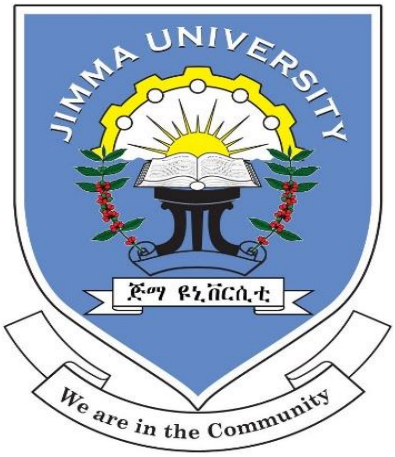
PROFILING AND QUANTIFYING RESIDUAL MALARIA TRANSMISSION IN KENYA AND ETHIOPIA

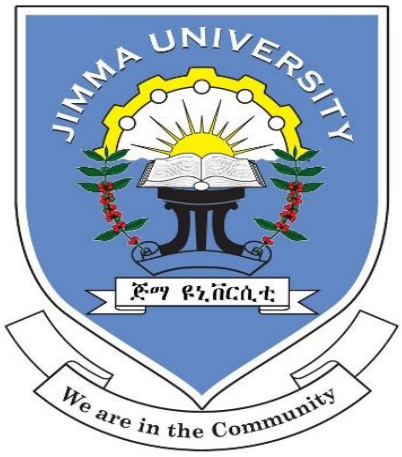


JIMMA UNIVERSITY

PhD CANDIDATE: TESHOME DEGEFA DEMIE

JANUARY 2022

JIMMA, ETHIOPIA



JIMMA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

PROFILING AND QUANTIFYING RESIDUAL MALARIA TRANSMISSION IN KENYA AND ETHIOPIA

CANDIDATE: TESHOME DEGEFA DEMIE

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Glory be to the almighty God!

LIST OF ACRONYMS AND ABBREVIATIONS

aHBR – Behaviour-adjusted human biting rate

ACT – Artemisinin-based combination therapy

ANOVA – Analysis of Variance

ATSB – Attractive toxic sugar bait

BBI – Bovine blood index

BGS – BioGents sentinel trap

BGM – BioGents malaria trap

CDC – Centers for Disease Control and Prevention

CI – Confidence interval

CSP – Circum-sporozoite protein

DDT - Dichloro diphenyl trichloroethane

DNA – Deoxyribonucleic acid

dNTP – Deoxynucleoside triphosphate

EIR – Entomological inoculation rate

ELISA – Enzyme-linked immunosorbent assay

EMM – Estimated marginal mean

FR – Forage ratio

GLM – Generalized linear model

GMEP – Global malaria eradication programme

GTS – Global technical strategy

HBI – Human blood index

HBLT – Human-odour-baited CDC light trap

HDNT – Human-baited double net trap

HLC – Human landing catch

ib/p/year – infective bites per person per year

IRS – Indoor residual spray

ITN – Insecticide-treated net

ITS2 - internal transcribed spacer 2

KEMRI – Kenya Medical Research Institute

LLIN – Long-lasting insecticidal net

LSM – Larval source management

MET – Mosquito electrocuting trap

MM-X – Mosquito magnet-x trap

NIH – National Institutes of Health

PBO – Piperonyl butoxide

PCR – Polymerase chain reaction

PSC – Pyrethrum spray catch

rDNA – Ribosomal DNA

SSA – sub-Saharan Africa

TIDRC – Tropical and Infectious Diseases Research Center

WHO – World Health Organization

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ABSTRACT

Background: Malaria is a serious vector-borne disease affecting hundreds of millions of people in sub-Saharan Africa (SSA). Kenya and Ethiopia are part of the SSA sharing the burden of the disease with about 75% and 52% of their total populations living in malaria-risk areas, respectively. In the past two decades, unprecedented success has been achieved in reducing the malaria burden in Africa, mainly due to the scale-up of vector control interventions such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Based on the progress made, several countries including Ethiopia have set goals to eliminate malaria. However, residual transmission due to outdoor and early-evening/morning biting vectors could pose a challenge to malaria control and elimination efforts. While monitoring malaria vector behaviour and residual transmission is crucial to evaluate the likely success of the existing interventions, such entomological monitoring has also been difficult in Africa due to lack of suitable, safe, efficient and well-standardized tools for surveillance of outdoor resting and host-seeking malaria vectors.

Objective: The aim of the study was to develop and evaluate surveillance tools for outdoor resting and host-seeking malaria vectors, and to determine vector species composition, abundance, behaviour, patterns of human exposure to vector bites, and residual malaria transmission in Kenya and Ethiopia

Methods: The study was conducted in Ahero and Iguhu sites in western Kenya and Bulbul *Kebele* in southwestern Ethiopia from September 2015 to December 2018. A new tool hereafter called sticky trap was developed for outdoor resting malaria vector surveillance. In addition, two exposure-free tools hereafter called human-odour-baited CDC light trap (HBLT) and human-baited double net trap (HDNT) were developed for outdoor host-seeking vector surveillance in Kenya and Ethiopia. A longitudinal entomological study was conducted from September 2015 to April 2016 to evaluate the performance of the new sticky trap as well as to assess the species composition and behaviour of mosquito vectors, and their role in indoor and outdoor malaria transmission in western Kenya. Twenty houses (for each trapping method) were randomly selected from each study site. Mosquitoes were collected using CDC light traps (indoor and outdoor), pyrethrum spray catches (PSC), pit shelters, the sticky pots, clay pots, exit traps and a prokopack aspirator. Furthermore, longitudinal entomological studies based on cross-over and

Latin Square experimental designs were conducted in Kenya and Ethiopia from November 2015 to December 2018 to evaluate the trapping efficiency of the HBLT and HDNT well as to determine vector species composition, behaviour, patterns of human exposure to vector bites and the magnitude of residual malaria transmission. Mosquitoes were collected using the HBLT, HDNT, CDC light traps, human landing catch (HLC) and PSC. Human behaviour data were collected using a semistructured questionnaire. Species within *Anopheles gambiae s.l.* and *Anopheles funestus* group were identified using polymerase chain reaction (PCR). Enzyme-linked immunosorbent assay (ELISA) was used to determine mosquito blood meal sources and sporozoite infections.

Results: Over the three years study period, a total of 31,862 female *Anopheles* mosquitoes (29,551 from western Kenya and 2,311 from Bulbul in southwestern Ethiopia) comprising at least seven species were collected. In western Kenya, *An. gambiae s.l.* was the predominant species accounting for 65.3% of the collected *Anopheles* mosquitoes, followed by *An. pharoensis* (14.1%), *An. coustani* (11.5%) and *An. funestus* group (9.2%), whereas in southwestern Ethiopia, *An. pharoensis* was the most abundant species accounting for 40.6% of the collected *Anopheles* mosquitoes, followed by *An. gambiae s.l.* (30.6%), *An. coustani* (28.2%), *An. squamosus* (0.3%) and *An. funestus* group (0.2%). PCR results showed that 98.9% *An. arabiensis* and 1.1% *An. gambiae* s.s. constituted *An. gambiae s.l.* in Ahero site, whereas in Iguhu, *An. gambiae s.s.* and *An. arabiensis* accounted for 87% and 13% of the *An. gambiae s.l.,* respectively. *Anopheles arabiensis* was the only member species of the *An. gambiae s.l.* in Bulbul. *Anopheles funestus s.s.* and *An. leesoni* accounted for 98.1% and 1.9% of the *An. funestus* group in western Kenya.

In western Kenya, *An. arabiensis* exhibited exophagic behaviour while *An. gambiae s.s.* and *An. funestus* showed endophagic behaviour. The human blood index (HBI) and bovine blood index (BBI) of *An. arabiensis* was 2.5% and 73.1%, respectively. *Anopheles gambiae* s.s. had HBI and BBI of 50.0% and 28.0%, respectively. The HBI and BBI of *An. funestus* was 60.0% and 22.3%, respectively. *Anopheles arabiensis* preferred to feed on cattle, *An. gambiae* s.s. showed preference for both humans and cattle, while *An. funestus* preferred humans over other vertebrate hosts. In Ahero site, *Plasmodium falciparum* sporozoite rates for *An. arabiensis, An. funestus* and *An. coustani* were 0.16%, 1.8% and 0.5%, respectively, respectively, whereas in Iguhu site, *P. falciparum* sporozoite rates for *An. gambiae* s.s. and *An. funestus* were 2.3% and 2.4%,

respectively. In Ahero, the estimated indoor and outdoor entomological inoculation rates (EIRs) were 108.6 infective bites/person/year (ib/p/year) (79.0 from *An. funestus* and 29.6 from *An. arabiensis*) and 43.5 ib/p/year (27.9 from *An. arabiensis* and 15.6 from *An. funestus*), respectively. In Iguhu, the estimated indoor and outdoor EIRs were 24.5 ib/p/year (18.8 from *An. gambiae* s.s. and 5.7 from *An. funestus*) and 5.5 ib/p/year (all from *An. gambiae* s.s.), respectively.

In southwestern Ethiopia, *An. arabiensis* and *An. pharoensis* were 2.4 and 2.5 times more likely to seek hosts outdoors than indoors, respectively. However, most (66%) of human exposure to *An. arabiensis* and 39% of exposure to *An. pharoensis* bites occurred indoors for LLIN non-users. For LLIN users, 75% of residual exposure to *An. arabiensis* bites and 84% of exposure to *An. pharoensis* occurred outdoors. The HBI and BBI of *An. arabiensis* were 19.2% and 65.4%, respectively while *An. pharoensis* had HBI and BBI of 16.7% and 66.7%, respectively, indicating that both species showed preference to feed on cattle. The overall sporozoite rates of *An. arabiensis, An. pharoensis* and *An. coustani* were 0.4%, 0.3% and 0.2%, respectively. The estimated indoor and outdoor EIRs of *An. arabiensis* were 6.2 and 1.4 ib/p/year, respectively, whereas *An. pharoensis* had an estimated outdoor EIR of 3.0 ib/p/year.

The new sticky pots showed a similar performance as pit shelters in terms of the relative abundance and host blood meal indices of malaria vector species. In terms of density per trap, a pit shelter caught on average 4.02 (95% CI: 3.06–5.27) times as many *An. arabiensis* as a sticky pot while a sticky pot captured 1.60 (95% CI: 1.19–2.12) times as many *An. arabiensis* as a clay pot. The HBLT captured two times as many *An. arabiensis* and *An. funestus* as the conventional CDC light trap, but it yielded a significantly lower density of *An. arabiensis* compared to HLC. The HDNT caught 6.5 times as many *An. arabiensis* as the CDC light trap. The mean density of *An. arabiensis* did not vary between the HDNT and HLC ($p = 0.098$). Moreover, there was a significant density-independent positive correlation between HDNT and HLC ($r = 0.69$).

Conclusions: *Anopheles gambiae s.s.* showed an increasing tendency to feed on cattle compared to historical data collected before the scale-up of vector control interventions in western Kenya while *An. funestus* exhibited anthropophagic and endophagic behaviour. *Anopheles arabiensis* was highly zoophagic and exophagic in both western Kenya and southwestern Ethiopia. Human exposure to *An. arabiensis* bites occurred mostly indoors for LLIN non-users, while most of the

residual exposure to both *An. arabiensis* and *An. pharoensis* bites occurred outdoors for LLIN users. Malaria transmission by *An. gambiae s.s.* and *An. funestus* occurred mostly indoors, *An. arabiensis* contributed to both indoors and outdoors malaria transmission while *An. pharoensis* exclusively contributed to outdoor transmission. This study revealed that the new sticky pots could be a useful and complementary tool for outdoor resting malaria vector surveillance, in settings where using pit shelters is not feasible and less productive. The present study also showed that both HBLT and HDNT caught a higher density of malaria vectors than the conventional CDC light trap. Moreover, the HDNT yielded a similar vector density as HLC, suggesting that it could be an alternative tool to HLC for outdoor host-seeking malaria vector surveillance. The findings of this study suggest that additional control tools targeting outdoor and early evening biting malaria vectors are required to complement the current control interventions to control residual transmission and ultimately achieve malaria elimination. Further studies are required to comprehend the role of the suspected vector, *An. coustani,* in malaria transmission.

CHAPTER ONE

1. GENERAL INTRODUCTION

1.1. Background

Malaria is an infectious vector-borne disease caused by five *Plasmodium* species; *Plasmodium falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi* (a zoonotic species mainly occurring in southeast Asia) (Kantele and Jokiranta, 2011). The disease remains one of the most serious infectious diseases, affecting hundreds of millions of people in Africa. In 2019, an estimated 229 million malaria cases and 409,000 malaria-related deaths were reported globally, with about 94% of the cases and deaths occurred in Africa (WHO, 2020b). *Plasmodium falciparum* is the predominant species and is responsible for most of the deaths from malaria in sub-Saharan Africa (SSA) (WHO, 2020b). Kenya and Ethiopia are part of the SSA sharing the burden of malaria, with about 75% and 52% of their total populations living in malaria-risk areas, respectively (PMI, 2020b, PMI, 2016a, FMoH, 2020).

In Kenya, malaria accounts for an estimated 16% of outpatient consultations and 6% of hospital admissions (PMI, 2020b). The western region of the country is the most affected area. This region includes areas around the Lake Victoria basin with malaria prevalence exceeding 20%, and western highlands of Kenya with malaria prevalence ranging from 5-20% (PMI, 2016b, Weiss et al., 2019). Malaria transmission intensity in Kenya is determined mainly by altitude, rainfall and temperature. Consequently, the prevalence varies considerably by season and across different geographic regions. *Plasmodium falciparum* is the most common species accounting for 92% of all malaria infections in the country, followed by *P. malariae* (6%) and *P. ovale* (2%) (MoH, 2016, MoH, 2019).

