may provide protection when individuals are not in bed [122, 186, 296].

While in this area, the majority of infective bites could be prevented by the use of mosquito nets while sleeping, a small proportion of the infective bites occurred outdoors before people retired to bed. Outdoor biting needs to be addressed. It could be impacted directly by tools designed for outdoor biting. There is also some evidence that mosquitoes biting outdoors go indoors at least once during their life and can be impacted by indoor interventions [292, 297].

5.6 Conclusion

In the study area, a substantial proportion of infective bites occurred indoors between 10PM and 5AM. Maintaining high levels of access and use of ITNs remains an important means to

reduce malaria transmission in this area. This study also contributes to the evidence of different biting rates and proportions of biting mosquitoes that are infective at different times and locations in the night. This finding has implications for estimating the actual risk of malaria transmission in a community.

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Author contributions

Conceived the current study: IHN, SJM, MAH, AR. Conceived and designed the entomological study: SJM, CM. Implemented the entomological study: SJM, CM, AS, DK, FT, NM, OGO, GL (Deceased), HN, IM, JB, JM. Conceived and designed the human behaviour study: MF, FO. Implemented the human behaviour study: MF, FO, SM, AL. Conducted or contributed to the analysis: IHN, SJM, MWH, AR. Drafted or edited the manuscript: IHN, SJM, CM, AS, DK, FT, OGO, JB, MWH, AR. All authors read and approved the final manuscript.

Data availability statement

The datasets used and or analysed in this study are available from SJM and MF upon reasonable request.

Competing interests

The authors declare no competing interests

Additional files

Supplementary Table 4. Proportions of *Anopheles* mosquitoes caught and infected and parous bites early, middle and late night estimated from different areas

	6-10PM	10PM-2AM	2-6AM
Giles, 1957† [74]*			
***An. gambiae* (Tanga, Tanzania)**			
Total catch	541	1575	1180
% of night's biting	16.4	47.8	35.8
% infected mosquitoes	3.6	2.7	1.3
% of infective bites	25	56	19
Maxwell et al, 1998 [56]			
***An. gambiae* (Muheza, Tanzania)**			
Total catch	23	189	211
% of night's biting	5.4	44.6	49.9
% infected mosquitoes	8.7	7.4	4.3
% of infective bites	8	56	36
***An. funestus* (Muheza, Tanzania)**			
Total catch	31	232	86
% of night's biting	9.8	66.5	24.6
% infected mosquitoes	0	3.0	2.3
% of infective bites	0	78	22
Bockarie et al, 1996 [75]			
***An. gambiae* (Bayama Sierra Leone)**			
Total catch	104	2016	4373
% of night's biting	1.6	31.0	67.3
Parity rate (%)	44.0	58.3	66.9
% of parous bites	1	28	71
***An. punctulatus* (Yauatong, Papua New Guinea)**			
Total catch	202	848	1100
% of night's biting	9.4	39.4	51.2
Parity rate (%)	39.8	54.8	63.2
% of parous bites	6	38	56
***An. punctulatus* (East Sepik, Papua New Guinea)**			
Total catch	535	1794	1839
% of night's biting	12.8	43	44.1
Plasmodium falciparum			
% infected mosquitoes	0.7	1.7	2.3
% of infective bites	5	40	55
Plasmodium vivax			
Sporozoite rate (%)	1.3	0.9	1.1
% of infective bites	16	37	47
Robert and Carnevale, 1991 [76]			
***An. gambiae* and *An. funestus* (Burkina Faso)**			
Total catch	5900	13000	10850
% of night's biting	19.8	43.7	36.5
% parous	22.8	25.2	24.6
% parous bites	18	45	37
% infected mosquitoes	0.05	0.08	0.19
% of infective bites	9	31	61
	5	36	59

* Sporozoite test on only gravid mosquitoes
† Indoor resting populations collected by hand
‡ Host seeking populations collected by CDC light traps
** Biting populations collected by the human landing catch

6. Tracking insecticide treated net use throughout the night to enhance evaluation of protection against malaria

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This chapter is a working paper

Summary

Tracking mosquito net use throughout the night is required to improve measurement of their effect on malaria transmission

Insecticide treated nets (ITNs) are an effective and widely used malaria intervention. Between 2000 and 2015, ITNs were estimated to have averted 68% of over 660 million *Plasmodium falciparum* malaria cases prevented by malaria control efforts in sub-Saharan Africa [241]. Regular use of ITNs has been associated with additional benefits including reduction of all-cause child mortality [97], enhanced child survival [21] and better education and development of children [22]. The global fight against malaria relies heavily on sustaining and enhancing the benefits of ITNs, but this requires good quality data to quantify accurately the effects of ITNs and to identify gaps in effective coverage that may require supplementary tools.

The World Health Organization recommends that at least 80% of the population at risk use ITNs [75]. However, this 'universal coverage' indicator suffers a lack of precision as it measures use in a binary yes/no manner. An ITN should be used throughout the night to realise

the maximum protection when asleep. It should be suspended above the sleeping space with its edges firmly tucked under the mattress to prevent mosquito entry. The ITN physically blocks or repels host-seeking mosquitoes attempting to bite a user.

As the direct personal protection is only afforded if ITNs are used during biting times of *Anopheles* mosquitoes, it is important to understand the behaviours of both the mosquitoes and the human host. The risk of malaria transmission is estimated in entomological surveys as the number of infective *Anopheles* bites per person per unit of time (entomological inoculation rate [EIR]). The hourly EIR, inside and outside dwellings, provides data on variations in transmission risk in time and space throughout the night [298]. To complement the entomological data, information on the use of nets by individuals at different times of the night is necessary to evaluate their protective effect in a given setting. Unfortunately, such granular data is not routinely collected.

ITN programs are presently evaluated largely from standard indicators designed to evaluate national malaria control programs and collected during malaria indicator surveys (MIS). In the MIS, ITN coverage is measured based on few standard indicators, including: (i.) *Household ownership* – proportion of households with at least one ITN; (ii.) *Access* – proportion of the population that could sleep under an ITN assuming one net is shared by up to two household members; (iii.) *Use* – proportion of the population that slept under an ITN the night before the survey [299]. Although these metrics are useful for tracking overall progress in ITN coverage, they are only rough estimates of the protection afforded by ITNs since the MIS-*use* indicator may not reflect the actual practice at different times of the night, week, month, or year. Hence, the current MIS data cannot necessarily be interpreted as the proportion of the population effectively protected by ITNs.

Suggestions have been made to improve the assessment of ITN use. For instance, the time of the night during which a person uses an ITN can be approximated by additional survey questions that elicit at what time individuals go to bed and when they wake up [169]. Alternatively, trained individuals can record the sleeping patterns of household members, as has been done elsewhere [188]. Ideally, the period of the night spent under an ITN would need to be assessed for each individual to calculate the protective effectiveness of an ITN for that person. Additionally, where infective mosquitoes bite (indoors versus outdoors) and where individuals spend their time, if they are not indoors and in bed would need to be assessed [61].

Susceptible mosquitoes should be killed as a result of interacting with ITNs as they seek a blood meal, or if they rest on the nets. In an area where mosquitoes mostly bite and rest indoors, and remain susceptible to insecticides, ITNs may therefore lead to a reduction in the overall mosquito numbers, and a decline in malaria transmission [260]. This 'community effect' of ITNs goes beyond the protection of the individual user, and may indirectly reduce the risk of malaria infection at times when ITNs are not used, or for individuals who do no sleep under an ITN.

In addition to the timing of net use and mosquito biting, improper installation as well as reduced physical integrity and bioefficacy of ITNs may undermine the effectiveness of ITN programs [247]. Lastly, resistance of *Anopheles* mosquitoes to insecticides used on ITNs and outdoor, early evening or diurnal biting need to be considered in comprehensive assessments of the effectiveness of ITNs.

For programmatic purposes, a refined assessment of the effective protective coverage of ITNs based on a combination of entomological data and information on human sleeping patterns collected during routine MIS would improve our understanding of persisting gaps in protection against malaria transmission and help to target complementary interventions.

Author Contributions

IHN developed the topic and wrote this article.

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Conflict of Interest

The author declares that this editorial was written in the absence of any motivation that could be considered as a potential conflict of interest.

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7. Efficacy of SumiShield™ 50WG for indoor residual spraying against resistant *Anopheles* mosquitoes in Ulanga, south-eastern Tanzania

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7.1 Abstract

Background: New insecticides for use against resistant *Anopheles* mosquitoes are needed for continued global progress in malaria control. SumiShield (clothianidin), a novel neonicotinoid

insecticide for indoor residual spraying (IRS) was assessed for non-inferiority in comparison with Actellic (pirimiphos-methyl), the standard IRS, against pyrethroid resistant *Anopheles* mosquitoes.

Method: Efficacy of SumiShield for IRS against *Anopheles arabiensis* mosquitoes was compared to Actellic over 8 months in 1) an experimental hut trial (EHT) to evaluate mosquito mortality and 2) in a cluster randomized community trial (cluster RCT) that evaluated sporozoite infection in Ulanga, south-eastern Tanzania between 2016 and 2017. Mosquitoes were pyrethroid resistant and pirimiphos-methyl susceptible.

Results: In the EHT, SumiShield was non-inferior to Actellic and was also associated with increased mortality after holding exposed mosquitoes for 72 hours, (odds ratio (OR): 1.22, 95 % confidence interval (95% CIs 1.05, 1.43)) and 168 hours (OR: 1.19, 95% CI 1.02, 1.38). There was no evidence that SumiShield was non-inferior compared to Actellic in the cluster RCT (OR: 1.16, 95% CIs: 0.46, 2.92) but there were low numbers of sporozoite positive mosquitoes leading to uncertainty in the estimates.

Conclusion: SumiShield was non-inferior to Actellic at killing mosquitoes in an experimental hut trial, and may be useful as an additional tool for insecticide resistance management. In order to demonstrate entomological effect of IRS on malaria parasite infection in mosquitoes much larger cluster RCTs would be needed as the sporozoite rate was extremely low in both arms.

Keywords: *Anopheles* mosquitoes, vector control, indoor residual spraying, SumiShield, clothianidin

7.2 Background

Targeting the disproportionately high burden of malaria in sub-Saharan Africa is at the core of the global plan to eradicate malaria [3, 75]. Intensified malaria vector control at the beginning

of the century has resulted in around two billion malaria cases averted between 2000 and 2021 [300]. This is mainly attributed to the scale up of vector control through universal coverage of insecticide treated nets (ITNs) and insecticide residual spraying (IRS). Maintaining effective ITN and IRS programs in malaria endemic areas is crucial to continue to reduce malaria transmission and forms an integral part of the global malaria control strategy [75].

Throughout sub-Saharan Africa, mosquito resistance to several classes of insecticides used for ITNs and IRS has emerged [301]. Combining ITNs with non-pyrethroid IRS has been shown to be additionally protective [186]. Only non-pyrethroid insecticides are recommended for IRS to combat the high levels of pyrethroid resistance among mosquito vectors [302]. Therefore, additional classes of insecticides are needed for IRS [108]. In addition to a different mode of action to that of pyrethroids and organophosphates, IRS preferred product characteristics also include a long residual life of between three to twelve months [108, 303].

Neonicotinoids constitute a new class of insecticides approved for public health use, and clothianidin has been shown to confer efficacy against pyrethroid resistant Afrotropical malaria vectors [108, 304]. The insecticides target the nicotinic acetylcholine receptor in the insect's central nervous system [305, 306]. Clothianidin is generally safe in humans [307].

Actellic is an organophosphate insecticide in widespread use for IRS which has extensive evidence of effectiveness against malaria [308-312] including evidence from a high quality RCT [313]. However, pyrethroid-organophosphate cross-resistance has been demonstrated [142] including among malaria vectors [314]. The objective of this study was to assess the non-inferiority of SumiShield (clothianidin), relative to Actellic that has proven public health benefit, against pyrethroid-resistant *Anopheles* mosquitoes following WHO guidance [315].

7.3 Methods

Study area

The study was conducted in Ulanga District, in the Kilombero river floodplain in south-eastern Tanzania (Figure 14). The area lies 270m above sea level with annual rainfall ranging 1200-1800mm, and peaks between October and November, and April and May. Temperatures range between 20ºC and 33ºC. [262]. The area is characterised by irrigated rice paddies, maize farms and banana fields. Malaria vectors occur year-round [151, 226]. The main malaria vectors are *Anopheles arabiensis*, constituting more than 95% of the *An. gambiae* complex and *An. funestus* sensu stricto (s.s.), constituting more than 93% of the *An. funestus* complex [151, 316, 317]. Both vector populations are highly resistant to pyrethroids (mortality is less than 20% with deltamethrin, lambda-cyhalothrin and permethrin) [226],but are susceptible to organophosphates including pirimiphos-methyl [318].

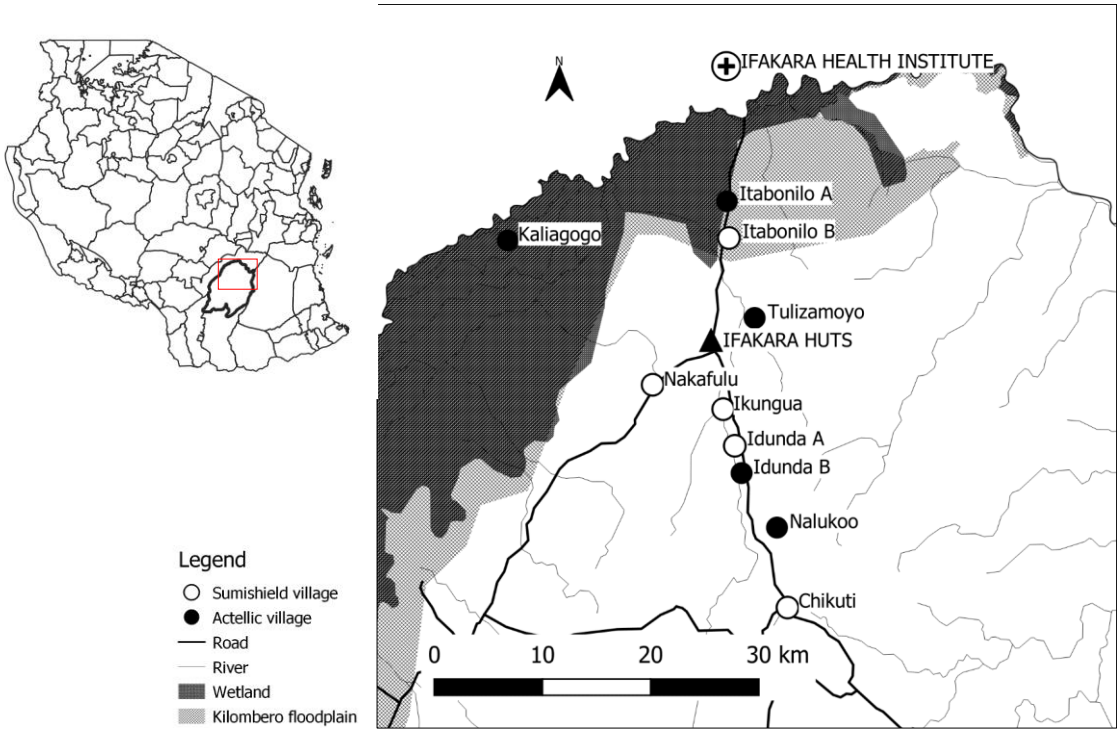

Figure 14. Study area.

Locations of Ulanga District in Tanzania and the study sites within Ulanga.

Study design

The comparative efficacy of SumiShield to Actellic was measured in two different trials and for two different entomological outcomes. Both *Anopheles* mortality and malaria parasite infection ('sporozoite rate') may be useful as proxies for the efficacy of vector control tools against malaria [319].

1.) An experimental hut trial (EHT) was conducted to measure mosquito mortality, the primary indicator of insecticide efficacy. Experimental huts allow evaluation of a range of vector control products under controlled conditions that resemble those in which mosquitoes enter a human habitation and interact with the product in normal use. Experimental huts have structural features that enable collection of mosquitoes that have entered the huts [320].

2.) A cluster-randomized community trial (RCT) measured the proportion of mosquitoes infected with sporozoites. Besides mortality, the reduction in mosquito infection by malaria parasites is useful for estimating the potential impact of insecticides applied at community scale against malaria transmission. An RCT permits examination of insecticide effect on wild infected mosquitoes in natural conditions in the community.

Experimental hut trial

The original Ifakara Huts design was used, located in Lupiro village (8.385°S and 36.670°E) (Figure 3). Each hut was fitted with four window exit traps and the eave entry points were fitted with baffles, consisting of netting barriers facing the inside of the huts but slanting upwards at approximately the same angle as the roofs to allow mosquito entry but restrict exit. The huts and the standard entomological operations associated with their use are described in detail elsewhere [321].

A single (investigator) blinded partially randomized design with two simultaneous 5 x 5 Latin Squares (LS) was followed. Five IRS treatment arms were assigned to five huts. However, only data on SumiShield™ and Actellic® 300CS are presented as these two insecticides are relevant to this study and were evaluated in both the EHT and the cluster RCT. The arms were: (1) SumiShield™ 50WG (50% Clothianidin) applied at 300 mg ai/m^2. (2) K-Othrine® 250 WDG (25% Deltamethrin) applied at 25 mg ai/m^2. (3) Actellic® 300CS (30% Pirimiphos-methyl) applied at 1 g ai/m^2. (4) Ficam VC WP-SB (80% Bendiocarb) applied at 400 mg ai/m^2. (5) Water. Spraying was done on the walls and doors as per WHO guidelines [322].

For each LS, five male adult volunteers, one volunteer per hut, rotated between the huts in each round of five nights. The volunteers entered the huts at 19:00hrs and remained inside until 06:00hrs, sleeping under untreated, deliberately holed bed nets with six 4x4 cm holes. Each morning of the study, dead and resting mosquitoes were collected from inside the net, followed

by the floor and walls of the hut and then from the exit traps. In total, 135 nights (27 rounds with five days of collection and two rest days per round) of data collection were conducted over a period of eight months.

Cluster randomized community trial

The community trial was conducted in Ulanga District in ten clusters (villages) (Figure 14) where relatively high malaria rates (9,328 deaths in 25 villages) had been reported between 2002 and 2012 [323]. The villages were formed into pairs by distance: (1) Itobanilo A and B, (2) Tulizamoyo and Chukuti, (3) Kaliagogo and Nakafulu, (4) Idunda A and B, (5) Ikungua and Nalukoo. Each village had around 200 structures (houses), and they were located at least two kilometers apart to minimise contamination [324]. One village of each pair was allocated to each study arm using the lottery method by pulling a number out of a hat. The two IRS treatment arms: (1) SumiShield^{TM} 50WG (50% Clothianidin) applied at 300 mg ai/m^2 and (2) Actellic$^{®}$ 300CS (30% Pirimiphos-methyl) applied at 1 g ai/m^2 were therefore assigned to five villages each. Spraying was conducted between December 2016 and February 2017 in a pairwise fashion to minimise temporal bias. The insecticides were applied on the walls and doors of all structures and to the ceiling of thatched houses as per WHO guidelines [322]. Written informed consent was obtained from the head of each participating household prior to the study. All participating households were geo-referenced and a short household characteristics questionnaire was conducted to note the key features of the house such as wall surfaces, roof surfaces, number of occupants and number of bed nets.

Longitudinal monitoring of indoor adult mosquitoes to measure the sporozoite rate were performed in 16 houses randomly selected per cluster. Mosquitoes were sampled at fortnightly intervals beginning one week before and during the intervention and for eight months using Centres for Disease Control light traps (CDC LTs) (John W. Hock Co., Gainesville, FL) inside

each selected house. Traps operated from 18:00hrs to 06:00hrs beside an occupied bed net [325] . The same set of houses were used over the eight months.

Additionally, monitoring through paired indoor-outdoor Human Landing Catches (HLC) [326] and CDC LTs was carried out in three houses per cluster over six nights per month for five months. This was done to in an attempt to calibrate the relative performance of the two methods to calculate the entomological inoculation rate (EIR) [327]. HLC was medically supervised by a clinical officer from the Lupiro Government dispensary [328] and was performed in each village by six participants during 12 hrs (18:00hrs to 06:00hrs) with a ten-minute break at the top of each hour to stretch and have a hot drink. Written informed consent was obtained from each participant prior to the study. On the morning of collection, mosquitoes were morphologically identified by eye and data recorded into forms.

Study quality control

Insecticide application procedure

The walls were sprayed to attain dosages as according to the manufacturer's recommendations. Micron CS10 compressor sprayers ceramic nozzles were fitted with matching 'Red Control Flow Valves (1 bar)' for a uniform discharge rate. A three-day training was conducted to familiarise the spray team with spraying and disposal of pesticides and the team repeatedly practiced application with the correct speed using water during a two day training beforehand. The lance of the sprayer was fitted with a 45cm long projecting guide to ensure the correct distance from the wall was maintained. Calibration of the spray pumps was done to ensure the application of the targeted dose. Separate sprayers were used for each kind of insecticide to avoid cross-contamination.

Verification of target dose

Four papers (Whatman® No.1) of 10 cm x 10 cm size were attached (using pins to hold them slightly away from the walls) at three different wall heights (top, middle and lower part of a

wall plus one randomly assigned), and were removed after the spray activity. Spray quality was assessed in each experimental hut and in eight randomly selected houses per cluster in the cluster RCT. Filter papers were analysed for pesticide residue using standard methods: 1) Clothianidin (CRA-W UHPLC-DAD method based on CIPAC 738/WG/M/3, CIPAC Handbook N, page 18 and 2. Pirimiphos methyl CRA-W PA-U10-RESMM005(GC-FID) method based on CIPAC 239/CS/M/3 (CIPAC 4778). Filter papers were placed individually in aluminium foil with appropriate label (house number, substrate, date, location of top, middle or bottom) and stored in a refrigerator at 4ºC until shipping for chemical analysis. The spots on walls where filter papers were placed were marked. Longitudinal cone bioassays were conducted in the houses avoiding the places where the filer papers had been located.

In addition, the volume of spray applied in each experimental hut and eight sample houses per cluster was determined gravimetrically by weighing unpressured sprayer before and after each experimental hut or house application and measuring the surface area sprayed to calculate volume/m^2.