In Ethiopia, malaria is seasonal with unstable transmission. The transmission patterns and intensity vary across the country due to the large diversity in altitude, rainfall, and population movement. Areas below 2,000 meters are considered malarious. These areas cover almost 75% of the country's landmass. Areas most affected include the lowlands and midlands of western Ethiopia, followed by areas in or near Rift Valley, which extend from the southwest of the country to the northeast (PMI, 2016a). The peak of malaria transmission follows the main rainfall season (July to September) every year. However, many districts in the south and west of

the country have a rainfall season beginning earlier in April and May or have no clearly defined rainfall season. Consequently, malaria transmission tends to be highly heterogeneous geospatially within each year as well as between years. *Plasmodium falciparum* and *P. vivax* are the most dominant malaria parasites in Ethiopia accounting for 65% and 35%, respectively (FMoH, 2020). *Plasmodium ovale* and *P. malariae* account for less than 1% (FMoH, 2016).

Malaria transmission involves complex interactions between *Plasmodium* parasites, female *Anopheles* mosquitoes, and people (Figure 1.1). Infection occurs when an infected female *Anopheles* mosquito injects sporozoites along with its anticoagulating saliva into the skin of a human while probing for a blood-meal. Sporozoites infect liver cells and mature into schizonts, which rupture and release thousands of merozoites within 7-10 days. Some parasite species such as *P. vivax* and *P. ovale* can enter a period of latency by forming non-replicating hypnozoites instead of schizonts. These hypnozoites enable long-term survival of the parasites and can lead to relapses. After replication in the liver, each exoerythrocytic form contains thousands of merozoites which are released into the bloodstream and rapidly invade erythrocytes (Sturm et al., 2006). These blood-stage parasites replicate asexually and destroy each red blood cell they infect, leading to the clinical symptoms of malaria (Bruce-Chwatt, 1980).

After several cycles of erythrocytic schizogony, some merozoites (those that are sexually committed) differentiate and mature into male and female gametocytes. The gametocytes are ingested by the *Anopheles* mosquito during a blood-meal. In the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile ookinetes, which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle. The time needed for the completion of the parasite life cycle in the mosquito varies according to the species and the ambient temperature and humidity, but is usually 7–21 days (WHO, 2010, Cowman et al., 2016).

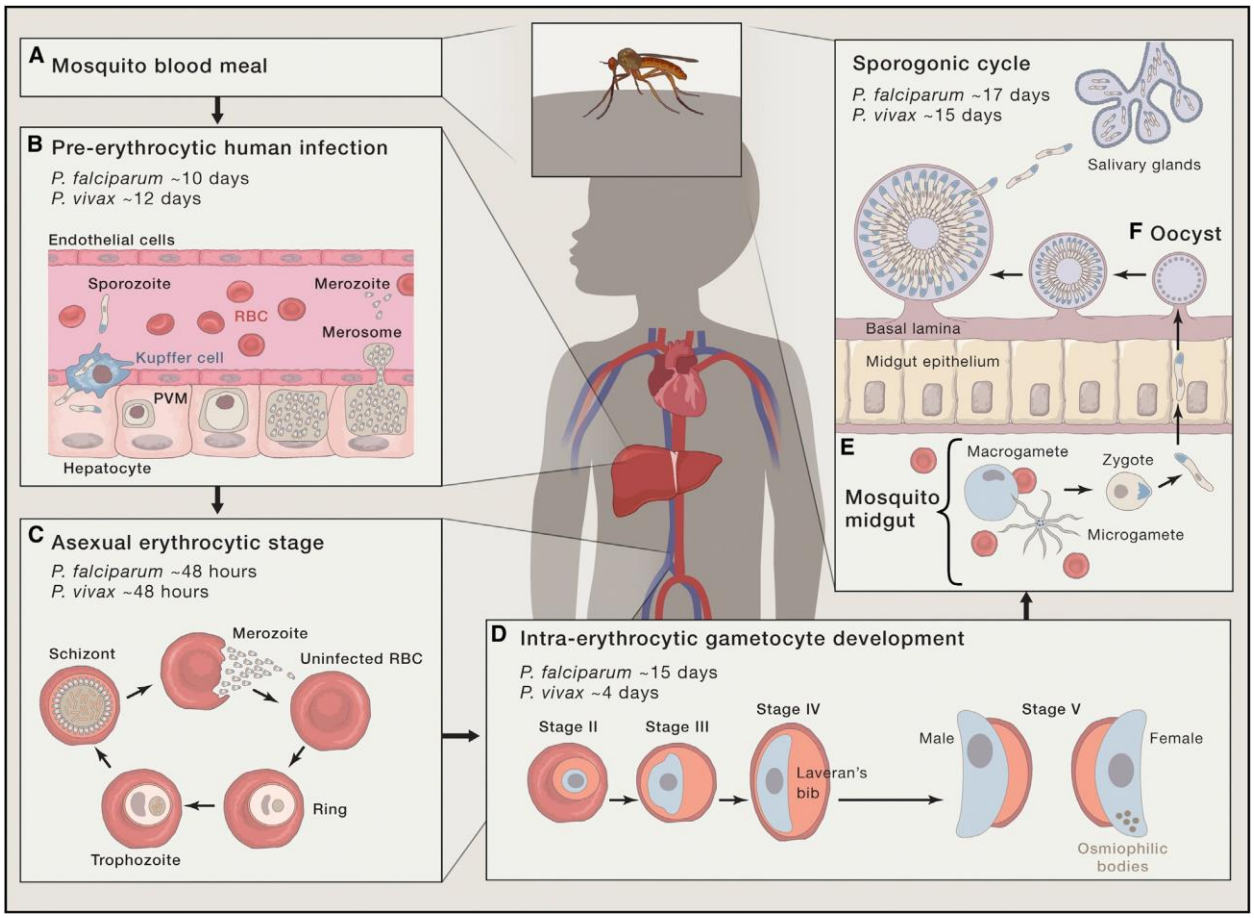

Figure 1.1. The life cycle of malaria parasites (Source: Cowman et al., 2016)

There are 480 recognized species of *Anopheles* mosquitoes and over 50 unnamed members of species complexes worldwide (Sinka et al., 2012, Harbach, 2021). About 70 of these species have the capacity to transmit human malaria parasites and 41 are considered to be dominant vector species, capable of transmitting malaria at a level of major concern to public health (Sinka et al., 2012). In Africa, *Anopheles gambiae, An. coluzzii*, *An. arabiensis* and *An. funestus* are the most efficient malaria vector species (Sinka et al., 2010).

1.2. Statement of the Problem

Malaria has had a profound effect on human lives for thousands of years, and remains one of the most serious life-threatening vector-borne infectious diseases in the world (Carter and Mendis, 2002, Vos et al., 2020). The SSA bears the highest burden of the disease with hundreds of millions of malaria cases and hundreds of thousands of malaria-related deaths occurring every year in the region (WHO, 2020b).

In the past two decades, unprecedented success has been achieved in reducing the malaria burden in Africa and elsewhere in the world (O'Meara et al., 2010, Weiss et al., 2019, WHO, 2015b, Battle et al., 2019, WHO, 2020b). Between 2000 and 2015, malaria incidence declined globally by 37%, and the reduction was higher (42%) in the World Health Organization's (WHO) African region (Figure 1.2) (WHO, 2015b). Similarly, the mortality rate due to malaria fell by 60% globally and by 66% in Africa during the same period. Between 2000 and 2019, an estimated 1.5 billion malaria cases have been averted globally, with over 7.6 million lives estimated to have been saved over the past two decades (WHO, 2020b). Most of the averted cases (82%) and deaths (94%) were from the SSA. The decline in the number of cases and deaths is attributable to the scale-up of key core malaria control interventions such as long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and artemisinin-based combination therapies (ACTs) (WHO, 2020b).

Similarly, increased coverage in malaria control and prevention interventions over the past two decades has resulted in significant declines in malaria morbidity and mortality in East Africa (Otten et al., 2009, Shargie et al., 2010, Taffese et al., 2018, Bhattarai et al., 2007). In Kenya for instance, national malaria prevalence declined from 11% in 2010 to 8% in 2015 (NMCP, 2016). In Ethiopia, the infection prevalence of *P. falciparum* was reduced by up to 15% in 2015 in children aged 2-10 years, compared to the baseline infection prevalence in 2000 (Figure 1.2) (Bhatt et al., 2015). Moreover, Ethiopia has achieved the Global Technical Strategy (GTS) target of a 40% reduction in malaria incidence by 2020, compared to the baseline data of 2015 (WHO, 2015a, WHO, 2020b). The estimated number of deaths per year due to malaria in all age groups declined in the country by 60.1% from 14,085 in 2000 to 5,626 in 2019 (WHO, 2020b). These achievements and gains have reawakened the notion of malaria elimination in many African

countries including Ethiopia (Campbell and Steketee, 2011, Woyessa et al., 2013, FMoH, 2017, WHO, 2015a).

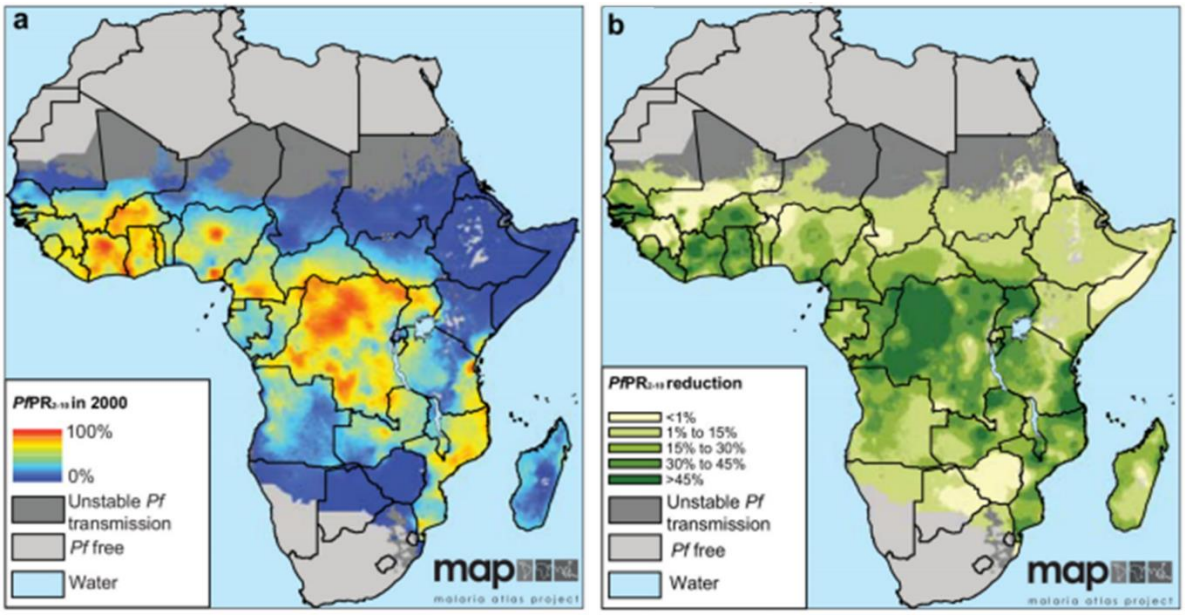

Figure 1.2. Changes in malaria prevalence in children in Africa between 2000 and 2015
a) Predicted *P. falciparum* infections in Children 2-10 years of age in 2000, b) absolute reduction in prevalence of *P. falciparum* infection in 2015 (Source: Bhatt et al., 2015).

However, the progress has stalled in the past five years and malaria transmission has continued to occur at a level of major public health concern in SSA, including Ethiopia, due to several challenges (WHO, 2020b). Among the main challenges are increased outdoor malaria transmission (Overgaard et al., 2012a, Durnez and Coosemans, 2013, Russell et al., 2011, Sherrard-Smith et al., 2019), insecticide resistance in mosquito vectors (Kawada et al., 2011, Yewhalaw et al., 2010, Mathias et al., 2011, Trape et al., 2011, Balkew et al., 2010, Yewhalaw et al., 2011, Hancock et al., 2020) and antimalarial drug resistance in *Plasmodium* species (Conrad and Rosenthal, 2019, Lu et al., 2017). Due to these challenges and other possible contributing factors such as environmental modifications (Kibret et al., 2019a), climate change (Endo et al., 2017, Endo and Eltahir, 2020), and population movement (Haile et al., 2017, Kagaya et al., 2019), increase in malaria incidence has been reported recently in several African countries (WHO, 2017b, Kagaya et al., 2019, Abiodun et al., 2020, WHO, 2020b).

Malaria transmission may persist even with the assumption of full coverage of interventions like LLINs and IRS (WHO, 2014b). The LLIN targets only indoor host-seeking mosquitoes while IRS targets only indoor resting mosquiotoes (Killeen et al., 2014). This leaves an opportunity for vectors that bite and rest outdoors (Fornadel et al., 2010a, Mahande et al., 2007a) to escape from contact with insecticide-treated surfaces and sustain residual transmission. Moreover, the long-term use of the LLINs and IRS has been shown to alter the behaviour of vectors by pushing them to adapt to feeding outdoors (Russell et al., 2011, Meyers et al., 2016, Kreppel et al., 2020), to bite earlier in the evening or morning when people are not protected (Fornadel et al., 2010a, Sougoufara et al., 2014), and feeding on animals when humans are not accessible (Lefèvre et al., 2009, Ndenga et al., 2016, Kreppel et al., 2020). Within *An. gambiae s.l.* species complex, these behavioural changes have been associated with a shift in the species composition towards vector species with more exophagic, exophilic and zoophagic tendency (i.e. from *An. gambiae* to *An. arabiensis*) in some places in East Africa (Bayoh et al., 2010, Russell et al., 2011, Derua et al., 2012, Kitau et al., 2012, Mwangangi et al., 2013a). These could pose challenges to the current indoor-based vector control tools as malaria transmission may occur outdoors, and in the early evening and morning hours.