Verification of residual efficacy of insecticides (biological efficacy by WHO cone bioassay)

A laboratory colony of *An. gambiae* (Ifakara strain), which is fully susceptible to all insecticides, was used for the cone bioassays. The WHO cone bioassays [315] were carried out monthly after spraying and were continued for up to eight months in both the EHT and the cluster RCT. Five cones were placed in each of the experimental huts and in eight selected houses in each cluster and ten unfed, two to five-day-old, female mosquitoes exposed for 30 minutes in each cone. Mosquitoes were returned to the Ifakara Health Institute (IHI) insectary for holding at approximately 27 ± 2ºC. Mortality of mosquitoes exposed in cones was measured at 24, 48, 72, 96, 120, 144, 168 hours post-exposure. In month eight, bioassays were performed with F1 generation *An. gambiae sensu lato* (s.l.) that are resistant to pyrethroids in one cluster per arm.

Insecticide susceptibility of target vector species

Insecticide susceptibility of wild mosquitoes was performed prior to the start of the study by WHO tube assay [329].

Verification of vector species composition

A proportion (<2%) of *An. gambiae* s.l. and *An. funestus* s.l mosquitoes were identified by polymerase chain reaction (PCR) [330, 331].

Entomological endpoints

Mosquito mortality

Mosquito mortality was estimated using the EHT. *Anopheles* mosquitoes were separated to species complex by morphological identification. The number of dead and live mosquitoes were recorded from the collections each morning. Live mosquitoes were placed in 330ml paper cups in IHI) insectary for holding at approximately 27 ± 2ºC, with no more than 20 individuals per cup and were provided with access to 10% sugar solution. Mortality was recorded every 24 hours (24, 48, 72, 96, 120, 144 and 168 hours) to assess delayed mortality.

***Anopheles* sporozoite infection**

Sporozoite infection was assessed from mosquitoes collected by CDC LT and HLC in the cluster RCT. The enzyme-linked immunosorbent assay (ELISA) was used for detection of *Plasmodium falciparum* circumsporozoite protein (Pf CSP) in the salivary glands of *Anopheles* samples [231]. The optical density of post-ELISA lysate were measured at 405 - 414nm after 45 minutes using ELISA plate reader machine [231]. Detection of *P. falciparum* parasites were performed from five thousand samples per arm selected at random from the storage freezer which is around half of the *An. arabiensis* and *An. funestus* mosquitoes collected between December 2016 and August 2017.

Data analysis

Mosquito mortality and malaria sporozoite infection

We estimated the odds ratio for mortality among the EHT mosquitoes for SumiShield compared to Actellic together with a 95% confidence interval (CI). We used logistic regression with fixed effects for intervention arm, number of nights after spraying and the hut, and a random effects for hut-night (observation). Due to low numbers of *An. funestus* mosquitoes, only *An. arabiensis* was included in the statistical analysis.

We estimated the odds ratio for sporozoite infection in mosquitoes in the SumiShield arm compared to the Actellic arm together with a 95% CI. We used logistic regression adjusting for month after spraying and mosquito species (*An. funestus* and *An. arabiensis*) as a fixed effect categorical variable, and cluster was included as a random effect.

To assess whether SumiShield was non-inferior to Actellic for both mortality and the proportion of mosquitoes infected, we set the margin of non-inferiority to a 7% difference in accordance with updated WHO recommendations[332]. If the new IRS has a greater effect on mortality than the comparator, then this would be represented by an OR of greater than 1. A lower effect would be represented by an OR of less than 1. Non-inferiority, where the effect of SumiShield is not unacceptably worse than Actellic, is shown if the lower bound of the confidence interval for the OR is not less than the margin of non-inferiority.

The statistical analyses were performed in Stata (16.1, StataCorp LLC, College Station, TX).

7.4 Results

Experimental hut trial

A total of 46,282 *Anopheles* mosquitoes, 99.5% (n = 46,063) of which were *An. gambiae sensu lato* (*s.l.*), were caught in the experimental huts.

There were 1247/1385 successful amplications with polymerase chain reaction (PCR) for *An gambiae sensu lato (s.l.),* of which all were *An. arabiensis.* There were 1776/2368 successful amplications for *An. funestus s.l.* of which 87% were *An. funestus* s.s. with smaller proportions of *An. rivulorum* (9%) and *An. leesoni* (4%). Therefore, it was decided to classify all *An. gambiae s.l.* as *An. arabiensis* and all *An. funestus s.l.* mosquitoes as *An. funestus s.s.* A median of 78 (range 10, 348) *An. arabiensis* per hut per night were collected in the Actellic arm and 61 (13, 327) in the SumiShield arm.

Chemical quantification showed that both arms were under sprayed but within the allowable margins of error: Actellic (39% under) SumiShield (23% under). Cone bioassays indicated that both SumiShield and Actellic were efficacious for six months (Round 5) using the 80% mortality threshold for a 72-hour holding time, and for eight months (Round 7) using 168-hour holding time (Supplementary Figure 1).

The cumulative proportion of EHT mosquitoes that were dead at 168 hours after exposure to the insecticide was slightly higher for SumiShield than for Actellic (Table 10). Delayed *Anopheles* mortality over 24-hour intervals following exposure to insecticides was also higher for SumiShield compared to Actellic. The estimated mortality measured at 168 hours for both products remained similar for the eight-month duration of the trial (Figure 15).

Table 10. Mosquito catches and mortality in the experimental hut trial at 168 hours

	An. arabiensis			*An. funestus*		
	Total Number (n)	Number per hut per night Median (Range)	Adj. % mortality (95% CIs)*	Total Number (n)	Number per hut per night Median (Range)	Adj. % mortality (95% CIs)*
Actellic	24,760	78 (10, 348)	46 (44, 49)	163	0 (0, 16)	39 (23, 57)
SumiShield	21,303	61 (13, 327)	53 (50, 56)	56	0 (0, 4)	55 (42, 68)

*Adjusted percentage mortality and 95% confidence intervals (Adj. % mortality (95% CIs)) were estimated from a logistic regression model with fixed effects for number of nights since spraying, hut and a random effect for hut-night.

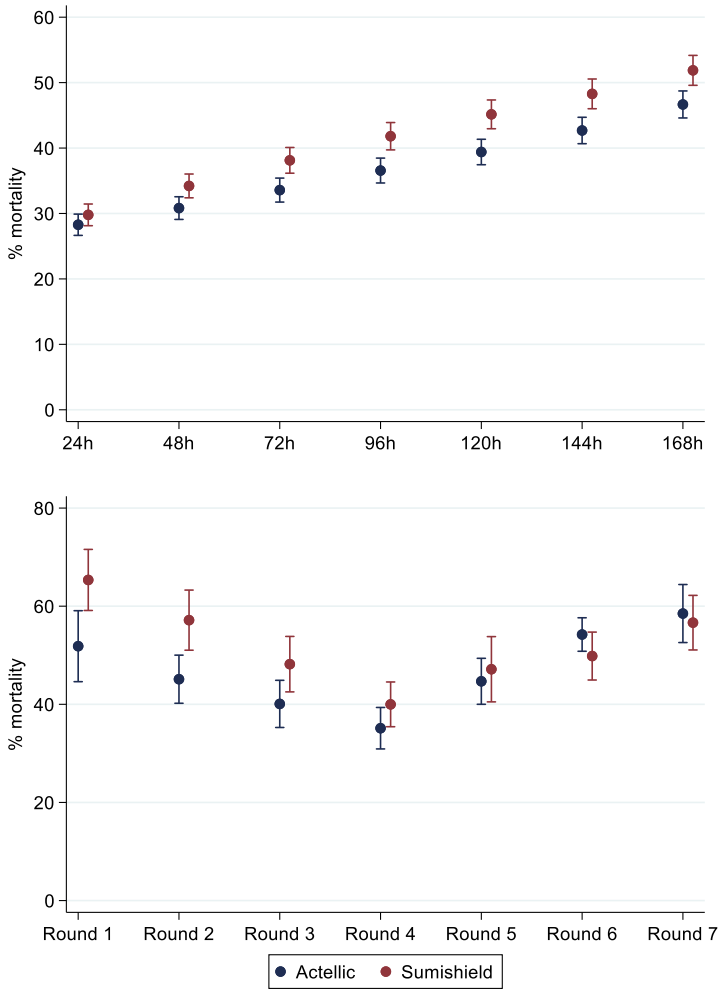

Figure 15. *Anopheles* mortality in the EHT.

Upper panel: cumulative mortality over 24-hour intervals of holding live exposed mosquitoes

Lower panel: mortality at 168 hours at intervals (Rounds) of 27 nights (5 days of collection and 2 rest days per rotation) after spraying

SumiShield was associated with increased *An. arabiensis* mortality at 72 hours (odds ratio (OR) 1.22, 95% CI 1.05, 1.43) and at 168 hours (OR: 1.19, 95% CIs 1.02, 1.38) relative to Actellic. The lower bound of the CI exceeded a 7% margin of non-inferiority (an odds ratio of 0.7 for 30% 72-hour mortality and 0.74 for 40% 168-hour mortality) indicating that SumiShield was non-inferior to Actellic for mortality in the EHT (Figure 16).

Cluster randomised community trial

The characteristics of the houses were similar in the two study arms (Supplementary Table 5). In total, 11,132 *Anopheles* mosquitoes were captured from the cluster RCT and tested for sporozoite infection, of which, 83% (n = 9,263) were *An. arabiensis*.

Gravimetric quantification showed that both arms were adequately sprayed within the allowable margins of error: Actellic (27% deviation) and SumiShield (12% deviation). Chemical evaluation showed that the Sumi-Shield arm was 40% under sprayed and the Actellic was 18% under sprayed. Cone bioassays with pyrethroid susceptible *An. gambiae* s.s. indicated that the residual efficacy passed the WHO threshold of 80% or higher mortality for Actellic up to four months with a 72-hour holding time and up to five months with longer holding times. The residual activity of SumiShield was seven months at 72-hour holding time and eight months at 168-hour holding times. For mosquitoes caught in the community trial, at eight months Actellic killed 68% (49-74%) and SumiShield killed 100% *An. arabiensis* mosquitoes at the 168-hour holding time.

Sporozoite infection was low in both *Anopheles* species and was similar in the SumiShield and in the Actellic arms (Table 11). Other studies in the same location have recorded a higher sporozoite rate among *An. arabiensis* (0.004) [333] than was observed in this study (0.001) with high IRS coverage. Non-inferiority was not shown for SumiShield compared to Actellic (OR: 1.16, 95% CIs 0.46, 2.92). The CI of the odds ratio were wide, reflecting uncertainty due to low numbers of infected mosquitoes.

Table 11. *Anopheles* malaria sporozoite infection in the cluster RCT

	An. arabiensis			*An. funestus*			Total		
	Total tested (n)	Number positive (n)	Adj. % positive (95% CIs)*	Total tested (n)	Number positive (n)	Adj. % positive (95% CIs)*	Total tested (n)	Number positive (n)	Adj. % positive (95% CIs)*
Actellic	5320	3	0.001 (0.000, 0.002)	1229	7	0.006 (0.003, 0.012)	6549	10	0.001 (0.000, 0.005)
SumiShield	3943	5	0.001 (0.001, 0.003)	640	4	0.006 (0.002, 0.016)	4583	9	0.002 (0.001, 0.006)

Percentage sporozoite infection (Adj. % positive (95% CIs)) estimates were obtained from generalised linear mixed effects models.

In summary, SumiShield was shown to have been non-inferior to Actellic with direction of effect towards higher mortality in the EHT. The village scale trial did not show evidence of non-inferiority for malaria sporozoite infection. The estimates were imprecise due to low sporozoite rates in both arms (Figure 16).

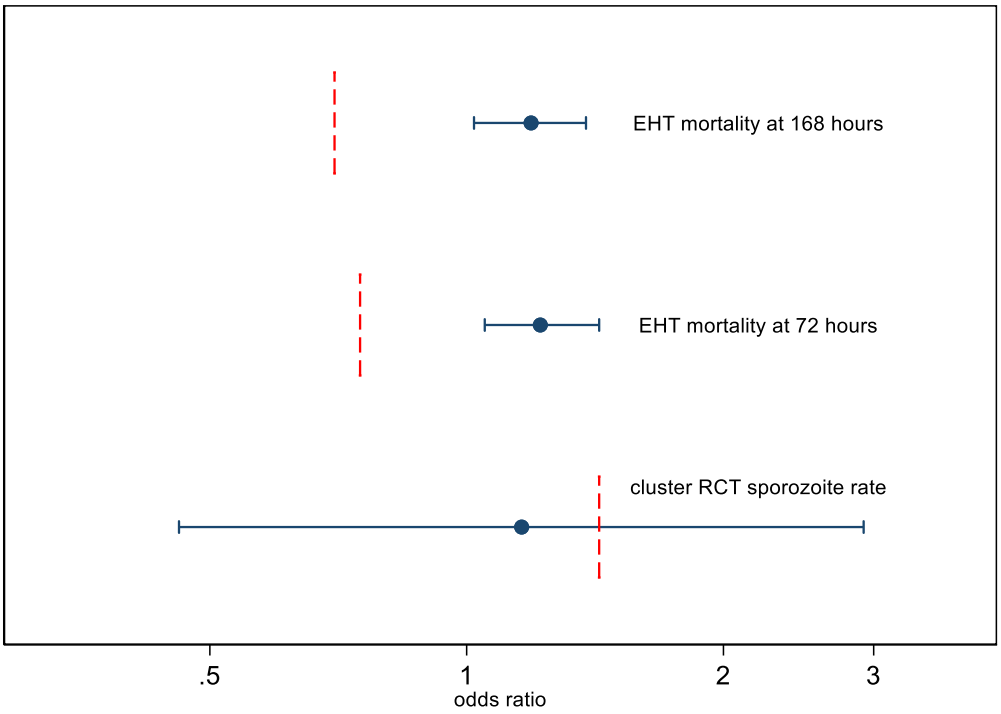

Figure 16. SumiShield efficacy.

Upper panel: Estimated odds ratio of SumiShield compared to Actellic of *Anopheles* mortality at 72 and 168 hours of holding after exposure to insecticides. An odds ratio of greater than one is in the direction of higher mortality for SumiShield. The solid line indicates the margin of non-inferiority (7% which is equivalent to an OR of 0.7 at 72 hours of holding and 0.75 at 168 hours of holding). If the lower bound of the CI is greater than this line, then there would be evidence of non-inferiority.

Lower panel: Estimated odds ratio of SumiShield compared to Actellic for *P. falciparum* sporozoite infection in mosquitoes. An odds ratio of less than one is in the direction of a lower sporozoite rate for SumiShield. The solid line indicates the margin of non-inferiority (7% which is equivalent to an OR of 1.07). Since the upper bound of the CI is not less than this line, there is no evidence of non-inferiority.

7.5 Discussion

We report a non-inferiority assessment of clothianidin, a novel neonicotinoid insecticide against pirimiphos-methyl, an organophosphate in common IRS use. In an EHT on mosquito mortality, clothianidin was found to be non-inferior to pirimiphos-methyl and was associated with higher mortality overall in the eight-month study. For *Anopheles* malaria parasite infection in the cluster RCT, non-inferiority of clothianidin compared to pirimiphos-methyl was not shown, possibly due to low number of infected mosquitoes overall.

Clothianidin has insecticidal and chemical properties that may explain a higher efficacy than Actellic as was observed in the EHT. Clothianidin's mode of action involves irreversible blockage of the nicotinergic acetylcholine receptors reducing the likelihood of cross-resistance [307, 334]. The more common vector control insecticides, pyrethroids, target voltage-gated sodium channels [335] while organophosphates inhibit acetylcholinesterase [336, 337]. Possible pyrethroid-organosphosphate cross-resistance mechanisms have been identified [142] although resistance to pirimiphos-methyl was not detected in the study area. Clothianidin has been shown to work well on different wall surfaces [338, 339]. A long-lasting residual effect against *Anopheles* mortality beyond 6 months was also observed in the cone bioassays in the present study even though both arms were undersprayed in both the EHT and the cluster RCT.

Experimental hut studies conducted across other parts of sub-Saharan Africa have similarly reported high efficacy and residual activity of clothianidin, [340, 341]. There was indication of a longer residual life of clothianidin relative to Actellic, a finding that has been reported recently in other locations [339, 340, 342]. A multi-country analysis reported 100% mortality of mosquitoes exposed to clothianidin including wild collected malaria vectors with multiple resistance mechanisms to pyrethroids, carbamates and organophosphates [343], although there are reports of resistance emerging [344].

A systematic review indicates a close link between entomological efficacy measured in experimental hut trials and the epidemiological efficacy of quick-acting, neuro-acting ITNs and IRS [187]. A concurrent pair of studies in India found clothianidin to have reduced the density of insecticide-resistant *An. culicifacies* in houses [345] and the proportion of parous females in village scale investigations [346]. The studies illustrate the link between experimental huts and community trials based on empirical data and may suggest that mosquito parity or age could be a useful entomological endpoint in RCT where malaria transmission is low due to high coverage of ITNs and IRS. Measuring mean mosquito population age is feasible through mark release recapture studies [347] and may also be a useful technique to measure the effect of IRS on mean mosquito population age in these studies.

Smaller field-scale settings comprising investigations in a small number of occupied houses in northern Tanzania [348], Democratic Republic of Congo [349], Ethiopia [340], southern Mozambique [338], Benin [350] and Ghana [351] similarly showed evidence of clothianidin residual efficacy similar to that measured in the EHT and cluster RCT reported here. Further evidence has also been shown in larger-scale and operational contexts where clothianidin demonstrated potential to reduce population-wide risk of endemic malaria transmission. In Madagascar, a three-year IRS program deployed to complement a pyrethroid-only ITN program was associated with substantial reductions in malaria case incidence in the communities where clothianidin was sprayed [352]. However, the same trend was not seen in Uganda [353] or Zambia [354] although there are a number of scenarios as to why it was ineffective As more data from IRS spraying with clothianidin becomes available it will be important to explore whether clothianidin and pirimiphos-methyl have a similar impact on malaria at a community scale.

Experimental hut studies have an advantage over community trials in that they allow for controlled exposure of a large number of wild mosquitoes to the insecticides, which can more easily attain adequate statistical power [320]. However, the effect of insecticides on mosquito infection cannot be measured in experimental huts since it requires application at the population level to impact on malaria transmission. The cluster RCT in this study did not demonstrate non-inferiority possibly because there was a very low number of infected mosquitoes, as would be expected if there was high coverage of an efficacious intervention within a community. The cluster RCT could have been improved by increasing the size of the area over which it was implemented, analysing more mosquitoes (only 50% were analysed due to budget constraints) and including a larger number of clusters. Conducting the study in an area with a high vectorial competence would also help maximise the probability of capturing sufficient mosquitoes carrying sporozoites [355].

A feature of the community trial was the setting in an area of high *An. arabiensis* abundance. *An. arabiensis* appears to be a less competent vector than *An. funestus* that tends to have higher parasite infection and mediates most of the malaria transmission in the study area [151, 226]. The study area mostly covered a region with rice farms ideal for *An. arabiensis* breeding but not *An. funestus*. A very low number of *An. funestus* were also caught in the EHTs as the huts are located close to rice paddies that tend to harbour more *An. arabiensis* breeding than *An. funestus* that prefers permanent water sources [356].

It was easier and cheaper to assess non-inferiority using the EHT than with the cluster RCT in this study. A review found general agreement between EHT and RCT results [357], and therefore EHT results provide some indication of what may be found in a larger RCT.

7.6 Conclusion

Clothianidin was found to be an effective product for killing pyrethroid resistant malaria vector mosquitoes and non-inferior to Actellic with data in the direction of higher mortality for clothianidin in an experimental hut trial. A larger-scale cluster RCT would be needed to assess non-inferiority of clothianidin relative to pirimiphos-methyl on malaria parasite infection in mosquitoes in this setting, as the sporozoite rate was extremely low in both arms.

Acknowledgements

We thank the communities for permitting study activities in their households.

Author contributions

JB managed the trial, contributed to study design and data collection, contributed to the manuscript editing. AS oversaw IRS. SJM designed the experiments, wrote the protocol, and supported the conduct of experiments, contributed to data analysis and manuscript writing.

JM and RY contributed to design and management of the trials and edited the draft manuscript.

NM, GL, HN, IM, JB collected data.

OP conducted chemical verification of IRS concentration

AR contributed to data analysis and writing

IN contributed to data analysis and drafted manuscript

MH contributed to writing

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Ethics approval and consent to participate

The studies received ethical clearance from the Medical Research Coordinating Committee of the Tanzanian National Institute of Medical Research reference number NIMR/HQ/R.8a/Vol.IX/1725 and NIMR/HQ/R.8a/Vol.IX/2270. Clearance by the Ifakara Health Institute Review Board (IHI-IRB) was issued under reference number IHI IRB 021/2016. A written informed consent was obtained beforehand from heads of all households that participated in the village trial.

Consent for publication

All authors have read the manuscript and consent for publication.

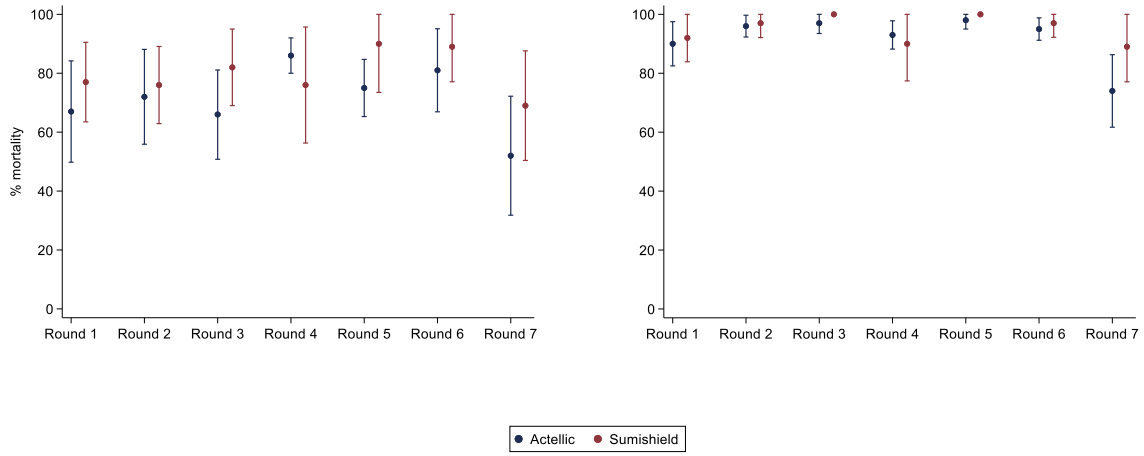
Competing interests

SJM and JM conduct evaluations of vector control products for a number of companies via service contracts to IHI.