Understanding and tackling residual malaria transmission requires monitoring and surveillance of local vector species composition, density, behaviour, and quantifying the magnitude of the residual transmission for surveillance driven control and to evaluate the impact of the existing vector control interventions (WHO, 2014a). However, surveillance of malaria vectors has been difficult in Africa due to lack of well standardized, efficient and safe surveillance tools (Service, 1977, Jamrozik et al., 2015).

An ideal vector surveillance system requires sampling host-seeking and resting mosquitoes both indoors and outdoors to provide an unbiased estimate of entomological indices (WHO, 1975). The gold standard method for sampling host-seeking vectors is human landing catch (HLC) as it provides a direct estimate of human exposure to infectious mosquito bites occurring indoors and outdoors (Service, 1977). However, this method is cumbersome, labour intensive and requires intense supervision to obtain reliable results (Qiu et al., 2006, Service, 1977). Alternative traps commonly used for monitoring indoor host-seeking vectors such as Center for Diseases Control and Prevention (CDC) light traps do not perform equally for outdoor mosquito collections

(Service, 1977, Costantini et al., 1998, Mboera, 2005, Kenea et al., 2017). Indoor resting mosquitoes are often sampled by pyrethrum spray catch (PSC), but it has no equivalent tool for outdoor resting mosquito sampling (Service, 1977). Hence, there is a pressing need to search for novel sampling tools and approaches for outdoor host-seeking and resting vector populations to perform effective vector surveillance, and to design appropriate complementary interventions based on local vector behaviour.

Furthermore, quantifying residual malaria transmission requires simultaneous monitoring of both local vector behaviour and human behaviour to better understand where and when the actual human exposures to infectious mosquito bites occur (Edwards et al., 2019, Finda et al., 2019, Monroe et al., 2019a). However, entomological surveillance activities in Africa in general and in Ethiopia in particular have almost exclusively relied on vector behaviour, with no or less attention paid to human habits and sleeping patterns. Therefore, the main aim of this study was to develop and evaluate surveillance tools for outdoor resting and host-seeking malaria vectors, as well as to assess vector behaviour, patterns of human exposure to vector bites, and residual malaria transmission in two East African countries, Kenya and Ethiopia.

1.3. Malaria Vector Bionomics

1.3.1. Life cycle

Anopheles mosquitoes have four different stages in their life cycle: the aquatic stages (egg, larva and pupa) and the adult stage. The preference of breeding habitat is different for different *Anopheles* species. Some species such as *An. arabiensis* and *An. gambiae* prefer breeding in small, temporary and sunlit water collections such as rain pools, puddles and hoof prints (Edillo et al., 2002, Minakawa et al., 2004). The breeding habitats of species such as *An. funestus* and *An. pharoensis* are usually large and permanent water bodies with emergent vegetation, such as swamps, large ponds and the edges of lakes (Nambunga et al., 2020, Kenea et al., 2011). Some species such as *An. merus* and *An. melas* prefer to breed in brackish water, while others prefer hot springs for breeding (Coetzee et al., 2013).

Adult female *Anopheles* mosquitoes lay their eggs on the water surface. Larvae hatch from the eggs within 1–2 days and float beneath and parallel to the water surface, where they breathe air. The larvae, which are active feeders on organic detritus and microorganisms, subsequently molt

into the second, third and fourth instars at intervals of about 2 days each. The fourth instar larvae develop into the non-feeding pupal stage, with adults emerging from the pupae within 2-3 days. The duration of the life cycle (usually 10-14 days) depends on the temperature, mosquito species and nutritional factors in their habitats (Service, 2012).

1.3.2. Mating, feeding and resting behaviour

A female mosquito mates only once after emerging from a pupa because she receives sufficient sperm cells from a single mating, which remain viable in the spermatheca and serve to fertilize all eggs that are laid during her lifetime (WHO, 2013c). Both male and female *Anopheles* mosquitoes feed on nectar to obtain energy for flight and dispersal as soon as they emerge from the pupae, but the female requires a blood-meal to obtain protein for egg development and maturation. In most tropical species, it takes 2-3 days to digest the blood-meal, but this depends on temperature and can take 7-14 days in a colder, temperate climates (Service, 2012).

After a blood-meal, the mosquito rests to digest the blood. Some *Anopheles* species prefer to rest inside houses during digestion of the blood-meal, while others prefer resting outdoors (Service, 2012). At the end of blood-feeding, the abdomen of a mosquito becomes dilated with a bright red appearance and subsequently changed to dark red. As the blood is digested and the white eggs in the ovaries are enlarged, the abdomen becomes whitish posteriorly and dark reddish anteriorly. Eventually, all blood is digested and the abdomen becomes dilated and whitish due to the formation of fully developed eggs. The mosquito then searches for suitable larval habitats to lay the eggs. After the oviposition, the female mosquito takes another blood-meal, and the cycle continues. This process of blood-feeding, resting for blood-meal digestion and egg development, and egg-laying is repeated several times throughout the lifetime of female *Anopheles* mosquitoes, and is referred to as the gonotrophic cycle (Service, 2012). The duration of a gonotrophic cycle depends on temperature (Service, 2012), availability of blood-meal sources and oviposition sites (Gu et al., 2006, Afrane et al., 2005), and it is important in determining the vectorial capacity of mosquitoes.

1.4. Malaria Vectors in Africa

There are over 144 species of *Anopheles* mosquitoes in Africa. Of these, about 20 are known to transmit malaria to humans (Irish et al., 2020).

1.4.1. Major vectors

There are eight dominant malaria vector species in Africa: *An. gambiae*, *An. coluzzii*, *An. arabiensis*, *An. melas*, *An. merus*, *An. funestus*, *An. moucheti* and *An. nili* (Wiebe et al., 2017, Sinka et al., 2010). These vector species are responsible for about 95% of the total malaria transmission in the continent (Manguin et al., 2008). The first five species are members of the *An. gambiae senso lato (s.l.),* while *An. funestus*, *An. moucheti* and *An. nili* are member species of *An. funestus* group, *An. nili group*, *An. moucheti s.l.*, respectively.

Anopheles gambiae s.l. comprises ten morphologically indigustishable sibling species including *An. gambiae*, *An. coluzzii*, *An. arabiensis*, *An. melas*, *An. merus*, *An. quadriannulatus*, *An. bwambe*, *An. amharicus*, *An. comorensis* and a recently discovered species from Gabon, *An. fontenillei* (Coetzee et al., 2013, Barrón et al., 2019). *Anopheles arabiensis* and *An. gambiae* are widely distributed in SSA (Figure 1.3). The distribution of *An. coluzzii* extends from northern Senegal in the west to east-central Africa and south to coastal Angola (Coetzee et al., 2013). *Anopheles melas* and *An. merus* are distributed in the western and eastern coasts of Africa, respectively (Deitz et al., 2012). The other sibling species of *An. gambiae s.l.* are confined to specific geographical locations (Figure 1.3).

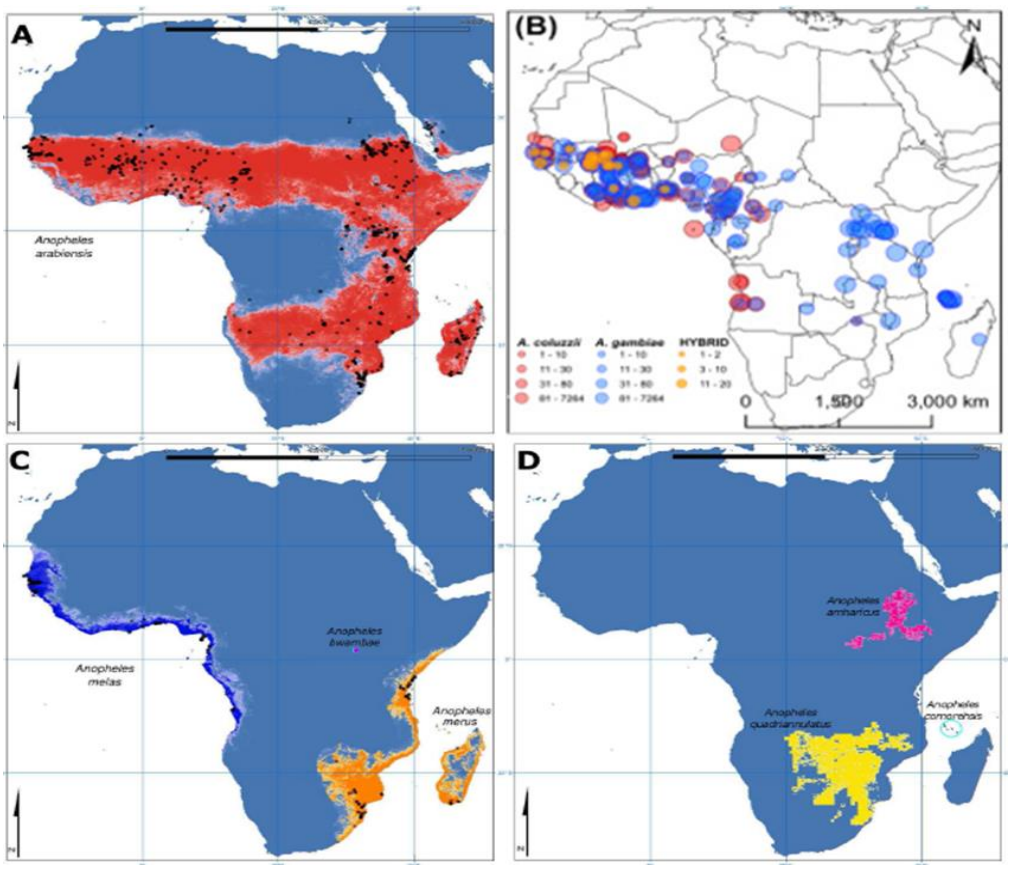

Figure 1.3. Geographic distribution of members of the *Anopheles gambiae* species complex A. *Anopheles arabiensis* (red); B. *An. gambiae* (blue) and *An. coluzzii* (pink); C. *An. melas* (Blue), *An. merus* (orange), and *An. bwambae* (cyan); D. *An. quadriannulatus* (yellow), *An. amharicus* (magenta) and *An. comorensis* (cyan circle) (Source: Sinka et al., 2010, Coulibaly et al., 2016).

Anopheles gambiae and *An. coluzzii* are known to be anthropophagic and endophagic vectors (Githeko et al., 1994b, Pappa et al., 2011, Akogbéto et al., 2018). *Anopheles gambiae* exhibits relatively higher endophagic tendency compared to *An. coluzzii* (Akogbéto et al., 2018). *Anopheles arabiensis* is described as a zoophilic, exophagic and exophilic species compared to *An. gambiae* (Githeko et al., 1994b). However, it is also known to have a wide range of feeding and resting behaviours, tending to be either endophilic or exophilic, anthropophagic or zoophagic, early biter or late biter, depending on geographical locations (White, 1974a, Sharp and le Sueur, 1991, Ameneshewa, 1996).

The role of *An. gambiae* and *An. coluzzii* as efficient vectors of malaria is reflected by their sporozoite rates reaching up to 10-13% in some African countries such as Benin (Akogbéto et al., 2018), Burkina Faso (Pombi et al., 2018) and Nigeria (Ebenezer et al., 2016). *Anopheles arabiensis* had relatively lower sporozoite infection rates, often less than 2% (Githeko et al., 1993, Mwangangi et al., 2013b, Massebo et al., 2013b, Taye et al., 2006), compared to that of *An. gambiae* and *An. coluzzii*. Nevertheless, its role in malaria transmission is increasing, replacing *An. gambiae* in some places (Lwetoijera et al., 2014, Pombi et al., 2018, Ebenezer et al., 2016).

Anopheles merus and *An. melas* have previously been considered as only minor vectors (White, 1974a). However, they have been reported later on to play a major role in malaria transmission (Ebenezer et al., 2016, Kipyab et al., 2013, Ridl et al., 2008, Temu et al., 1998). *Anopheles merus* has been incriminated as a vector of malaria in coastal areas of Tanzania (Thomson, 1951, Temu et al., 1998), Madagascar (Tsy et al., 2003), Mozambique (Cuamba and Mendis, 2009) and Kenya (Mbogo et al., 2003, Kipyab et al., 2013). Similarly, *An. melas* was shown to play a significant role in malaria transmission in west African countries such as The Gambia (Bryan, 1983), Senegal (Diop et al., 2002), Equatorial Guinea (Ridl et al., 2008) and Nigeria (Ebenezer et al., 2016). Both *An. merus* and *An. melas* exhibit opportunistic feeding behaviour (both anthropophilic and zoophilic) depending on host availability, with a tendency to bite and rest outdoors (Sinka et al., 2010). *Anopheles bwambae,* a species known to occur in geothermal springs in western Uganda, has also been identified as a local malaria vector in the area (White, 1985). *Anopheles quadriannulatus*, which is found in southeast Africa (Coluzzi, 1984) and *An. amharicus*, which has been described in Ethiopia (Hunt et al., 1998, Coetzee et al., 2013) are not considered vectors of human malaria as they are generally zoophilic (Coluzzi, 1984).

Anopheles funestus group comprises at least 12 sibling or closely related species. These include *An. funestus* s.s., *An. funestus-like, An. aruni* , *An. confusus, An. parensis* , *An. vaneedeni, An. longipaplis* type A & C, *An. leesoni* , *An. rivulorum* , *An. rivulorum-like, An. brucei* and *An. fuscivenosus* (Dia et al., 2013, Coulibaly et al., 2016). Of these, *An. funestus s.s.* is the most anthropophagic and endophagic, and is an efficient vector of malaria in many African countries (Coetzee and Fontenille, 2004). It had high sporozoite infection rates, exceeding that of *An. gambiae* in some some countries such as Côte d'Ivoire (Adja et al., 2011), Tanzania

(Lwetoijera et al., 2014) and Kenya (Shililu et al., 1998, Ndenga et al., 2006). The geographical distribution of *An. funestus* sibling species in Africa is shown in Figure 1.4.

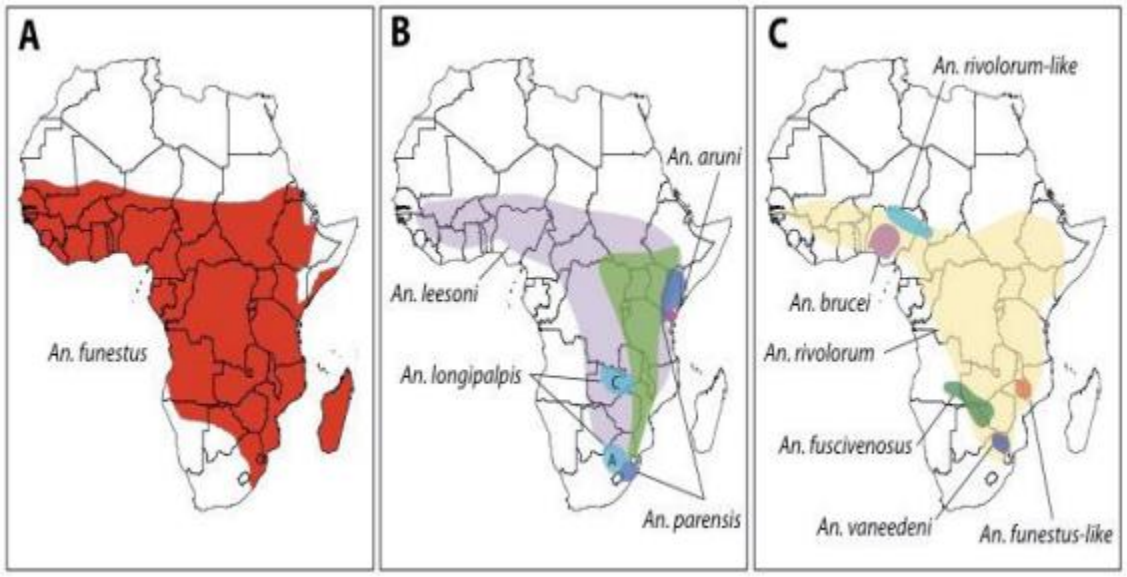

Figure 1.4. Distribution of member species of the *Anopheles funestus* group in Africa
A. *Anopheles funestus*; B. *An. leesoni*, *An. longipalpis* (type A & C), *An. aruni* and *An. parensis*, C: *An. rivolorum*, *An. rivolorum-like*, *An. funestus-like*, *An. vaneedeni*, *An. fuscivenosus* and *An. brucei* (Source: Dia et al., 2013).

Anopheles nili group and *An. moucheti* are major vectors of malaria in forested and humid areas in Africa (Ndo et al., 2010, Antonio-Nkondjio et al., 2008). *Anopheles nili* group comprises four morphologically similar species, including *An. nili s.s.*, *An. somalicus*, *An. carnevalei* and *An. ovengensis* (Gillies and Coetzee, 1987). Of these, *An. nili s.s.*, is a widespread and efficient vector of malaria in African countries such as Cameroon (Carnevale et al., 1992), Senegal (Dia et al., 2003), Côte d'Ivoire (Adja et al., 2011) and Benin (Ossè et al., 2019). It is highly anthropophagic, but exhibits both endophagic and exophagic behaviours (Carnevale et al., 1992, Dia et al., 2003, Adja et al., 2011). *Anopheles moucheti* is an important vector of malaria in Cameroon (Antonio-Nkondjio et al., 2002, Antonio-Nkondjio et al., 2006), Nigeria (Awolola et al., 2002), Gabon (Gillies and Coetzee, 1987) and Equatorial Guinea (Ridl et al., 2008).

1.4.2. Secondary vectors

Secondary African malaria vectors are responsible for about 5% of the total malaria transmission in the continent (Afrane et al., 2016a). Within the *An. funestus* group, *An. rivulorum*, *An. leesoni*, *An. parensis* and *An. longipaplpis* have been incriminated as vectors of malaria in Tanzania (Temu et al., 2007) and Kenya (Ogola et al., 2018) based on polymerase chain reaction (PCR). Moreover, sporozoite positive *An. parensis* and *An. vaneedeni* were reported in South Africa (Burke et al., 2019, Burke et al., 2017). Similarly, *An. pharoensis*, *An. coustani*, *An. zeimanni,* and *An. squamosus* have been incriminated as secondary vectors of malaria in different African countries (Sinka et al., 2010, Afrane et al., 2016a, Antonio-Nkondjio et al., 2006, Goupeyou-Youmsi et al., 2020, Stevenson et al., 2016, Gillies, 1964).

1.5. Malaria Vectors in Kenya

More than 48 species of *Anopheles* mosquitoes have been documented in Kenya (Gaffigan et al., 2015, Kyalo et al., 2017). Of these, four species are incriminated as major vectors of malaria (PMI, 2019b), with at least four additional species considered as secondary vectors in the country (Afrane et al., 2016a, Ogola et al., 2018).

1.5.1. Primary vectors

The major malaria vectors in Kenya are from the *An. gambiae s.l.* species complex (*An. gambiae*, *An. arabiensis* and *An. merus*) and *An. funestus* (PMI, 2019b). *Anopheles gambiae* and *An. funestus* are highly anthropophagic and endophagic, and the most efficient vectors in western part of the country (Shililu et al., 1998, McCann et al., 2014, Githeko et al., 1993, Githeko et al., 1996, Githeko et al., 1994b). *Anopheles arabiensis* had lower sporozoite rates compared to *An. gambiae* and *An. funestus*, with zoophagic feeding behaviour (Githeko et al., 1993, Githeko et al., 1996, Githeko et al., 1994b). However, the density of *An. gambiae* population has declined in some parts of western Kenya following the scale-up of vector control interventions with a proportionate increment in its sibling species, *An. arabiensis* (Bayoh et al., 2010).

1.5.2. Secondary vectors

Anopheles pharoensis and *An. coustani* have been incriminated as secondary vectors of malaria in Kenya based on enzyme-linked immunosorbent assay (ELISA) (Mukiama and Mwangi, 1989,

Mwangangi et al., 2013b). In Mwea Irrigation Scheme, central Kenya, *P. falciparum* sporozoite rate of 1.3% was reported for *An. pharoensis* (Mukiama and Mwangi, 1989) while in Taveta district, coastal Kenya, *P. falciparum* sporozoite rate of up to 1.78% was reported for *An. coustani* (Mwangangi et al., 2013b). Moreover, *An. rivulorum* and *An. longipalpis* have been incriminated as vectors of malaria in the country using PCR (Gillies and Smith, 1960, Ogola et al., 2018). Recently, involvement of new unnamed *Anopheles* species in malaria transmission has also been reported in western Kenya (Laurent et al., 2016, Zhong et al., 2020).

1.6. Malaria Vectors in Ethiopia

More than 44 species of *Anopheles* mosquitoes have been documented in Ethiopia (Gaffigan et al., 2015, Kyalo et al., 2017). Few of these species are incriminated as primary and secondary vectors of malaria, while most species are considered non-vectors.

1.6.1. Primary vector (s)

In Ethiopia, *Anopheles arabiensis,* the species responsible for over 95% of malaria transmission in the country, is the principal vector of malaria (Abose et al., 1998). *Anopheles arabiensis* shows variable feeding and resting behaviours with both anthropophagic and zoophagic, and exophagic and endophagic behaviours (Tirados et al., 2006, Habtewold et al., 2001, Taye et al., 2016). For instance, Tirados *et al*. reported its anthropophagic and exophagic behaviour in the Konso district in southern Ethiopia (Tirados et al., 2006). On the other hand, Habtewold *et al.* documented zoophagic behaviour of *An. arabiensis* from another locality in the same region (Habtewold et al., 2001). Other studies conducted in southern parts of the country have also reported zoophagic behaviour for this species (Fettene et al., 2004, Massebo et al., 2015).

The *Plasmodium* sporozoite infection rates of *An. arabiensis* varied from place to place in Ethiopia. In 1977, Krafsur reported a sporozoite rate of 1.87% in Gambella, western Ethiopia (Krafsur, 1977), whereas Nigatu *et al.* documented a sporozoite rate of 0.77% in the same area in 1994 (Nigatu et al., 1994). *Anopheles arabiensis* had *Plasmodium falciparum* sporozoite rates of 1.18-1.67% around Ziway irrigation schemes in central Ethiopia (Kibret et al., 2010, Kibret et al., 2014), 0.3-05% in southern Ethiopia (Taye et al., 2006, Massebo et al., 2013b), 0.2% in south-central Ethiopia (Animut et al., 2013) and 1.5% in Jimma area, southwestern Ethiopia (Degefa et al., 2015). *Plasmodium vivax* sporozoite rates of 1.76%, 1.7% and 0.3% were

recorded for this species in southern (Taye et al., 2006), south-central (Animut et al., 2013) and southwestern (Degefa et al., 2015) parts of Ethiopia, respectively.

1.6.2. Secondary vectors

Anopheles pharoensis is one of the secondary vectors of malaria in different parts of Ethiopia. In Gambella, *Plasmodium* sporozoite rate of 0.47% was documented for *An. pharoensis* (Nigatu et al., 1994). *Anopheles pharoensis* sampled from around Koka reserviour dam and Ziway irrigation schemes in central Ethiopia had *P. falciparum* sporozoite rates of 0.47-0.81% (Kibret et al., 2010, Kibret et al., 2014, Kibret et al., 2012). In central Ethiopia, *P. vivax* sporozoite rate of 1.4% was documented for this species (Animut et al., 2013). Similarly, *An. nili* and *An. funestus* have been shown to play roles as secondary vectors of malaria with sporozoite rates of up to 1.57% and 1.23% documented in Gambella region for *An. nili* and *An. funestus*, respectively (Krafsur, 1977, Krafsur, 1970).

1.6.3. Potential vectors

Anopheles coustani has been considered as a suspected vector of malaria in Ethiopia. This species was found to be positive for *P. falciparum* sporozoite in Jimma area (Degefa et al., 2015). *Anopheles demeilloni* was also found to be positive for *P. falciparum* sporozoite in southern Ethiopia (Daygena et al., 2017). However, the sporozoite detection in both species was based on ELISA. Hence, further investigation using more specific molecular techniques like PCR is needed to incriminate them as vectors of malaria. Moreover, *Plasmodium* positive *An. cinereus* was recently reported from northwest Ethiopia, suggesting that this species could also have a role in malaria transmission (Lemma et al., 2019).

1.6.4. Invasive vector

Anopheles stephensi, species known to be an efficient vector of urban malaria in Asia and the Mediterranian region (Sinka et al., 2011), was reported in Eastern Ethiopia for the first time in 2016 (Carter et al., 2018). This species was reported from the horn of Africa for the first time in Djibouti in 2012 (Faulde et al., 2014), and it was confirmed to have a potential role in malaria transmission in the country (Seyfarth et al., 2019). Both *P. falciparium* and *P. vivax* have been detected in *An. stephensi* in Ethiopia (Amenu et al., 2020). Its distribution is expanding to multiple regions of Ethiopia (Balkew et al., 2020), and is resistant to several classes of

insecticides (Yared et al., 2020), suggesting that this species could be a challenge to malaria control and elimination efforts in Ethiopia.

1.7. Malaria Vector Control

Appropriate malaria control strategies vary with local malaria endemicity. Countries with stable endemic malaria use strategy of prevention by LLINs and IRS. In addition, intermittent preventive treatment, especially for malaria prevention and treatment in pregnant women, and control strategy and treatment by early and effective case management are also strategies of prevention in endemic countries. Countries with unstable malaria use IRS, LLINs, larviciding, environmental management, and treatment through early and effective case management. Countries with regions considered as free of malaria use control strategies of prevention for travelers going to malarious areas. These include chemoprophylaxis and personal protective measures against mosquitoes and treatment by early and effective management in suspected cases and diagnosis to confirm cases (WHO, 2011, WHO, 2015a).

In SSA, LLINs and IRS remain the frontline interventions for malaria vector control, with larval source management (LSM) considered as a supplementary intervention depending on the target vector and local situation (WHO, 2019a). Several other potential vector tools have also been developed and evaluated in SSA.

1.7.1. Long-lasting insecticidal nets

Insecticide-treated net, particularly the LLIN is one of the key malaria control interventions used against indoor biting malaria vectors. Since 2000, the LLIN coverage has increased tremendously in SSA and resulted in a drastic decline in malaria in the region (UNICEF, 2020). Between 2000 and 2015, the massive scale-up of the LLINs averted more than 450 million malaria cases, accounting for 68% of the total malaria cases averted in SSA as a result of all malaria control interventions (Bhatt et al., 2015). The WHO recommends universal coverage of LLINs for population living in malaria risk areas to achieve the GTS targets of reducing malaria incidence and mortality rates by at least 90% by 2030 compared with the year 2015 (WHO, 2015a, WHO, 2020b). By 2019, 68% of the households in SSA had at least one LLIN, increasing from about 5% in 2000 (WHO, 2020b).

Currently, LLINs are impregnated with pyrethroid insecticides, because of their favourable safety, low cost, and rapid insecticidal activity. However, their effectiveness is threatened by widespread pyrethroid resistance in African malaria vectors (Hemingway et al., 2016). Consequently, the WHO recommends piperonyl butoxide (PBO) LLINs for areas of pyrethroid resistance (WHO, 2017a). The PBO LLINs have been shown to significantly reduce malaria prevalence compared to conventional LLINs (Staedke et al., 2020).

1.7.2. Indoor residual spraying

The use of IRS as malaria vector control intervention was first demonstrated during the World War II when Dichloro-diphenyl-trichloroethane (DDT) was successfully used for killing indoor resting mosquito vectors. In most African countries, DDT and pyrethroid had been commonly used for IRS operation until 2010, resulting in a remarkable decline in vector density and malaria transmission (WHO, 2006, WHO, 2007, Tangena et al., 2020). However, the widespread resistance of mosquito vectors to these insecticides limited their use for IRS (WHO, 2011, Yewhalaw et al., 2011). Consequently, they were partly replaced by carbamates starting from 2011 and by organophosphates since 2013 in many African countries (Tangena et al., 2020).