Additional files

Supplementary Table 5. Baseline characteristics of the RCT trial

	Actellic	**SumiShield**
Baseline factors		
Household size		
≤ 5 members	537 (77%)	428 (80%)
> 5 members	160 (23%)	110 (20%)
Total households	697 (100%)	538 (100%)
Age of householders		
≤ 5 years	411 (15%)	413 (20%)
5 < years ≤ 18	894 (33%)	586 (29%)
> 18 years	1371 (51%)	1020 (51%)
Total participants	2676 (100%)	2019 (100%)
ITNs access coverage		
No ITNs	19 (3%)	14 (3%)
1 ITN/≤2 persons	457 (66%)	302 (56%)
1 ITN/>2 persons	221 (31%)	222 (41%)
House modification		
Not screened & open eaves	39 (6%)	31 (6%)
Screened & open eaves	52 (8%)	28 (5%)
Not screened & closed eaves	324 (46%)	281 (52%)
Screened & closed eaves	282 (40%)	198 (37%)
House walls		
Mud	283 (41%)	251 (47%)
Sticks	7 (1%)	35 (7%)
Sticks and plaster	51 (7%)	42 (8%)
Plaster	17 (2%)	2 (1%)
Burned bricks	339 (49%)	208 (39%)
Animals in/around house		
No animals	312 (45%)	163 (30%)
Poultry, cats, dogs	314 (44%)	222 (41%)
Goat, donkey, cattle	4 (1%)	5 (1%)
Both animal groups	67 (10%)	148 (28%)



Supplementary Figure 1. Residual activity of insecticides against susceptible An. gambiae s.s from the EHT

Left panel: 72-hour holding time.

Right panel: 168-hour holding time.

Data show *Anopheles gambiae* s.s. (pyrethroid susceptible) % mortality and 95% confidence intervals after a 30 minute exposure to insecticides applied to experimental huts after a 30 minute cone bioassay exposure. Data were pooled from seven cone bioassays conducted monthly from August 22nd 2016 to February 22nd 2017. Huts were sprayed on July 22nd 2016 (Month 0).

Supplementary Table 6. Residual activity of insecticides on susceptible *An. gambiae* s.s.

		Actellic	SumiShield		
Hours	Rounds	% mortality* (95% CIs)	% mortality* (95% CIs)	Odds ratio (95% CIs)	p value
24	1	**90.9 (87.0, 94.7)**	**88.3 (84.5, 92.1)**	0.87 (0.28, 2.67)	0.807
	2	**78.2 (71.9, 84.5)**	**90.2 (87.0, 93.4)**	2.63 (1.12, 6.20)	0.027
	3	**75.8 (68.1, 83.5)**	**80.7 (76.9, 84.4)**	1.37 (0.38, 5.00)	0.632
	4	46.2 (28.0, 64.4)	66.8 (58.0, 75.5)	2.37 (1.06, 5.31)	0.036
	5	53.0 (43.6, 62.5)	65.7 (57.4, 74.0)	2.09 (0.97, 4.52)	0.06
	6	34.4 (22.7, 46.1)	30.3 (21.0, 39.7)	0.97 (0.62, 1.51)	0.891
	7	39.6 (29.4, 49.8)	53.9 (45.0, 62.8)	2.74 (1.50, 4.99)	0.001
	8	14.5 (6.1, 22.9)	30.0 (21.5, 38.4)	1.59 (0.86, 2.95)	0.138
168	1	**100**	**100**		
	2	**99.2 (98.2, 100)**	**100**		
	3	**98.3 (96.7, 100)**	**100**		
	4	**100**	**100**		
	5	76.9 (64.2, 89.6)	**98.6 (97.2, 100)**	21.17 (6.35, 70.54)	<0.0001
	6	57.4 (38.9, 75.9)	**81.3 (66.6, 96.0)**	1.98 (0.20, 19.89)	0.562
	7	78.8 (69.9, 87.6)	**98.1 (95.3, 100)**	8.14 (1.38, 47.91)	0.02
	8	37.6 (16.2, 59.1)	**88.1 (79.2, 97.0)**	26.02 (6.20, 109.18)	<0.0001

Residual activity of Actellic and SumiShield applied to baked brick structures with fully susceptible *An. gambiae* s.s. mosquitoes. Estimates in **bold** are at or above WHO residual activity criteria for IRS (>=80%) * control corrected

8. Discussion and conclusion

8.1 Discussion

The array of issues raised around the challenges faced by malaria control following changes in vector populations can be consolidated in the context of this study in two ways. (i.) Whether sleeping under insecticide treated nets (ITNs) indoors during the night still confers effective malaria protection to individuals in malarious areas in light of concern of increased *Anopheles* outdoor biting. (ii.) Whether vector control that relies primarily on insecticides can still confer sufficient malaria protection in the context of widespread and multiple resistance by *Anopheles* populations to the insecticides in current use. Going by the World Health Organisation (WHO) vector control strategy, the protection of ITNs and indoor residual spraying (IRS) of insecticides, the core interventions, against bites from local *Anopheles* populations and malaria risk should be assessed under universal coverage [75]. In this thesis, the evaluation of ITNs and IRS occurred under settings where both interventions exceeded the WHO-recommended population-wide use of not less than 80% for effective malaria protection.

8.1.1 Malaria protection by ITNs in light of *Anopheles* outdoor biting

The assessment of ITN effectiveness in Chapter 5 estimated that in the study area, roughly four in every five infective *Anopheles* bites, the incidence of bites by *Anopheles* mosquitoes carrying malaria sporozoites, assessed between 6PM and 6AM occurred when nearly everyone was indoors and using an ITN. Such a high estimate of the potential protection from malaria by ITNs is attributed to the preference to bite and rest indoors by the major Afrotropical malaria vectors where also people spend most of the hours during the nights indoors [61, 62]. The remainder of infective *Anopheles* bites were estimated to occur, in the order of decreasing magnitude, when people were outdoors, indoors before retiring to bed and due to failure to use an ITN during sleep. These findings show that an assessment that takes into consideration human and mosquito behaviours can provide a more detailed and more accurate measure of the

protection and limitations of using ITNs. This also highlights potential limitations of the commonly used approaches that measure net use as a binary yes/no variable and that derive transmission risk merely from densities of host-seeking *Anopheles* populations captured by mosquito traps.

As increasing efforts have been made to protect people indoors across malarious areas, the greatest concern for progressing towards elimination besides insecticide resistance has been *Anopheles* outdoor biting [68]. Outdoor biting assessed in the context of this thesis, where humans spent most of the night hours indoors represented roughly 12% of all nightly infective *Anopheles* bites estimated to occur among children below school-age and 18% among the rest of household members. A recent study in an area in Burkina Faso reported similar findings [261]. The role of *Anopheles* outdoor biting on onwards malaria transmission continues to stimulate debate and uncertainty among malariologists for malaria elimination, particularly because there is a current dearth of tools and limited understanding of approaches that can be applied broadly to protect people outdoors [68, 160, 355]. However, the actual protection afforded by ITNs has also been subject of debate due to suspected increase in *Anopheles* outdoor biting or shift in species composition in favour of mosquitoes that mostly bite and rest outdoors (exophilic species) where use of ITNs is high [148, 356]. A factor that has tended to hinder proper determination of the actual role of *Anopheles* outdoor biting on onwards malaria transmission has been the tendency to rely on the incidence of malaria vector bites alone to estimate malaria risk. In 2019, a modelling study projected that outdoor biting was likely to impede malaria elimination even if universal coverage by ITNs and IRS was attained in SSA due to increased proportions of *Anopheles* bites received outdoors [156]. The study predicted an additional 10.6 million malaria cases annually attributed to outdoor biting. However, is the problem of *Anopheles* outdoor biting only as simple as the proportion of nightly mosquito bites estimated to occur outdoors? Evidence both from this thesis and in literature necessitate

cautious interpretation of the potential threat posed by *Anopheles* outdoor biting with increased ITN use in the recent times.

Firstly, previous studies have shown that the nightly incidence of infective *Anopheles* bites does not always follow the same hourly patterns as that of overall bites [283]. Both early evening and late night biting by infective *Anopheles* mosquitoes have been shown in different studies [283]. The timing of infective bites (rather than any bites) by *Anopheles* mosquitoes is epidemiologically relevant considering that humans usually spend night hours indoors and that the later the night, the more people are indoors and in bed where they often use ITNs [188]. Therefore, the time and location of infective *Anopheles* bites should be assessed relative to the human population exposed in order to determine accurately the risk of malaria transmission in a population. *Anopheles* biting densities demonstrated in Chapter 5 were similar throughout the night. However, infective bites tended to occur around the middle of the night when most people were using ITNs, explaining the estimated high protective potential of ITNs. Human exposure outdoors was highest between 6PM and 9PM because a higher proportion of the population was outdoors despite a relatively lower incidence of infective *Anopheles* bites around this time. Beyond 10PM, when the proportion of humans outdoors dropped dramatically, infective outdoor bites carried little epidemiological significance despite appearing to rise towards the middle of the night.

Secondly, unlike indoor biting where mosquitoes almost exclusively bite humans, *Anopheles* outdoor biting is in addition influenced by the availability of alternative blood meal sources. *An. arabiensis*, the species most implicated in outdoor biting has dynamic behaviours that are often characterised by a less discriminate choice of blood meal resources, with many studies reporting animals as a source of their blood meal in addition to humans [357]. There are studies that suggest a close link between *An. arabiensis* preferences for the animal blood meal

(zoophagy) and its exophilic tendencies. A study evaluating a new trap for sampling outdoor biting mosquitoes caught a disproportionately larger number of *An. arabiensis* mosquitoes when using cattle as a bait than it did with a human bait [224]. Whether occurring merely as an opportunistic event [358], particularly in the rural African contexts where typically animals such as cattle are kept outdoors during the night [359], or due to survival selection, the *Anopheles* exophilic-zoophagic tendencies may lower proportions of *Anopheles* bites on humans. A study observed significantly lower human blood index (HBI, the proportion of mosquito blood meals that are of human origin) for both *An. arabiensis* and *An. funestus* s.l. at households with livestock compared to those without [360], suggesting that *Anopheles* exophagic-zoophagic biting tendencies might not in fact be limited to the highly exophilic species. A review and meta-regression corroborates findings of lower *An. funestus* HBI from outdoor collections relative to indoor collections [361]. These findings are of particular relevance to the study area in this thesis, where *An. funestus* has been shown to pose greater malaria transmission threat contributing to roughly 90% of the entomological inoculation rate [226, 280]. Overall, reduced human-biting lowers rate of malaria parasite ingestion during *Anopheles* blood-feeding events. Mixed feeds also tend to diminish the density of gametocytes in the mosquito stomach which in turn lowers the chance of fertilisation of the female gamete thereby impeding mosquito infection [362]. In Chapter 5, similarly lower proportions of infective *An. funestus* bites were estimated from catches outdoors relative to those indoors. Garrett-Jones and colleagues observed that host-selection patterns of 'opportunistic' *Anopheles* feeders may be heavily influenced, even from village to village or from month to month, by the changing availability of alternative hosts, particularly cattle [363]. Therefore, whereas biting densities may not be highly varied in a season or in a given area the risk of mosquitoes carrying sporozoites may be subject to changes over smaller spatial

and temporal scales where malarious communities rear animals and where mosquitoes are for instance forced to exit houses for reasons such as when houses get too warm [263].