1.7.3. Larval source management

Larval source management is the management of aquatic habitats that are potential larval habitats for mosquitoes, to prevent the completion of development of the immature stages (Tusting et al., 2013, WHO, 2013b). It includes habitat modification, habitat manipulation, larviciding and biological control using predators (WHO, 2013b). In the early twentieth century when LSM was the only tool available to contain malaria, environmental management and larviciding contributed significantly to malaria vector control in the world (Shousha, 1948, Killeen et al., 2002). Several studies have also shown that LSM is effective in reducing malaria morbidity and mortality in Africa when integrated with LLINs and IRS. In Tanzania for instance, environmental management through cleaning drains significantly reduced both larval density and the risk of malaria infection (Castro et al., 2009). In Kenya, shading habitats with Napier grass reduced the density *An. gambiae s.l.* larvae by over 75% (Wamae et al., 2010). In Ethiopia, increasing water drawdown rates around reservoirs by 10-20 millimeters per day was shown to reduce mosquito larval density by 70%-84% (Kibret et al., 2018, Kibret et al., 2019b).

1.7.4. Other potential vector control tools

Several other tools and approaches for malaria vector control have also been developed and evaluated. These include biological control strategies using either mosquito predators (Kumar and Hwang, 2006, Ohba et al., 2010, Chobu et al., 2015), microbial larvicides (Walker and Lynch, 2007, Derua et al., 2019) or enthomopathogenic fungi (Scholte et al., 2006, Blanford et al., 2005, Litwin et al., 2020), zooprophylaxis (Bulterys et al., 2009, Lyimo et al., 2012, Iwashita et al., 2014), attractive toxic sugar baits (ATSB) (Fiorenzano et al., 2017), spatial repellents (Sibanda et al., 2018, Mapossa et al., 2021), and genetic control approaches based on sterile insect technique (SIT), *Wolbachia* or gene drive technologies (Alphey, 2014).

Among the predators, larvivorous fish belonging to the genus *Gambusia* and *Poecilia* have been demonstrated to be very effective in reducing mosquito larval populations in many parts of the world, and in a variety of habitats (Kumar and Hwang, 2006, Kweka et al., 2011, Ohba et al., 2010, Chobu et al., 2015). However, there is a concern that larvivorous fish could also affect non-target organisms (Rupp, 1996), highlighting the need to carefully consider the ecological cost of introducing predators.

Microbial larvicides such as *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*), which could selectively kill mosquito larvae with negligible effect on non-target organisms, are promising alternatives to use as supplementary vector control intervention (Walker and Lynch, 2007, Derua et al., 2019). Application of *Bti* and *Bs* on natural habitats has been found to be effective in controlling malaria vectors in different African countries such as Kenya (Afrane et al., 2016b, Kahindi et al., 2018), Tanzania (Geissbühler et al., 2009), Ghana (Nartey et al., 2013), Burkina Faso (Dambach et al., 2014), Senegal (Diédhiou et al., 2017), Botswana and Zimbabwe (Mpofu et al., 2016), significantly reducing the density of mosquito larvae and pupae by 60-100%, with residual efficacy of up to 3-5 months in some settings (Derua et al., 2019).

Zooprophylaxis involves the use of animals to divert host-seeking mosquito vectors from humans (WHO, 1982). Studies conducted on the impact of zooprophylaxis on malaria control reached on different conclusions. Several studies reported a positive effect (Fritz et al., 2009, Iwashita et al., 2014, Kaburi et al., 2009, Mahande et al., 2007b); some studies have found no effect (Tirados et al., 2006, Habtewold et al., 2004, Tirados et al., 2011), while others have

reported a negative effect (Deressa et al., 2007, Ghebreyesus et al., 2000), depending on the type of zooprophylaxis. Some studies have shown that zooprophylaxis is only effective when humans are indoors and cattle are kept outdoors (Tirados et al., 2011, Seyoum et al., 2002), while treating animals with insecticides such as ivermectin, deltamethrin and fungus was found to significantly reduce survival rates of malaria vectors in several studies (Fritz et al., 2009, Mahande et al., 2007b, Lyimo et al., 2012). On other hand, keeping cattle indoors in human shelters was shown to increase the risk of malaria infection (Deressa et al., 2007, Ghebreyesus et al., 2000).

The ATSB works based on the "lure and kill" strategy, in which the innate behaviour of mosquitoes to search and feed on sugar sources is exploited (Beier et al., 2018, Fiorenzano et al., 2017). It can be made by mixing low-toxic substances such as boric acid (1%), ivermectin (0.01%) or other insecticides in 10% sugar solution to attract and kill mosquitoes (Maia et al., 2018, Fiorenzano et al., 2017). In Côte d'Ivoire, the use of ATSB in addition to LLINs increased the mortality rates of wild pyrethroid-resistant *An. gambiae* from 19% with LLIN alone to 39% with added ATSB (Furnival-Adams et al., 2020). In Morocco, field studies demonstrated over 70% reduction of mosquito populations after three weeks of ATSB application (Khallaayoune et al., 2013). In Mali, spraying ATSB reduced the abundance of *An. gambiae* by 90% compared to pre-intervention (Müller et al., 2010). In Tanzania, over 95% of *An. arabiensis* were knocked down 48 hours post-sugar feeding on 10% sucrose solutions containing 0.01% ivermectin (Tenywa et al., 2017). This highlights that ATSB could be one of the promising supplementary interventions for controlling malaria transmission in Africa.

1.8. Malaria Elimination Efforts in Africa

1.8.1. Past efforts

A world free of malaria has long been a major goal of the WHO. In 1955, the WHO embarked on the Global Malaria Eradication Programme (GMEP) to achieve this ambitious goal (Nájera et al., 2011). The programme mainly focused on vector control, with DDT-based IRS used in large scale. This intervention, together with other malaria control measures, led to malaria elimination from several countries in Europe, Asia and the Caribbean (WHO, 2006). Although most of the Africa continent was not included in the GMEP because of logistics issues, pilot eradication projects were initiated from 1950s to 1970s in African countries such as Benin, Burkina Faso,

Burundi, Cameroon, Kenya, Liberia, Madagascar, Nigeria, Rwanda, Senegal, Tanzania and Ethiopia (Garrett-Jones and Ferreira Neto, 1964, De Zulueta, 1964, WHO, 2006, NMCT et al., 2014). These projects had resulted a significant reduction of vector density and malaria cases although the transmission could not be interrupted (WHO, 2006).

The GMEP ended in 1969 without achieving the target, and was replaced by a long-term malaria control programme, due to the resistance of *Anopheles* mosquitoes to DDT and consequently dwindled political and financial support for the eradication campaigns (Sadasivaiah et al., 2007). After the failure of the GMEP, the WHO launched another pilot project in Garki district in northern Nigeria in 1969 (1969-1976), in which propoxur was used for IRS together with mass drug administration (Molineaux and Gramiccia, 1980). However, malaria transmission could not be interrupted although parasite positivity rates were reduced to a low level. Finally it was concluded that the Garki project was failed due to exophilic behaviour of mosquito vectors (Molineaux and Gramiccia, 1980).

1.8.2. Recent progress

After almost 60 years, the world once again began to consider the feasibility of eradicating malaria. Significant declines in the global malaria case incidence and mortality rates between 2000 and 2015, and an increasing number of countries certified malaria-free generated renewed enthusiasm to rid the world of malaria. In 2015, the WHO endorsed a bold plan to reduce malaria incidence and mortality rates by 90% and to eliminate the disease from at least 35 countries by 2030 (WHO, 2015a). African countries have also been intensifying the existing malaria control interventions to achieve this goal. Such efforts helped Algeria to eliminate malaria and Cape Verde to reach on zero malaria case status in 2019 (WHO, 2020b). Several other African countries including Ethiopia have also set goals to eliminate the disease by 2030 (WHO, 2020b, FMoH, 2020).

1.9. Challenges of Malaria Control and Elimination

Despite the efforts made in scaling up the control interventions, the progress towards malaria elimination has been hindered in SSA since 2015, with an increase in malaria incidence has been reported in several African countries in the past five years (WHO, 2020b). This could be due to various factors (Guyant et al., 2015, WHO, 2020a, Lubinda et al., 2021). Among the factors,

residual malaria transmission and widespread insecticide resistance in mosquito vectors have been thought as major threats to the vector control and malaria elimination efforts (Carnevale and Manguin, 2021, Hancock et al., 2020).

1.9.1. Residual malaria transmission

Residual transmission comprises all forms of malaria parasite transmission that are beyond the reach of standard ITNs and IRS (Sherrard-Smith et al., 2019) or transmission that persists after universal coverage of LLINs (>80%) and maximal coverage of IRS has been achieved using insecticides to which the local vectors are susceptible (Killeen, 2014a, WHO, 2014a). It includes outdoor transmission, and transmission sustained by early evening and/or early morning biting mosquito vectors. The first concern about the possible existence of residual malaria transmission was emerged in the 1970s, after the failure of malaria elimination programme conducted by WHO in Garki, Nigeria. Indoor residual spraying with propoxur and mass drug administration had been used in full force in the area for several years, but malaria persisted. In hindsight, researchers speculated that the Garki project was doomed by elusive mosquitoes resting outdoors (Molineaux and Gramiccia, 1980, Maxmen, 2011). Since then, outdoor malaria transmissions have been reported from several malaria endemic regions in Africa (Killeen et al., 2006, Maxwell et al., 1998, Okello et al., 2006, Shililu et al., 2004) and elsewhere in the world (Van Bortel et al., 2010, Durnez et al., 2013, Prakash et al., 2005).

Outdoor malaria transmission and transmission due to early biting vectors have been reported in many malaria-endemic settings of Africa regardless of ITN and/or IRS use. For example, in Kpone-on-sea area of Ghana, 78.6% of all infectious mosquito bites occurred outdoors (Tchouassi et al., 2012). In Bioko Island of Equatorial Guinea, an outdoor entomological inoculation rate (EIR) of up to 922 infective bites per person/year (ib/p/year) was recorded while indoor EIR was 652 ib/p/year in the same area (Overgaard et al., 2012a). Similarly, higher outdoor malaria transmission compared to indoor was documented in Taveta district of Kenya, with EIRs of 56.81 and 31.13 ib/p/year for outdoor and indoor collected vectors, respectively (Mwangangi et al., 2013b). In northeastern Tanzania, 12% of malaria transmission occurred in the evening before sleeping time (Maxwell et al., 1998). Such persistent transmission may continue to occur in SSA despite high coverage of LLINs and IRS.

Several factors are thought to uphold residual malaria transmission in Africa. These include behavioural heterogeneity among vector species, pre-existing vector behavioural resilience, insecticide-induced shift in vector species composition and behaviour, increasing role of secondary vectors in residual transmission, and human behaviour. These factors are reviewed in detail in the following sections.

1.9.1.1. Behavioural heterogeneity of malaria vectors

While LLINs and IRS could play a key role in controlling endophagic, night biting and endophilic vectors, there is a pre-existing behavioural heterogeneity among malaria vector species that could attenuate the effectiveness of these vector control tools (Durnez and Coosemans, 2013). In Africa, the three most efficient malaria vector species, *An. gambiae*, *An. coluzzii* and *An. funestus* are endophilic, anthropophagic, endophagic, and bite late at night (Sinka et al., 2010), hence they could be targeted by the existing vector control interventions. In contrast, *An. arabiensis* is more plastic in its behaviour, exhibiting more often exophily, zoophagy, exophagy, and early evening biting tendency (Sinka et al., 2010, Mahande et al., 2007a, Massebo et al., 2013a), behaviours which are beyond the reach of LLINs and IRS (Table 1.1). Like other vector species, *An. arabiensis* can readily feed on humans to sustain intense malaria transmission (Fornadel et al., 2010a), but often enough on animals to evade the effect of LLINs, and to maintain residual transmission (Sherrard-Smith et al., 2019).

Table 1.1. Definition of mosquito behavioural choices

1.	Anthropophagy	The tendency of mosquitoes to prefer feeding on humans
2.	Anthropozoophagy	The tendency of mosquitoes to prefer feeding both on humans and animals
3.	Endophagy	The tendency for mosquitoes to prefer biting indoors
4.	Endophily	The tendency for mosquitoes to prefer resting indoors
5.	Exophagy	The tendency for mosquitoes to prefer biting outdoors
6.	Exophily	The tendency for mosquitoes to prefer resting outdoors
7.	Zoophagy	The tendency for mosquitoes to prefer feeding on animals

However, different factors can influence the behaviour of mosquito vectors. For example, host availability could play an important factor in the final host choice. In Burkina Faso for instance, a double choice experiment showed that 88% of *An. coluzzii* (the then *An. gambiae s.s.* molecular M form) chose human-odour baited trap and only 12 % for cattle odour trap. In contrast, the human blood index of indoor-resting *An. coluzzii* collected in the same locality was only 40% (Lefèvre et al., 2009), showing that *An. coluzzii* population can adapt to feeding on cattle in case of a lower availability of human hosts. In Equatorial Guinea, *An. gambiae* was observed to be partly exophagic and early-biting because of a high bed net use (Reddy et al., 2011). In those cases, the frequency of human-vector contact is lowered although humans are bitten in the evening. Consequently, the longevity and vectorial capacity of these exophagic or zoophilic vectors are slightly or not affected by ITNs and residual malaria transmission continues.

1.9.1.2. Vector behavioural resilience

Behavioural resilience is defined as a pre-existing behaviour of mosquitoes that results in evasion of insecticide contact, rather than resistance which infers the ability to do so (Killeen, 2013, Govella et al., 2013). Several malaria vector species around the world exhibit a pre-existing tendency to exit houses soon after entering (Killeen and Chitnis, 2014, Killeen et al., 2014). For example, in Latin America, vector species such as *An. darlingi*, *An. punctimacula* and *An. nunetzovari* enter houses but then rapidly exit, regardless of whether or not they have successfully fed upon humans (Elliott, 1972). In Africa, the population of *An. arabiensis* is known to be capable of avoiding exposure to fatal doses of insecticide through behavioural evasion, by entering but then rapidly exiting houses containing IRS and LLINs (Kitau et al., 2012, Okumu et al., 2013b, Okumu et al., 2013a).

Mosquito populations which are normally susceptible to control with LLINs or IRS, due to the fact that they usually feed and rest indoors, may also choose to avoid physical contact with insecticides if they can detect them with their sensory organs (Muirhead-Thomson, 1960, Kouznetsov, 1977). Such stimulant insecticides induce early exit behaviour, ultimately attenuating mosquitoes' exposure to lethal doses (Killeen et al., 2011, Killeen and Moore, 2012, Muirhead-Thomson, 1960, Kouznetsov, 1977, Achee et al., 2012). Behaviour-modifying insecticides which require physical contact with mosquitoes to induce avoidance response are

known as contact irritants, while those that mosquitoes can sense in the air at a distance from the treated surface are called spatial repellents (Achee et al., 2012, WHO, 2013a). Vector species may prefer to feed and rest indoors, but the presence of LLINs or IRS with such irritant or repellent insecticides may induce them to leave houses prematurely (Durnez and Coosemans, 2013, Muirhead-Thomson, 1960, Achee et al., 2012, Pates and Curtis, 2005, Killeen, 2014b).

1.9.1.3. Insecticide-induced shift in vector species composition and behaviour

1.9.1.3.1. Species shift

Historically, malaria transmission in many African countries has been dominated by vector species that primarily feed and rest indoors where they can be efficiently targeted with domestic insecticides (Gillies and DeMeillon, 1968, Gillies and Coetzee, 1987). However, there is growing evidence that the widespread use of LLINs and IRS is driving vector species composition towards those species with more flexible behaviours (Table 1.2). For instance, *An. gambiae* has been historically viewed as the most significant vector of malaria in many African countries (Gillies and DeMeillon, 1968, Gillies and Coetzee, 1987, Davidson, 1966, Lindsay et al., 1998). However, following the widespread use of insecticidal interventions, this species is in a significant decline in many areas with the majority of the remaining transmission being dominated by *An. arabiensis* (Braimah et al., 2005, Bayoh et al., 2010, Russell et al., 2011).

In Tanzania for instance, a major shift in *An. gambiae s.l* sibling species composition has taken place in different parts of the country from predominantly *An. gambiae* to predominantly *An. arabiensis* (Russell et al., 2011, Derua et al., 2012, Kitau et al., 2012). In Niger, nation-wide distribution of LLIN caused a marked decrease in *An. funestus* abundance, without effect on *An. gambiae s.l.* (Labbo et al., 2012). Large scale ITN use in different parts of Kenya significantly decreased the proportion of indoor-resting *An. funestus* (Mwangangi et al., 2013a, Lindblade et al., 2006) and *An. gambiae* (Mutuku et al., 2011, Mwangangi et al., 2013a, Lindblade et al., 2006, Bayoh et al., 2010) while the proportion of *An. arabiensis* has increased. Similarly, the implementation of vector control interventions reduced the proportion of *An. gambiae* in Uganda (Musiime et al., 2019), *An. gambiae* and *An. coluzzii* in Senegal (Sougoufara et al., 2016) and *An. quadiannulatus* in Zambia (Chinula et al., 2018), with proportionate increment in *An. arabiensis* population in each country. On the other hand, no evidence of species shift was

observed in Bioko Island of equatorial Guinea (Reddy et al., 2011) and in some parts of Kenya (Mathenge et al., 2001, Futami et al., 2014) regardless of the use of ITNs or IRS.

1.9.1.3.2. Shift to early evening or morning biting

The widespread use of LLINs and IRS has been shown to change the biting cycle of malaria vectors to early evening and/or morning hours in different parts of Africa and elsewhere in the world (Sougoufara et al., 2014, Moiroux et al., 2012, Charlwood and Graves, 1987, Taylor, 1975). In Tanzania, the widespread use of ITNs increased the proportion of early biting *An. gambiae* (Braimah et al., 2005) and *An. funestus* (Russell et al., 2011). In southern Benin, a significant change in host-seeking behaviour of *An. funestus* to early morning was observed after achieving a universal coverage of LLINs (Moiroux et al., 2012). In one of the study sites for example, up to 26% of the *An. funestus* bites were observed after 6:00 am (Moiroux et al., 2012). The use of ITNs resulted in a shift towards earlier biting of *An. gambiae s.l.* in Kenya (Mbogo et al., 1996). In Senegal, six times more *An. funestus* were caught in broad daylight than at night after the implementation of LLINs (Sougoufara et al., 2014).

1.9.1.3.3. Shift to exophagy

Several studies revealed that many of the most regionally important vectors of malaria throughout the world showed a shift in feeding behaviour from endophagic to exophagic after the long-term use of indoor-based vector control interventions (Taylor, 1975, Suwonkerd et al., 1990, Nutsathapana et al., 1986, Lourenço-de-Oliveira et al., 1989, Li et al., 1989, Zhang and Yang, 1996, Molineaux and Gramiccia, 1980, Russell et al., 2011, Reddy et al., 2011, Cano et al., 2004). Similarly, shifts to exophagy have been reported for dominant malaria vector species in Africa (Table 1.2). In Nigeria for example, extensive use of IRS resulted in a threefold increase of the proportion of outdoor biting *An. gambiae s.l.* (Molineaux and Gramiccia, 1980). In Equatorial Guinea, several years of vector control by IRS and LLINs resulted in an increased proportion of outdoor biting *An. gambiae* and *An. melas* (Reddy et al., 2011), as compared to historical data collected in the same region (Cano et al., 2004). Also in Tanzania, high ITN use resulted in an increased proportion of outdoor biting *An. funestus* (Russell et al., 2011). Similarly, increased proportion of outdoor biting *An. funestus* was documented in southern Benin after achieving universal ITN coverage (Moiroux et al., 2012).

1.9.1.3.4. Shift to zoophagy

The widespread use of ITNs and/or IRS has also been shown to alter the host preference of vectors in several malaria endemic countries (Table 1.2). In Kenya, the long-term use of ITN caused a shift in host selection of *An. gambiae s.l.* and *An. funestus* from humans towards cattle or other vertebrate hosts (Bøgh et al., 1998, Mutuku et al., 2011). Similarly, shift to zoophagy was reported for *An. gambiae* in a study conducted in Burkina Faso (Lefèvre et al., 2009). In Tanzania, the proportion of human blood meals taken by *An. arabiensis* was reduced in favour of cattle blood meals following massive distribution of LLINs (Kreppel et al., 2020). However, in some other studies, the use of ITNs and/or IRS caused no shift in host selection (Fornadel et al., 2010a, Magesa et al., 1991), for example, for *An. arabiensis* in Zambia (Fornadel et al., 2010a).

1.9.1.3.5. Shift to exophily

The long-term use of ITN and/or IRS reduced the indoor resting fraction of *An. gambiae s.l* in Benin (Padonou et al., 2012), Niger (Labbo et al., 2012) and Kenya (Mutuku et al., 2011). Similarly, the widespread distribution of ITNs reduced the indoor resting fraction of *An. funestus* in Kenya (Mutuku et al., 2011). In Tanzania, both *An. arabiensis* and *An. funestus* showed a shift towards exophily following massive distribution of LLINs (Kreppel et al., 2020). The outdoor resting fractions of these populations could persist and sustain residual malaria transmission despite a good coverage of ITN and/or IRS.

Table 1.2. Review of the impact of indoor-based vector control interventions on malaria vector species composition and behaviour in Africa

Country	**Vector control intervention**	**Collection methods**	**Species shift**	**Shift to early biting**	**Shift to exophagy**	**Shift to zoophily**	**Reference**
Benin	LLIN	HLC	-	*An. funestus*	*An. funestus*	-	(Moiroux et al., 2012)
Burkina Faso	ITN	PSC	-	-	-	*An. coluzzii*	(Lefèvre et al., 2009)
Equatorial Guinea	LLIN, IRS	HLC	-	-	*An. coluzzii*	-	(Reddy et al., 2011)
Kenya	ITN	PSC, Clay pots	*An. gambiae* to *An. arabiensis*	-	-	-	(Mutuku et al., 2011)
Kenya	ITN	PSC, larvae collection	*An. gambiae* to *An. arabiensis*	-	-	-	(Bayoh et al., 2010)
Kenya	LLIN	HLC, light trap, PSC	*An. gambiae* and *An. funestus* to *An. arabiensis* and *An. merus*	-	-	*An. gambiae s.l.* *An. funestus*	(Mwangangi et al., 2013a)
Kenya	LLIN	PSC, exit trap, Clay pots	-	-	-	*An. gambiae*	
Niger	LLIN	HLC, light trap, PSC	*An. funestus* to *An. gambiae s.l.*		*An. gambiae s.l* *An. funestus*	-	(Labbo et al., 2012)
Senegal	LLIN	HLC, PSC	-	*An. funestus*	-	-	(Sougoufara et al., 2014)
Senegal	LLIN	HLC	*An. coluzzii* and *An. gambiae to An. arabiensis*	-	-	-	(Sougoufara et al., 2016)

Country	Vector control measure	Collection methods	Species shift	Shift to early biting	Shift to exophagy (exophily*)	Shift to zoophily	Reference
Tanzania	ITN	Light trap, bed net trap	-	*An. gambiae s.l.*	-	-	(Braimah et al., 2005)
Tanzania	ITN	HLC	*An. gambiae* to *An. arabiensis*	*An. gambiae s.l* *An. funestus*	*An. gambiae s.l* *An. funestus*		(Russell et al., 2011)
Tanzania	LLIN	Light trap, backpack aspirator	-	-	*An. arabiensis** *An. funestus**	*An. arabiensis*	(Kreppel et al., 2020)
Tanzania	LLIN	Light trap	*An. gambiae* to *An. arabiensis*	-	-	-	(Derua et al., 2012)
Uganda	LLIN, IRS	HLC	*An. gambiae* to *An. arabiensis*	*An. gambiae s.l.*	*An. gambiae s.l.*	-	(Musiime et al., 2019)
Zambia	IRS	HLC, light trap, resting box	*An. quadriannulatus* to *An. arabiensis*	-	-	-	(Chinula et al., 2018)

Note: ITN: insecticide treated net, LLIN: long-lasting insecticidal net, IRS: indoor residual spraying, HLC: human landing catch, PSC: pyrethrum spray catch, *shift to exophily

1.9.1.4. Role of secondary vectors

Most of the secondary malaria vectors are exophagic and exophilic; hence they could play a significant role in residual malaria transmission. (Gillies, 1964, Afrane et al., 2016a). Recent studies have shown that secondary vectors are increasing in number with several mosquito species recently incriminated as vectors (Mustapha et al., 2021, Zhong et al., 2020). Moreover, the vectorial capacity of some of the secondary vector species is increasing, even exceeding that of *An. arabiensis* in some places (Abduselam et al., 2016, Goupeyou-Youmsi et al., 2020). In Maevatanana district of Madagascar for instance, *An. coustani* played a major role in malaria transmission, causing 61.2 ib/p/six months while the primary vector *An. arabiensis* caused only 36 ib/p/six month in the same district (Goupeyou-Youmsi et al., 2020).

1.9.1.5. Human behaviour

The effectiveness of malaria vector control interventions depends not only on vector behaviour but also on human behaviour and sleeping patterns. Many people in Africa usually engage in activities that keep them away from ITN protection at peak vector biting times (Monroe et al., 2019b), increasing the risk of residual malaria transmission. Outdoor sleeping is a habitual practice during special events such as weddings, funerals, and religious and cultural rituals in some African countries, and these could expose people to infectious mosquito bites (Monroe et al., 2019a, Moshi et al., 2018, Monroe et al., 2015). In northwest Ethiopia for instance, a study showed that people who slept in outdoor sites were 2.76 times more likely to be infected with malaria as compared to people sleeping indoors (Aschale et al., 2018).

Human sleeping habit is likely to change according to season and geographical locations, with more people staying outside for a longer period of time when the nights are hot and in areas where houses are uncomfortably warm (Sherrard-Smith et al., 2019). In such circumstances, the risk of exposure to infectious mosquito bites could increase and hence malaria transmission continues to occur despite high coverage of indoor-based vector control interventions.

1.9.2. Insecticide resistance

According to the WHO, insecticide resistance is defined as the ability of mosquitoes to tolerate exposure to a standard dose of insecticide, which would prove lethal to the majority of individuals in a normal population of the same mosquito species (WHO, 2016). The extensive use of DDT for the control of agricultural pests and disease vectors led to the first emergence of

resistance in malaria vectors in Greece in 1951 (Livadas and Georgopoulos, 1953). In 1960s, DDT resistance in *An. gambiae* was reported in different African countries, and detrimentally affected malaria eradication plan (Webb Jr, 2014). Since 1990s, resistance of major African malaria vectors to pyrethroids, the only class of insecticides currently used for LLINs, has also been reported in many African countries (Chandre et al., 1999, Hancock et al., 2020), and becomes one of the major challenges of malaria control and elimination efforts in SSA.

At present, insecticide resistance among major African malaria vectors was reported in all of the four classes of insecticides recommended for use by the WHO (organochlorines, pyrethroids, carbamates and organophosphates), although the distribution and intensity of the resistance vary between different countries (Yewhalaw and Kweka, 2016, Ondeto et al., 2017, Alemayehu et al., 2017, Messenger et al., 2017).