The relative epidemiological importance of *Anopheles* outdoor biting is also influenced by dynamics around human-vector contacts indoors. It is generally understood that high malaria risk occurring indoors across many malaria endemic areas is based on the predominance of indoor biting and resting by the major Afrotropical malaria vectors [61, 62]. The typical rural African hut settings often with large household occupancies tend to attract a lot of mosquitoes into the houses [364] In this houses, mosquitoes may even bite people when they are resting during the day [64]. Higher blood meal taking rate has been shown to shorten the extrinsic incubation period of *Plasmodium falciparum* [365] and also some arboviruses [366]. Therefore, indoor resting as mosquitoes are lured to stay close to human hosts where they may frequently take blood meals can increase the risk of malaria infection inside the house. The observations support reports of higher *An. funestus* capacities to transmit malaria than does its usual companion, the more exophilic *An. arabiensis* [226, 280]. Considering that indoor resting increases probability of insecticidal contacts, in the contexts of higher use of interventions indoor malaria risk may be underestimated if mosquito sampling methods miss engorged and potentially infected mosquitoes that may be knocked down by insecticides before they can be sampled [363].

Taken together, it is rather foreseeable that relying simply on biting densities rather than considering explicitly the probability that the mosquito bites actually pose the risk of human inoculation with malaria parasites can be misleading when characterising malaria risk. It is therefore crucial that the predicted significance of possible increase in proportions of *Anopheles* outdoor biting be considered cautiously and in setting specific contexts where use of interventions and ITNs in particular is accounted for. Of importance is to take into

consideration the roles played by different vector species and potential implications of possible shifts in local species composition. In essence, in an area where both indoor and outdoor *Anopheles* biting occurs alongside high coverage of efficacious ITNs, there may be reduced risk of malaria overall even when the outdoor biting experience appears to rise.

8.1.2 Linking human-*Anopheles* behaviours in assessing malaria protection by ITNs

As already discussed in the preceding section, human-vector interaction is crucial for determining the effectiveness of ITNs and for identifying gaps that require supplementary protection measures [367]. Chapter 5, Hourly changes of the proportions of the human population outdoors, indoors but out of bed and during sleep as well as incidence of infective *Anopheles* bites illustrated in Chapter 5 illustrated. The study also illustrated use of ITNs Chapter 5, although roughly everyone in the study population had access to a bed net, since the study was conducted just after a distribution campaign, the actual hours spent under ITNs were varied. Some people for instance did not immediately get under ITNs even after retreating to their sleeping spaces with possibly nets hanging but not spread to cover them. In other cases, individuals were observed to spend a few hours before sleeping chitchatting yet with the ITNs lowered and covering them [188]. Although in the context of the study of this thesis the risk of malaria was not substantial indoors before sleeping times, due to relatively shorter periods spent in this location and a relatively lower indoor incidence of infective *Anopheles* bites around the early evening hours, in other settings this may not be the case [299, 368]. Therefore, the period before bedtime, when people may already be inside their houses but not ready to sleep could be an important target for strengthening the benefits afforded by use of ITNs through appropriate education and communication strategies.

There is perhaps no better way of understanding the effectiveness of ITNs besides measuring the hourly incidence of infective *Anopheles* bites that occurs for all of the times when humans

are under ITNs relative to the overall exposure at night. The common approach to approximating the protection afforded by ITNs through the typical malaria indicator surveys that measures use of ITNs as a binary variable certainly fails to gather the details of ITN use by individuals from hour-to-hour throughout the night. The approach used in the study in this thesis enabled hour-by-hour assessments of infected *Anopheles* biting behaviours outdoors and indoors and proportions of the human populations outdoors, indoors but out of bed and during bed time.

Although the importance of understanding human-vector interaction is well established, there is often a tendency to estimate and interpret malaria risk from mosquito data alone. For example, the absolute risk of malaria estimated by the entomological inoculation rate is measured from *Anopheles* biting rates and the 'sporozoite rate', the portion of infected mosquitoes. However, mosquitoes are sampled on assumption that humans are present to receive each bite at the respective locations and times. In reality, people move from outdoors to indoors and then to bed during the night. Therefore, the absolute risk of malaria may not be consistent with the actual exposure of humans, which only depends on human-vector contact. Some standardised approaches have been proposed that can be used to measure human-vector interaction and help provide critical information on exposure across settings and over time [169, 257]. Individuals within households can be trained to observe nightly activities of members of their households for instance [188]. In Chapter 5, study revealed that nearly everyone in the study population was indoors, in sleeping spaces and under a mosquito net between 10PM and 5AM. Therefore, *Anopheles* bites measured outdoors during these hours would be unlikely to lead to malaria transmission due to the relative absence of human hosts during this period. Similarly, if we were to assume that everyone used nets effectively and that the nets could avert 100% of potential *Anopheles* bites, there would be zero exposure to malaria during the hours between 10PM and 5AM in the study area. Nonetheless, human exposure

outdoors between 6PM and 10PM would require supplementary personal protection measures. A slightly higher proportion of bites preventable by nets for children below school-age than the rest of household members was because the latter group tended to spend longer times under nets during the evenings than the former group.

8.1.3 Malaria protection by IRS under increased *Anopheles* pyrethroid-resistance

Like ITNs, IRS effect protection against malaria by inducing *Anopheles* mortalities so that mosquitoes do not survive long enough to carry malaria sporozoites and infect humans [186]. Insecticides also weaken mosquito ability to sustain parasite growth so that even resistant strains exhibit reduced capacity to carry malaria sporozoites [88]. Once applied on walls, no effort is needed from household members for IRS to function whilst potentially more dangerous indoor resting populations of mosquitoes are well targeted as they perch on treated wells. However, only a limited choice of insecticides exist for use to control malaria vectors and the situation is worsened by multiple and widespread resistance by *Anopheles* mosquitoes. The non-inferiority of SumiShield (clothianidin) relative to Actellic (pirimiphos-methyl) against pyrethroid-resistant *An. funestus* and *An. arabiensis* populations demonstrated in Chapter 7 brings new optimism for malaria vector control in a difficult era of increased insecticide resistance [369-371]. This is particularly because presently, ITNs use only pyrethroids of the five classes of public health approved insecticides. As a regulation to prevent and mitigate insecticide resistance, the WHO discourages use of pyrethroids or insecticides with similar modes of action to pyrethroids in IRS [108].

High clothianidin bioefficacy singly or in a mixture with deltamethrin (Fludora Fusion) against resistant *Anopheles* populations has been reported in other studies across sub Saharan Africa in both experimental huts and community trials [348, 350, 372]. Lack of evidence of clothianidin effect on *Anopheles* malaria parasite infection in this thesis was because of a sample size limitation of the community trial and is not to be mistaken for absence of sufficient

insecticidal activity. The major Afrotropical malaria vectors comprising the *An. funestus* and the *An. gambiae* species complexes are known to mostly rest indoors on walls and ceilings and are therefore often highly vulnerable to IRS insecticides. Exposure to insecticides has also been associated with reduced *Anopheles* vectorial capacity even in resistant strains [88]. Evidence so far of high clothianidin efficacy measured by entomological outcomes is expected to translate to reduction in disease transmission based on existing literature of IRS programmes with effective insecticides. For example during the global malaria eradication programme (GMEP) that succeeded in eliminating malaria completely from some parts of the World [106]. In the Pare-Taveta Malaria Scheme, intensive applications of IRS managed to decimate to near extinction the *An. gambiae* s.s. populations that initially contributed substantially to malaria transmission in the area. As a consequence, substantial reductions in the incidence of malaria cases overall, and a delay in resumption of intense transmission even after interrupting the programme were achieved [373]. A systematic review and mathematical modelling study showing that entomological data from experimental huts could be used to infer epidemiological impact of insecticidal interventions [187] support the link between *Anopheles* mortality and reductions in incidence of malaria. A recent operational-scale implementation of IRS with use of pirimiphos-methyl and Fludora Fushion in Madagascar was found to have caused reductions of up to 30% in the incidence of malaria from routine health data [374].

Pyrethroids are the sole insecticides approved for use in ITNs, therefore under the context of increasing *Anopheles* resistance to pyrethroids, the role of IRS for malaria control has become paramount [375]. The global strategy for insecticide resistance management advocates use of insecticides such as clothianidin that have a different mode of action to that of pyrethroids. Using insecticides for both ITNs and IRS in an effective manner and for a long period has often been hard to attain [305] particularly because the insecticides approved for public health are also used for agricultural pest control [376, 377] hence widespread use of the same insecticides

in the same locations is often common. However, a few lessons from the past are of crucial importance for managing the present and future careful use of malaria vector insecticides. Over the years, use of insecticides for malaria vector control has relied mostly on four classes of insecticides that are primarily nerve poisons. Prior to the era of widespread ITN use, IRS campaigns relied primarily on pyrethroids and the organochlorine DDT. Although insect nerve poisons are highly effective, there is often a danger of cross-resistance, where one or more resistance mechanisms affect multiple insecticides, and has tended to occur typically because of over-relying on pyrethroids. For instance, knockdown resistance that alters normal function of the voltage-sensitive sodium channel is associated with reduced efficacy of both DDT and pyrethoids [378], while elevated expression of cytochromes mediating enzyme metabolism is also shown to affect the two insecticide classes [379]. The strategic limiting for IRS use of both pyrethroids and DDT in order to mitigate spread of primarily knockdown resistance and to comply with DDT ban left a narrow choice of IRS insecticides among carbamates and organophosphates that function by a different mode of action.

The organophosphate pirimiphos-methyl is among the insecticides that have been preferred for IRS across many areas reporting pyrethroid resistance. Besides remaining unaffected by knockdown resistance, pirimiphos-methyl also has shown evidence of long residual efficacy capable of protecting households through a malaria season in many settings [380]. High operational costs of applying population-wide IRS programmes necessitate use of insecticides that can be applied through bi-annual campaigns. Although in Ulanga, the local *An. funestus* and *An. arabiensis* populations are thus far fully susceptible to pirimiphos-methyl, concerns elsewhere [381] of cross-resistance with pyrethroids raise alarm for continued use alongside ITNs. A study of a Cuban resistant *Aedes aegypti* population revealed high cross-resistance against pyrethroid and organophosphate insecticides due to presence of detoxifying esterases, monooxygenases and glutathione-s-tranferases [142]. Similar observations have been made in

Anopheles populations across sub Saharan Africa [371, 382]. Although pirimiphos-methyl has remained largely effective across most parts, some studies have reported reduced residual efficacy with the short-lasting insecticide effect leaving households with little protection during malaria seasons [109].

It is generally an expensive and lengthy process to develop a new insecticide. Average costs are estimated at USD 250 million for a single synthetic pesticide and the development period may be ten years; therefore, fewer and fewer new chemical active ingredients may be launched over the next 10-20 years [383]. In order to mitigate further spread of *Anopheles* resistance it will be important to observe correct and efficient use of available insecticides in order to maintain the effectiveness of current vector control efforts while novel vector control tools are under development [384]. The recommended strategy is to employ a wider range of different insecticides using approaches such as mosaics, combinations, mixtures and rotations to deploy IRS and to limit overuse of the same insecticides or those with similar modes of actions in the same locations [108]. Clothianidin and Fludora Fusion will certainly add value to the current toolbox of IRS insecticides and its use in careful combinations with organophosphates such as pirimiphos-methyl and carbamates such as bendiocarb may help restrict further spread of *Anopheles* pyrethroid resistance. Indications of resistance to clothianidin attributed to unregulated use of the neonicotinoids for agricultural pest control neonicotinoids in Yaoundé, Cameroon [377] must be investigated and appropriate precautions taken. Nonetheless, the necessary supportive infrastructure including agile surveillance systems to monitor and detect emergence of insecticide resistance in a timely manner will be required.

8.1.4 Human protection against residual malaria risk occurring outdoors

Outdoor biting has proven to be the 'Achilles' heel' of malaria vector control due to lack of effective and widely applicable tools that can protect humans outdoors [68]. Across most malarious parts, there is increased concern over onward malaria transmission attributed to

outdoor *Anopheles* biting following strengthening of interventions for human protection indoors [156, 254, 385]. If malaria elimination is to be attained, the incidence of locally contracted malaria cases has to be reduced completely to zero; hence, every single mosquito bite that poses risk of human infection has to be stopped. The problem of malaria exposure occurring outdoors can be minimised under high coverage with ITNs and IRS with efficacious insecticides. This is because even the highly exophagic *Anopheles* populations such as the *An. arabiensis* tend to visit houses where they can make contact with insecticides [291, 386]. Therefore, promoting widespread use of ITNs and IRS can be a 'double edged sword' strategy for targeting *Anopheles* outdoor biting as well, through the communal effects of the indoor-based interventions.

Several intervention measures have been shown to have potential to protect human exposure outdoors in various contexts and could help close the gap in effective malaria vector control. A study of long-lasting permethrin-impregnated clothing for protecting outdoor workers found that individuals using the intervention had lower antibody titres antibody titres to mosquito salivary gland extracts suggesting protection against bites [387]. Where appropriate, protective clothing could be an important addition to the toolbox of outdoor preventive measures because the intervention is also relatively easier to use. Aerial, spatial and topical mosquito repellents with effective chemicals and deployment approaches may be additional outdoor interventions. For instance, Ogoma and colleagues demonstrated that a low technology emanator consisting of hessian stripes treated with the volatile pyrethroid transfluthrin and that could be set around sitting spaces could confer long term protection against outdoor biting vectors of not just malaria but other mosquito-borne pathogens such as lymphatic filariasis and arboviruses [388]. With evidence of outdoor *Anopheles* biting of animals in livestock keeping communities, zooprophylaxis, the purposeful use of livestock (as dead-end hosts) to divert mosquitoes away from humans [133], with animals such as cattle could be exploited as a supplementary vector

control tool. Strategies employing cattle treatment with endocticides, insecticides applied directly to hosts to kill blood-feeding mosquitoes, such as ivermectin, diflubenzuron, eprinomectin and fipronil could yield equivalent, and in some cases improved, efficacy over ITNs and IRS in controlling malaria transmission [389]. House modifications may also help improve not just the reduction of *Anopheles* biting indoors but may as well help provide some level of protection against outdoor biting in the peridomiciliary spaces. Mmbando and colleagues demonstrated that fitting houses with transfluthrin-treated eaves ribbons either alone to keep mosquitoes away from people in and around houses [390] or together with odour-baited traps via a push-pull system both to repel and to divert mosquitoes away from people [391] could help protect against mosquito bites indoors and outdoors and help alleviate malaria transmission. An area of house settings that has hardly been explored for control of vector-borne disease control is the artificial lighting condition, yet there is compelling evidence that insects including mosquitoes are highly influenced by light cues [392] and that *Anopheles* may be attracted to variable light wavelengths [393]. Studies may help to understand the association between lighting outdoor resting spaces such as verandas with fluorescent or incandescent light bulbs and *Anopheles* biting. Measures with potential to cause significant reductions in mosquito densities such as targeting male mosquito swarms [394] or larval sources [395] could be exploited to improve protection of humans alongside the standard vector control interventions. In particular, where *Anopheles* breeding sites and larval sources are well characterised, larval source management with larviciding, environmental modification or manipulation and biological control as appropriate based ecological and geographical conditions [395].

8.1.5 Reconciling *Anopheles* human-biting estimates from different mosquito traps

Man biting by *Anopheles* mosquitoes is the fundamental biological and epidemiological basis of indigenous malaria transmission. Based on the degree of mosquito bites and human inoculation with malaria parasites, the intensity of malaria transmission and the vectorial

capacities of *Anopheles* mosquitoes can be determined. Sampling human-biting mosquito populations is therefore of critical relevance to monitor changes in *Anopheles* biting intensities for evaluating vector control programmes. The human landing catches (HLC) method is used to estimate mosquito biting directly from captures of mosquitoes as they attempt to bite an exposed human. HLC is considered the gold standard for measuring *Anopheles* human biting rates. However, the method has some limitations including high operational costs, difficulties in standardising catches from different catchers and ethical concerns. The latter is related to the deliberate exposure of human catchers to potentially infective mosquito bites. These ethical concerns are theoretically solvable by use of exposure-free traps such as the US Centres for Disease Control and Prevention light trap (CDC LT) and the human decoy trap (HDT). Of critical importance is that potential limitations of *Anopheles* biting data obtained by the exposure-free traps be reconciled with estimates obtained by HLC. In this thesis both the CDC LT and the HDT tended to underestimate human biting by both *An. funestus* and *An. arabiensis* host-seeking captures compared to the HLC with the largest differences occurring under conditions of lower mosquito densities. HDT was in the settings of this thesis not effective in catching *Anopheles* mosquitoes primarily due to operational challenges. For example, difficulties in transporting the relatively large size of the trap during studies and in maintaining a source of heat typically warm water throughout the trapping exercise can pose operational challenges. The HDT also tends to work better with animal baits and is therefore in principal expected have lower effectiveness for measuring human biting [224].

A few points are therefore necessary to consider while employing traps for sampling *Anopheles* populations. Surveys to obtain a precise estimate of the entomological inoculation rate (EIR) should employ HLC, which appears to be best placed to provide direct accurate measurements. For practical reasons, as HLC maybe resource-intensive to implement, HLC would be most suitable if the exercise does not involve very large operations. If HLC cannot be used, the CDC

LT, which in this thesis was comparable to HLC indoors under high *Anopheles* densities and which in other studies has shown effectiveness in sampling major Afro-tropical malaria vectors, may be used provided the trap yields catches that are not largely different from those of HLC in the settings under consideration. Regression models can be used to resolve density-dependence bias of traps with reference to HLC. Use of HLC or HLC-standardised mosquito trap metrics is the approach of choice for calibrating new or improved tools for sampling human-biting *Anopheles* populations. For routinely monitoring changes in behaviours of *Anopheles* mosquitoes, including identification of possible indicators of insecticide resistance such as persistence of mosquito densities despite applications of interventions that may also require covering large areas, appropriate trap choice from a range of effective mosquito traps can be employed for sampling mosquitoes. A few options found to be effective include CDC LT, suna trap and furvella tent trap that have been used for sampling Afrotropical vectors in different areas. Similarly, exposure-free traps would be most recommendable for the typically largescale mosquito sampling enterprise required for evaluating vector control interventions such as community trials. While a wide choice of mosquito traps exists appropriate for sampling *Anopheles* populations for general purposes of estimating densities and for purposes such as monitoring changes in species composition, exposure-free traps have not tended to perform well for sampling human host-seeking mosquitoes outdoors. Perhaps the difficulty in achieving much success by use of host/odour-baited traps outdoors may be linked to the general issue of *Anopheles* human-biting 'distraction' due to alternative blood meal resources from the presence of animals outdoors especially that mimicking and/or delivering host-cues effectively can be a challenge [396]. On the other hand, animal baiting of traps [224, 397, 398] can improve mosquito sampling effectiveness outdoors where mosquitoes are not needed for obtaining explicit human exposure metrics such as the EIR and can be particularly highly effective for sampling highly exophilic species such as the *An. arabiensis*.

8.1.6 Implications of findings of this thesis

The findings in this thesis have important implications for policy, research and development as well as for the local communities for strengthening malaria vector control by both ITNs and IRS in the context of the present challenges of increased *Anopheles* insecticide resistance and outdoor biting.

Implications for policy

Recommendations for policy would include: (i.) ITNs have great potential to protect individuals across malaria endemic areas even with increased *Anopheles* behavioural avoidance characterised by a shift to biting outdoors. Therefore, high coverage with efficacious ITNs in the communities needs to be promoted across all malarious areas by appropriate catch-up campaigns and maintained through suitable keep-up channels to address attrition and loses of ITNs from households. The period before bedtime, when people may already be inside their houses but not ready to sleep could be an important target for strengthening the benefits afforded by use of ITNs through appropriate education and communication strategies. (ii.) Considering that ITNs employ only pyrethorids of the five insecticide classes approved for public health use, IRS has greater significance in the contexts of increasing *Anopheles* pyrethroid-resistance as the capacity to employ insecticides that have different modes of action may help mitigate further spread of resistance and secure the effectiveness of ITNs. Clothianidin has proven potential for inclusion in the IRS insecticides toolbox for targeting resistant *Anopheles* populations. Careful use of the limited number of insecticides on the IRS shelf through deployment strategies such as mosaics, rotations, mixtures and combinations as recommended by the WHO to curb risk of *Anopheles* resistance need to be upheld in regulations guiding field practise. Finally, insecticide use for malaria vector control and for agricultural pest control need to be coordinated and regulated to enhance adherence to good practise and avoid overuse of insecticides locally.

Strategies to promote acceptance and use of ITNs and IRS in the population, which target problematic perceptions, tradition, culture and habits that may hinder use of vector control tools [189, 399] through effective health education and communication channels should be encouraged [400].

Implications for research

The epidemiological significance of *Anopheles* outdoor biting in general and the recent observation of possible shift towards increased proportions of malaria vectors biting outdoors due to wide coverage of the indoor-based insecticidal interventions and potential effects on vector control needs to be interpreted with caution. Across most areas where malaria is endemic, there is hardly a single *Anopheles* species responsible for spreading malaria. Malaria vector systems in the high burden settings in Africa are typically comprised of at least one sibling species both from the *An. gambiae* and from the *An. funestus* complexes. The relatively highly exophilic and zoophagic *An. arabiensis* almost invariably occurs alongside predominantly endophilic populations such as *An. funestus* or *An. gambiae* s.s. It is well understood that *Anopheles* vectorial capacities vary from species to species and across space and time. In addition, evidence from a wide range of sources suggests that biting behaviours of infected *Anopheles* mosquitoes, which are often the older mosquitoes, tends to be different from the rest that may be mainly younger. Clearly, the mere biting incidence of *Anopheles* mosquitoes is not adequate to characterise properly the risk of malaria transmission but the better approach should consider species composition locally as well as biting behaviours of infected mosquitoes indoors and outdoors ideally over shorter time intervals such as hours throughout the night.