1.10. Malaria Vector Surveillance in Africa

Surveillance of malaria vectors is a prerequisite to determine various entomological indices (entomological indicators of malaria transmission) including vector density, biting behaviour, feeding behaviour (blood meal indices and host preference), resting behaviour, human biting rate (HBR), sporozoite rate and EIR for surveillance driven control and to evaluate the impact of the control interventions. The success of the control interventions is often measured in terms of reduction in malaria transmission intensity (Lines et al., 1991). Malaria transmission intensity is measured by EIR, the number of infective mosquito bites received by a person per unit time, and is calculated by multiplying HBR by sporozoite rates. The magnitude of the HBR in turn depends on vector density and blood-meal indices, as well as on human behaviour (Burkot et al., 2018, WHO, 2013c, Monroe et al., 2020).

Sampling vector populations is a cornerstone in malaria surveillance system. The sampling tools and procedures usually differ depending on the type of entomological indices to be measured (Service, 1977). The vector species may occur as indoor-host seeking, outdoor host-seeking, indoor resting and outdoor resting fractions, each requiring different sampling tools and techniques (WHO, 1975). Various tools and techniques have been developed for sampling malaria vectors. These tools and techniques together with their advantages and limitations are reviewed in the following sections.

1.10.1. Techniques of sampling indoor and outdoor resting malaria vectors

Sampling indoor and outdoor resting mosquito vectors is a prerequisite to obtain information about the usual resting places of mosquito vectors, and to determine the effect of control interventions on indoor and outdoor resting vector density and species composition (WHO, 2013c). Moreover, samples of both indoor and outdoor resting vector collections are important to determine vector feeding behaviour and host preference through blood meal analysis by estimating HBI and blood meal indices of other vertebrate hosts (Garrett-Jones, 1964).

1.10.1.1. Hand collection

Hand collection involves the use of mouth aspirators or sucking tubes to sample mosquitoes resting inside houses and/or from their natural outdoor resting sites such as vegetation, cracks on stone walls, holes in rocks, and crevices in the ground. Hand collection provides information about usual resting places, resting density, and seasonal changes in vector density. It also provides live specimens for susceptibility and bioassay testing (WHO, 2013c). The limitation of the hand collection method is that it is time-consuming and unlikely to capture all resting mosquitoes, hence it is not an appropriate method to use for routine monitoring of vector density as it may not indicate the actual mosquito density (WHO, 2013c, Service, 1977). Furthermore, mosquito collection using mouth aspirators requires methodical and attentive work that is highly dependent on individual skill and motivation (Douglas, 1984).

1.10.1.2. Electronic aspirators

In efforts to reduce the level of skill needed to use mouth aspirators, several battery-powered aspirators have been developed and evaluated (Husbands and Holten, 1967, Meek et al., 1985, Nelson and Chamberlain, 1955). The most commonly used electronic mosquito aspirators are backpack aspirator, developed in 1990's by CDC (Clark et al., 1994), and a prokopack aspirator devised by Vazquez-Prokopec *et al*. in 2009 (Vazquez-Prokopec et al., 2009). The aspirators can be used for both indoor and outdoor resting mosquito collections (Maia et al., 2011). In Tanzania, the prokopack aspirator was found to be more efficient than mouth aspirator, yielding about 1.5 times more mosquito density compared to the manual aspirator (Charlwood et al., 2018). According to another study done in southern Tanzania, prokopack and backpack

aspirators showed a similar performance, although the prokopack aspirator showed a better consistency when used by different collectors (Maia et al., 2011).

However, both aspirators do have limitations. Backpack aspirator is relatively heavy (weighs up to 12 Kg), hence it may not be suitable to use for routine vector surveillance (Maia et al., 2011). Both backpack and prokopack aspirators rely on batteries, and hence it may not be feasible to use them in rural African settings where there is no access to electricity for charging the batteries (Maia et al., 2011, Vazquez-Prokopec et al., 2009).

1.10.1.3. Pyrethrum spray catch (PSC)

Pyrethrum spray catch involves using a pyrethrin space spray to knock down mosquitoes resting inside a house and collecting them on white sheets spread on the floor and other flat surfaces in the house (WHO, 2013c). It is considered the gold standard method for monitoring indoor resting vector density. Moreover, PSC is an ideal method to obtain engorged mosquitoes for monitoring vector feeding behaviour through blood meal analysis (Githeko et al., 1994b, Ndenga et al., 2016, Animut et al., 2013, Massebo et al., 2013a). Furthermore, PSC can be used for indirect estimation of HBR (WHO, 2013c).

However, since PSC is used only for indoor resting mosquito collection, it misses mosquito vectors that leave houses immediately after feeding due to the excito-repellent effect of LLINs and IRS (Muirhead-Thomson, 1960). Moreover, PSC is less sensitive in settings where mosquito populations are exophagic and exophilic (Mahande et al., 2007a). Thus, it may result in a false impression of the effectiveness of vector control measures by underestimating vector density when vector populations are exophagic and exophilic. In Guinea-Bissau for instance, reliance on indoor resting collection alone in vector surveillance concealed the presence of *An. arabiensis* population in the country (Gordicho et al., 2014). This suggests the need to complement PSC with another tool that could trap a fraction of vectors that rest outdoors in order to have a good estimation of resting vector density, blood meal indices and HBR.

1.10.1.4. Exit traps

Malaria vector species such as *An. arabiensis* exhibit a tendency to enter houses at night to bite and then leave the houses soon after feeding without resting indoors (Mboera, 2005, Pates and

Curtis, 2005, Fornadel and Norris, 2008, Tirados et al., 2006, Killeen et al., 2016). This fraction of mosquito vectors, together with those that do rest indoors but eventually leave houses to lay eggs, can be monitored by using exit traps placed over windows (WHO, 1995). Mosquitoes are trapped by window exit traps as they leave houses, thus allowing vector density to be monitored. Data from exit traps provide information about exophilic versus endophilic resting behaviour and physiological status of the mosquito population. The exit trap may also be used to test the behavioural avoidance responses of malaria vectors to different insecticides sprayed on the wall of houses or used to impregnate bed nets (Lindsay et al., 1991, Quinones et al., 1997).

Exit trap has been reported to be useful for monitoring malaria vector density trends in some African countries such as South Africa (Mouatcho et al., 2007), Equatorial Guinea (Sharp et al., 2007, Ridl et al., 2008), Kenya (Wong et al., 2013) and Ethiopia (Abraham et al., 2017). However, its trapping efficiency is likely affected by variations in house designs. The trap showed poor sensitivity in African settings where most houses had open eaves and without ceilings (WHO, 1995, Govella et al., 2011, Sikaala et al., 2013).

1.10.1.5. Pit shelters

Traditionally, mechanical aspiration of mosquitoes from artificial pit shelters has been used as a method for sampling outdoor resting malaria vectors (WHO, 1995, Service, 1977). Pit shelters have the advantage of providing concentrated places for collections and representative samples that can be used for quantitative work (WHO, 2013c). Pit shelter was proved to be an effective method of sampling outdoor resting population of *An. arabiensis* for blood meal analysis in different parts of Ethiopia (Tirados et al., 2006, Massebo et al., 2015, Ameneshewa, 1996), Eritrea (Shililu et al., 2004) and Tanzania (Ijumba et al., 2002).

However, sampling inside pit shelters is difficult to standardize. It is also difficult to maintain pit shelters, especially during the rainy season as the pits could be filled with water. Moreover, dangerous animals such as snakes may also be encountered in the pits, causing a risk to mosquito collectors.

1.10.1.6. Resting boxes

Resting boxes have also been used to sample mosquitoes since it was first observed that mosquitoes tend to congregate in dark, sheltered resting places (Crans, 1989). It was assumed that resting boxes could provide unbiased samples of endophilic and exophilic mosquito populations when the traps are placed both indoors and outdoors (Menon and Rajagopalan, 1977). However, the number of adults resting in the resting boxes depends on the availability of alternative resting sites (Service, 1977), hence the resting boxes may not be as productive as the traditional outdoor trapping method. A study conducted in Burkina Faso showed that resting boxes yielded a positive correlation with pit shelters in sampling *An. gambiae s.l.* However, the daily performance of the resting boxes was five times lower in terms of mosquito density per trap (Pombi et al., 2014).

1.10.1.7. Clay pots

Clay pots have also been developed for outdoor resting mosquito collection (Odiere et al., 2007). In western Kenya, clay pots were successfully used to collect outdoor resting female and male *An. arabiensis* and *An. gambiae* (Odiere et al., 2007, Machani et al., 2020). The advantage of the clay pots is that they are small and portable so that they could be deployed in large numbers and in different settings. However, retrieving mosquitoes resting within the pots needs active aspiration by collectors (Odiere et al., 2007), which may lead to collection bias due to variation in skill among collectors. Moreover, mosquitoes could escape at any time before collection when the pots are disturbed by animals or children playing in the area.

1.10.2. Techniques of sampling indoor and outdoor host-seeking malaria vectors

1.10.2.1. Human landing catch (HLC)

The HLC consists of a volunteer person (male) exposing his legs and collecting mosquitoes with an aspirator when they land on his legs (Service, 1977, Mboera, 2005). This is the most direct method available for estimating human exposure to mosquito bites and obtaining samples of host-seeking, human-biting mosquitoes (Lines et al., 1991, Service, 1993a, Davis et al., 1995, Mboera, 2005), and is therefore accepted as a gold standard method (Service, 1977, Service, 1993a). Since mosquitoes are caught in the act of biting human host (Lines et al., 1991, Service, 1977, Davis et al., 1995, Mboera, 2005), the number of mosquitoes caught can be considered to

reasonably represent the human biting rate, and the sample of mosquitoes obtained to have the same distribution of age, physiological status and infection status as those to which attack people at that time and place. Moreover, HLC can be performed both inside and outside houses, and therefore provides important information on when and where humans are exposed to vector bites, as well as the degree of exophagy of vector populations. Such information on the indoor and outdoor biting pattern of mosquitoes have major implication for malaria epidemiology, both in terms of host-vector contact and the choice of effective vector control strategy (Pates and Curtis, 2005).

Nonetheless, HLCs do have major drawbacks. It is arduous, uncomfortable and labour intensive technique, requiring such intense supervision that it is difficult to sustain on large scale. Close supervision is required because the collector needs not only to remain awake but also constantly vigilant for the data to be reliable (Service, 1977, Mboera, 2005). Moreover, there may be substantial differences between biting rates experienced by different collectors due to variation in individual attractiveness (Lindsay et al., 1993) and skill in catching mosquitoes (Service, 1977, Mboera, 2005, WHO, 1995). A greater concern arises from the fact that it increases the risk of exposure of participants to mosquito-borne infections (Mboera, 2005, Service, 1977).

1.10.2.2. CDC miniature light trap

In an attempt to search for an alternative trap to HLC, different designs of light traps were developed and their reliability in estimating EIR has been evaluated under different settings (Sudia and Chamberlain, 1962, Service, 1970). Of the various designs, CDC miniature light traps are the most commonly used alternative method for sampling host-seeking African malaria vectors (Sudia and Chamberlain, 1962). The traps are battery-powered with a fan, light bulb, and a mosquito collection cup. Mosquitoes attracted to the traps, by host odour and light, are drawn in at the top and forced downward by the fan into the collection cup, from which they cannot escape. In the first evaluation, it was noted the trapping efficiency of the CDC light traps increased when the traps were placed close to hosts (Odetoyinbo, 1969), and subsequent experiments proved that its sampling efficiency has improved dramatically by setting the trap beside human hosts protected by bed net (Garrett-Jones et al., 1975, Magbity et al., 2002). Since then, the CDC light traps have been used by setting indoors beside human occupied bed nets as a successful standard practice for monitoring vector density, and for estimating HBR, sporozoite

rate and EIR (Lines et al., 1991, Davis et al., 1995, Githeko et al., 1994a, Mathenge et al., 2004, Mbogo et al., 1993).

Several researchers have evaluated the trapping efficiency of the CDC light traps against the gold standard HLC to find a conversion factor that may be used to infer HBR from the number of mosquito vectors caught by the light traps, but they reached on different conclusions. In many studies conducted in different African countries, the CDC light traps yielded significantly lower vector density compared to HLC (Lines et al., 1991, Govella et al., 2011, Githeko et al., 1994a, Kenea et al., 2017, Le Goff et al., 1993), but positive correlations were reported between the two traps in most of the studies (Lines et al., 1991, Githeko et al., 1994a, Kenea et al., 2017). In other studies, the light traps captured significantly higher vector density (Davis et al., 1995, Mathenge et al., 2004, Fornadel et al., 2010b, Costantini et al., 1998) (Table 1.3).

However, CDC light traps have also several limitations. The conversion factors that have been suggested for CDC light traps versus HLC vary between different countries and even within a country in different geographical locations (Table 1.3), thus there is no well-established consensus on which conversion factor to use for estimation of the HBR from mosquito collections by CDC light traps. In some studies, the trapping efficiency of CDC light trap was found to be density-dependent, and its trapping efficiency was shown to be affected by seasonal variation and trap position (Le Goff et al., 1993, Overgaard et al., 2012b, Mbogo et al., 1993, Mboera et al., 1998, Service, 1993a). Moreover, some studies have documented higher sporozoite rates for mosquitoes captured by CDC light traps as compared to that of HLC (Mbogo et al., 1993, Mboera, 2005), which may lead to an overestimation of EIR. Furthermore, CDC light traps have been reported to be less effective and unreliable for sampling outdoor host-seeking malaria vector populations in most studies conducted in Africa (Service, 1993a, Overgaard et al., 2012b, Kenea et al., 2017, Costantini et al., 1998).