The indoor and outdoor incidence of bites by infected *Anopheles* mosquitoes measured directly from catches of host-seeking populations and the proportions of the sporozoite-infected

mosquitoes preferably over hourly time points should be adjusted for the proportion of the exposed human population in the respective hours and locations. Human exposure to malaria defined as the proportion of infective *Anopheles* bites occurring outdoors, indoors but out of bed and during bed time can then be use to properly quantify the potential of interventions such as ITNs and the gaps of effective vector control that need targeting by supplementary tools. The actual protection from malaria infection afforded to humans by ITNs can be taken to be the proportion of infectious *Anopheles* bites occurring during all the hours of the night that humans are beneath ITNs. Accordingly, field methods of assessing use of ITNs need to take into account the hours of the night spent under ITNs as opposed to the current assessments that measure use of ITNs overall.

In view of the intensive resource demands for conducting community trials for evaluating vector control tools, use of entomological data obtained from experimental huts for projecting epidemiological metrics of malaria transmission should be a focus of research in order to enhance the evidence base to inform the potentially cost-saving use of experimental huts alone [187, 322]. Interpretation of mosquito data from experimental huts need to take into account the hut locations and potential influence of the surrounding ecological characteristics on the breeding of different local *Anopheles* species. Where community trials must be conducted, they would need to be large and cover wide areas and longer periods to generate data of sufficient sizes that allow adequate statistical power and would need to include numerous population clusters to enhance collection of sufficient numbers of infected mosquitoes in order to detect effects of interventions.

The EHT caught a disproportionately larger number of *An. arabiensis* mosquitoes compared to the more dangerous *An. funestus* in the area, than has been demonstrated by collections from communities. In the studies highlighted in Chapter 4 of this thesis [271] and previously [280], higher numbers of *An. funestus* than *An. arabiensis* were caught. Whereas three other studies

[151, 188, 226] estimated higher *An. arabiensis* than *An. funestus* densities, there were much larger relative *An. funestus* proportions compared to the EHT in this thesis. Possible explanations of differences in relative densities of the local malaria vectors estimated from the communities could range from the exact times of the year that mosquito collections are made to the traps used as well as vector control coverage at specific times of surveys. The differences in relative densities of the local vector species estimated between the EHT and community surveys might be explained by ecological features around the locations of the Ifakara experimental huts [324], where surrounding rice farms (Figure 3) may provide favourable breeding sites for the *An. arabiensis*. Locations of huts with respect to mosquito ecology may be a factor to consider when installing huts and when interpreting mosquito data from EHTs.

Implications for the local communities

Our study in Chapter 5 highlights high potential of ITNs to prevent malaria under condition that they are of good quality and are used well and at all times during nightly sleep. Everyone in the household should use an ITN despite age and gender. ITNs should ideally be obtained from campaigns or from accredited shops. The ITNs need to be properly installed in the sleeping spaces to limit entry of mosquitoes. Shared ITNs should not be crowded so that chances of body contact with the fabric of the nets are minimised as mosquitoes can still bite through the pores of the net despite irritancy from insecticides [401]. Before new ITNs are received in the households, the available ITNs should be used consistently even when old and torn and where possible should be repaired for instance to seal holes, as even old nets still confer some level of protection [402].

8.1.7 Limitations and areas for improvements

A key feature from the community trial for evaluating IRS was the lack of sufficient statistical power to detect IRS effect on *Anopheles* sporozoite infection due to a sample size limitation.

Community trials need to be implemented over a long period and cover a sufficient numbers of population clusters to attain adequate statistical power to calculate mosquito infection endpoints. Locations of huts with respect to mosquito ecology may be a factor to consider when installing huts and when interpreting mosquito data from EHTs.

Assessments of effectiveness of ITNs in Chapter 5 assumed that ITNs prevent 100% of all *Anopheles* bites occurring during times when humans are under them. In reality, ITNs in their best state prevent only a proportion of mosquito bites referred to as the blood-feeding inhibition, the nightly proportion of mosquitoes collected from a hut where an individual spent all night under an ITN that are without a blood meal [322, 403]. ITN feeding inhibition from intact and bio-efficacious ITNs is often high averaging greater than 90% but often declines with deterioration of ITNs for instance when they get holes [402]. Humans were also assumed to incur balanced risk of infective *Anopheles* bites. Individual-to-individual variations in human attractiveness of *Anopheles* mosquitoes have been studied and may be influenced by malaria infection in the human host [404] and size and smell, with a previous study suggesting an underlying genetic component detectable by mosquitoes through olfaction [405].

8.2 Conclusion

Studies covered in this thesis have together demonstrated that in a typical sub Saharan African setting where malaria transmission is endemic and where transmission is primarily by *An. funestus* and *An. arabiensis* that bite both indoors and outdoors, ITNs afford high protection against malaria transmission. Malaria control programmes should therefore ensure high household ownership and use of efficacious ITNs across all malarious areas. Strategies that promote high use of ITNs in the households by each member over all the times when they are in their sleeping spaces at night need to be encouraged to guarantee optimal protection by ITNs for everyone in the population.

IRS with the use of effective insecticides such as clothianidin and that function by a different mode of action from that of pyrethroids can be employed to mitigate the spread of *Anopheles* resistance to pyrethroids and help complement ITNs to drive a higher effectiveness for vector control and greater impact on malaria transmission. Appropriate IRS deployment strategies need to be employed to ensure that pyrethroid insecticides are not used alongside ITNs, and that insecticides with similar modes of action are not used in the same locations but instead insecticides with dissimilar modes of action are used alternatingly or in combinations.

Sampling tools for estimating human biting by local malaria vector populations need to be considered on a case-by-case basis appreciating fundamental limitations of exposure-free mosquito traps for specific entomological survey tasks in different settings. HLC should be preferred over exposure-free traps where the purpose of surveying mosquitoes is to quantify absolute estimates of malaria risk more specifically the EIR. HLC-standardised entomological metrics estimated from catches by exposure-free mosquito traps may be used for evaluating malaria vector control interventions and for monitoring changes in behaviours in *Anopheles* populations including possible shifts in species composition, biting behaviours and occurrence or spread of insecticide resistance.

9. References

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Curriculum vitae

Personal information

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Education

Doctor of Philosophy	Subject: Epidemiology Awarded: September, 2023 Results: Cum Laude Thesis: Monitoring and control of Afrotropical malaria vectors Institution: University of Basel, Basel, Switzerland and Swiss Tropical and Public Health Institute, Allschwil, BL, Switzerland
Master of Science	Subject: Applied Parasitology Awarded: 2013 Results: Distinction Thesis: The role of routine outdoor nocturnal activities on residual malaria transmission among adults in Dar es Salaam, Tanzania Institution: University of Nairobi, Nairobi, Kenya and Ifakara Health Institute, Dar es Salaam, Tanzania
Bachelor of Science	Subject: Zoology Awarded: 2009 Results: First Class Honours Thesis: The effects of recombinant cyclophilin-A on the establishment and progress of experimental trypanosome infection in mice Institution: University of Nairobi

Work and responsibilities

Doctoral research fellow	Program: Phase II and III non-inferiority trials of SumiShield™ Program head: Sarah Moore (PhD) Institution: Ifakara Health Institute-Vector Control Product Testing Unit (VCPTU) Specific duties and skills: 1. Data curation 2. Data analysis and archiving 3. Authorship of peer-reviewed publications Time: 2018-2023
Research officer	Program: Malaria transmission consortium (MTC), Tanzania Program head: Gerry F. Killeen (PhD) Institution: Ifakara Health Institute, Dar es Salaam, Tanzania

	Specific duties and skills: 1. Supervision of malaria indicator household surveys 2. Supervision of clinical teams conducting malaria tests in houses 3. Supervision of mosquito monitoring in villages 4. Data curation 4. Data analysis and archiving 5. Authorship of peer-reviewed publications Time: 2011 to 2013
Student leader	Institution: Swiss Tropical and Public Health Institute (Swiss TPH), Switzerland Specific duties and skills: 1. Represented student welfare at Swiss TPH 2. Organised student academic and recreational activities 3. Coordinated weekly student scientific meetings Time: 2020 to 2021

Key competencies and experiences

Data collection, management, analysis	Household surveys involving one-on-one interviews Use of palm digital assistants (PDAs) for data collection Application of Microsoft Access and Excel for data handling Data cleaning with STATA and R Data analysis with STATA and R Statistical modelling including use of mixed effects models
Scientific writing	Writing peer-reviewed publications in English scientific journals Literature review and knowledge synthesis

Awards and scholarships

Young Investigator Award (YIA)	Awarded by: American Society of Tropical Medicine and Hygiene (ASTMH) Type: Recognition of meritorious scientific work by young researchers Awarded: 2021
Swiss Excellence Scholarships for International Students	Awarded by: Swiss Federal Commission for Scholarships (FCS) Type: Fully-funded PhD Scholarship Awarded: 2018
University of Nairobi-Ifakara Health Institute joint Masters Scholarship	Awarded by: University of Nairobi, Kenya and Ifakara Health Institute, Tanzania Type: Fully-funded Master of Science Scholarship Awarded: 2009

Selected publications

1. Msellemu, D., **Namango, H.I.**, et al., *The epidemiology of residual Plasmodium falciparum malaria transmission and infection burden in an African city with high coverage of multiple vector control measures.* Malar J, 2016. **15**(1): p. 288.

2. Mmbando, A.S., Kaindoa, E.W., Ngowo, H.S., Swai, K.J., Matowo, N.S., Kilalangongono, M., Lingamba, G.P., Mgando, P., **Namango, H.I.**, Okumu, F.O., Nelli, L., *Fine-scale distribution of malaria mosquitoes biting or resting outside human dwellings in three low-altitude Tanzanian villages.* PloS one, 2021. **16**(1): p. e0245750.

3. **Namango, H.I**, et al., *The Centres for Disease Control light trap (CDC-LT) and the human decoy trap (HDT) compared to the human landing catch (HLC) for measuring Anopheles biting in rural Tanzania.* Malaria Journal, 2022. **21**(1): p. 181.

4. **Namango, H.I**, et al., *A matter of timing: Biting by malaria-infected Anopheles mosquitoes and the use of interventions during the night in rural southeastern Tanzania.* Submitted to PLOS Global Public Health.

5. **Namango, H.I**, et al., *Efficacy of SumiShield^{TM} 50WG for indoor residual spraying against resistant Anopheles mosquitoes in Ulanga, southeastern Tanzania*. To be submitted to Parasites and Vectors.

6. **Namango, H.I**. *Tracking insecticide treated net use throughout the night to enhance evaluation of protection against malaria*. To be submitted as an editorial to Malaria Journal

7. Liaga, E.A., O. Menang, and **I. Namango**, *Sub-Saharan Governments' Response to COVID-19 and the Second Order Crises.* Horn Bull, 2020. **3**(3).

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