Table 1.3. Review of the the comparison of CDC light traps and human landing catches in sampling indoor host-seeking African malaria vectors

Country	Mosquito species	Relative Ratio[#] (LT vs. HLC)	Conversion Factor*	Correlation Coefficient	References
Ethiopia	*An. arabiensis*	0.35	2.86	0.31	(Kenea et al., 2017)
Burkina Faso	*An. gambiae s.l.*	1.08	0.93	0.62	(Costantini et al., 1998)
Cameroon	*An. gambiae*	0.54	1.85	NA	(Le Goff et al., 1993)
Kenya	*An. arabiensis*	0.60	1.67	0.75	(Githeko et al., 1994a)
Kenya	*An. gambiae s.l.*	1.86	0.54	0.73	(Mathenge et al., 2004)
Kenya	*An. gambiae s.l.*	1.18	0.85	NA	(Wong et al., 2013)
Kenya	*An. funestus*	0.56	1.79	0.49	(Githeko et al., 1994a)
Kenya	*An. funestus*	1.91	0.52	0.20	(Mathenge et al., 2004)
Kenya	*An. funestus*	0.69	1.45	NA	(Wong et al., 2013)
Tanzania	*An. gambiae s.l.*	0.67	1.5	NA	(Lines et al., 1991)
Tanzania	*An. gambiae s.l.*	1.18	0.85	NA	(Davis et al., 1995)
Tanzania	*An. gambiae s.l.*	0.052	19.2	NA	(Govella et al., 2011)
Tanzania	*An. gambiae s.l.*	0.33	3.0	NA	(Okumu et al., 2008)
Tanzania	*An. funestus*	0.67	1.5	NA	(Lines et al., 1991)
Tanzania	*An. funestus*	1.32	0.76	NA	(Davis et al., 1995)
Tanzania	*An. funestus*	0.82	1.22	NA	(Okumu et al., 2008)
Zambia	*An. arabiensis*	1.91	0.52	0.51	(Fornadel et al., 2010b)
Zambia	*An. funestus*	1.53	0.65	NA	(Sikaala et al., 2013)

Note: [#]Relative catch ratio of CDC light traps (LT) to human landing catches (HLC), *indicates the estimated multiplication factor for estimation of HBR if CDC light trap is to be used, NA: not available i.e. correlation coefficient was either not determined or the exact number was not mentioned in the literatures

1.10.2.3. Human or animal baited traps

Another approach for sampling outdoor host-seeking malaria vectors is the use of host bait to attract and catch mosquitoes. This involves enclosing human or animal bait in nets, cages or traps which permit the entrance of mosquitoes but prevent their escape (Service, 1993b). There are different types and designs of host-baited traps. The most common are human-baited bed net trap, Mbita trap and Tent traps.

In human-baited bed net traps, the usual procedure involves a man sleeping under a mosquito net that is either raised a few centimetres from the ground or has one or two panels rolled back or horizontal slits to provide an entrance for host-seeking mosquitoes (Service, 1977). The person acting as bait can be enclosed within a fully protective inner net to prevent him from being bitten. Mosquitoes trapped within the net can be collected either by the person acting as bait or by another person at intervals throughout the night. Human-baited double net traps have been shown to have good trapping efficiency when compared to HLC in Asia (Tangena et al., 2015, Gao et al., 2018). In Lao PDR for instance, a human-baited double net trap collected a similar number of *Anopheles* mosquitoes as an outdoor HLC (Tangena et al., 2015). However, they have been found to be insensitive for sampling malaria vectors in some settings in Africa (Service, 1977, Le Goff et al., 1997).

While human-baited double net traps were initially designed as a safer alternative to outdoor HLC, they also had major drawbacks. In some studies, two persons were used to conduct a double net trap i.e. one individual acting as a bait and the other as a collector (Gao et al., 2018), and such procedure is almost as labour intensive as conducting HLC. In another circumstance when one person is used both as bait and collector (Tangena et al., 2015), there might be a possibility of exposure to infectious mosquito bites during the collection process. This suggests the need to further modify its design to use it as a routine surveillance tool for outdoor host-seeking malaria vectors.

The Mbita trap was conceived primarily for sampling unfed host-seeking mosquitoes, based on the host-seeking behaviour of mosquitoes around human-occupied bed nets. It is conically shaped, resembling a bed net made of cotton cloth with its circular upper part consisting of a netting funnel with a small inner aperture kept open by a small metal ring. These structural features allow the entrance of mosquitoes but limit their exit (Mathenge et al., 2002). The Mbita trap does not expose volunteers to mosquito bites, allows them to sleep throughout the sampling period, and requires neither skilled personnel nor electrical power (Mathenge et al., 2002). Initial evaluation of the Mbita trap in Kenya showed the trap to be relatively sensitive and provided catches which were proportional to those by HLC (Mathenge et al., 2002, Mathenge et al., 2004). However, other studies have reported very poor performance for this trap (Mathenge et al., 2005, Laganier et al., 2003, Braimah et al., 2005, Okumu et al., 2008). In Madagascar for instance, the

Mbita trap yielded a mean mosquito density of 1.0 per trap-night while HLC collected on average 15.4 mosquitoes per person-night in the same villages (Laganier et al., 2003). In western Kenya, the Mbita trap caught about half of the number of *An. gambiae s.l.* caught in the HLC (Mathenge et al., 2005).

Several designs of human-baited tent traps have also been developed and evaluated for outdoor biting malaria vector surveillance. These include Ifakara tent traps (Govella et al., 2009, Govella et al., 2011) and Furvela tent trap (Charlwood et al., 2017). Although tent traps have been shown to possess a potential for monitoring Afrotropical malaria vectors, they do have their own limitations. The use of Ifakara tent traps may raise ethical concerns due the risk operators' exosure to mosquito bites during the collection process (Govella et al., 2009). Moreover, there is uncertainty about wether the tent traps best reflect indoor biting or outdoor biting mosquito densities (Govella et al., 2011).

1.10.2.4. Odour-baited traps

Host odours play a major role in attracting host-seeking mosquitoes (Takken and Knols, 1999). Carbon dioxide (CO_2), human sweat and skin residues such as ammonia and L-lactic acid are known to attract host-seeking malaria vectors (Healy and Copland, 2000, Takken and Knols, 1999), and hence can be used as a strategy to attract and sample mosquito vectors. Several designs of traps such as mosquito magnet-x (MM-X) trap (Schmied et al., 2008, Njiru et al., 2006), BG-Sentinel (BGS) trap (Kröckel et al., 2006, Batista et al., 2017), BG-Malaria (BGM) trap (Batista et al., 2017), Suna trap (Hiscox et al., 2014, Mburu et al., 2019), host decoy trap (Abong'o et al., 2018, Hawkes et al., 2017) and odour-baited entry trap (Costantini et al., 1993, Duchemin et al., 2001) have been developed for sampling outdoor host-seeking mosquitoes by incorporating such chemical attractants.

The MM-X trap uses different attractants, carbon dioxide and counterflow technology to capture mosquitoes (Kline, 1999). The trap has the potential to attract and catch outdoor host-seeking African malaria vectors (Njiru et al., 2006, Schmied et al., 2008). However, it was not evaluated and optimized against the gold standard HLC in African settings. Outside Africa, the MM-X trap caught a significantly lower number of *Anopheles* mosquitoes compared to the HLC (Jeyaprakasam et al., 2021).

The BGS (BioGents HmGb, Regensburg, Germany) is a simple suction trap that uses upward-directed air currents and visual cues to attract mosquitoes. It has a dispenser system, BG-Lure, which releases artificial human skin odours (Kröckel et al., 2006). The BGM trap is a modification of BGS trap, hung upside down at 40cm above the ground, and has an electrical fan which produces an upward suction that captures mosquitoes approaching the trap (Batista et al., 2017). A study done in Tanzania showed that both BGM and BGS traps caught significantly lower number of *An. gambiae s.l.* than HLC, but the BGM yielded a higher density of *An. funestus* compared to HLC (Batista et al., 2017).

The Suna trap is an odour-baited trap which has been developed for sampling host-seeking mosquitoes both indoors and outdoors (Hiscox et al., 2014). To attract mosquitoes, the trap uses a synthetic blend of chemicals found on human skin (Mukabana et al., 2012) and CO_2 produced through a process of yeast and molasses fermentation (Mweresa et al., 2014). In Malawi, the Suna trap caught a similar number of *Anopheles* mosquitoes as the HLC both indoors and outdoors (Mburu et al., 2019). The trap does not require human labour once set in the evening as it can collect mosquitoes throughout the night until morning (Hiscox et al., 2014). However, the Suna trap yielded lower mosquito density compared to the HLC in another study (Verhulst et al., 2015), suggesting the need to evaluate the trap in different African settings and standardize to use it for routine malaria vector surveillance.

1.10.2.5. Mosquito electrocuting trap

Mosquito electrocuting trap (MET) is a recently developed tool for sampling host-seeking malaria vectors (Maliti et al., 2015). It consists of four $30\ \text{cm} \times 30\ \text{cm}$ grid panels made of wooden frames that can be assembled into a square trapping box with the bottom and top open (Maliti et al., 2015). Stainless steel wires are embedded to run from the top to bottom of each frame at a spacing of 5 mm. A volunteer person sits on a stool with his lower legs positioned inside the trapping box. Adjacent embedded wires are differentially charged as negative or positive, such that mosquitoes approaching human bait will be shocked on contact with both wires. Knockdown mosquitoes due to the electric shock can easily be collected afterwards (Maliti et al., 2015, Meza et al., 2019, Sanou et al., 2019).

The MET is an exposure-free and promising alternative tool to HLC for surveillance of outdoor host-seeking malaria vectors (Maliti et al., 2015). The trap has shown a positive correlation with the gold standard HLC (Sanou et al., 2019). However, it yielded significantly lower mosquito density compared to the HLC in some settings (Sanou et al., 2019). Moreover, MET use may raise ethical concerns due to possible risk of human contact with the electric grid (Maliti et al., 2015).

1.11. Rationale of the Study

Despite high LLINs and IRS coverage, malaria incidence in many African sites is resurging following a short-time reduction, and disease transmission is persisting in most African countries despite the scale-up of vector control interventions (Weiss et al., 2019). Such persistent malaria transmission has been thought to occur primarily due to outdoor malaria transmission and widespread insecticide resistance in mosquito vectors because the present first-line malaria vector control measures do not target outdoor biting and outdoor resting vectors. This shows the need to regularly monitor vector behaviour and outdoor malaria transmission to evaluate the likely success of the current vector control interventions, and to design complementary control strategies based on the local vector behaviour. However, quantifying the magnitude of outdoor malaria transmission has been difficult due to lack of well standardized and robust tools for outdoor biting and outdoor resting malaria vector surveillance. This study is aimed at developing and validating new/alternative surveillance tools for monitoring outdoor biting and outdoor resting malaria vectors. Moreover, the study generated evidence on vector behaviour and residual malaria transmission dynamics in different eco-epidemiological settings in East Africa.

1.12. Conceptual Framework

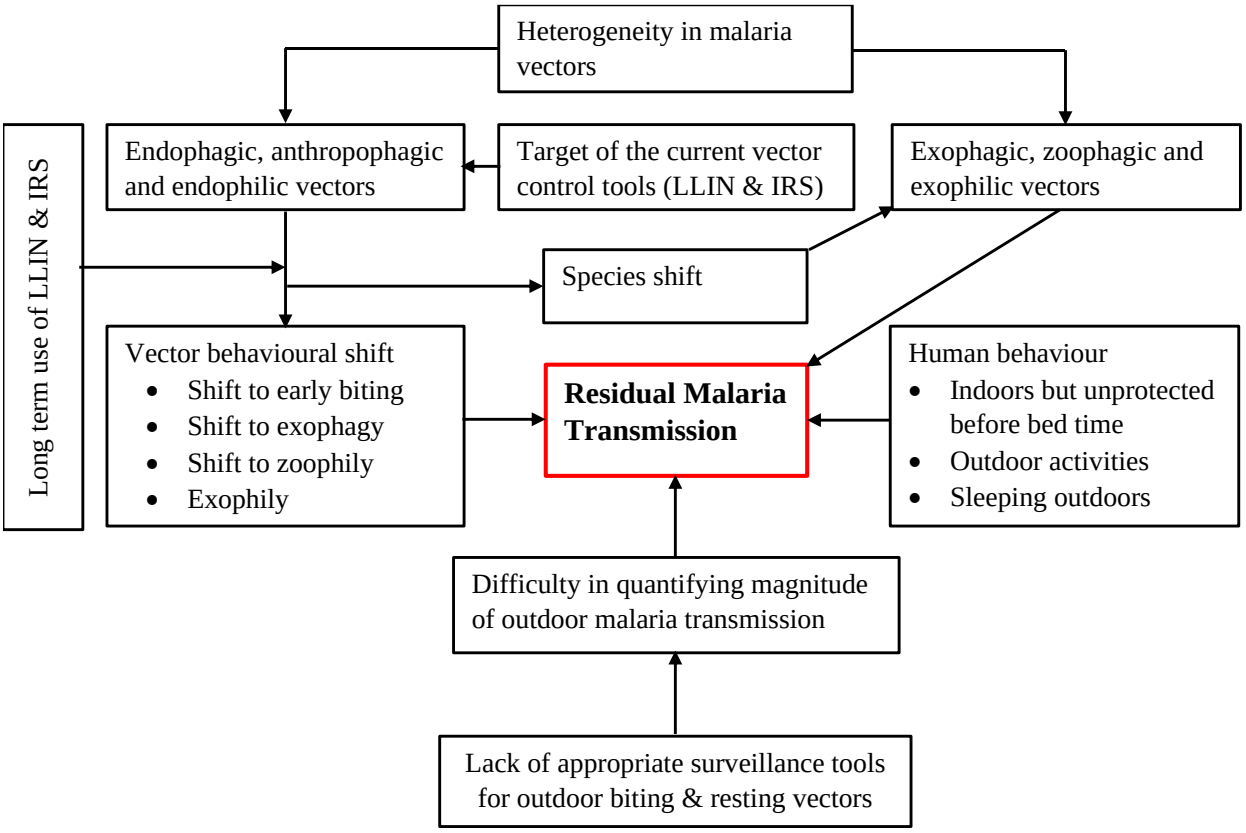

Figure 1.5. Conceptual framework

1.13. Objectives

1.13.1. General objective

To develop and evaluate surveillance tools for outdoor resting and outdoor host-seeking malaria vectors, and to determine vector species composition, abundance, behaviour, patterns of human exposure to vector bites, and residual malaria transmission in Kenya and Ethiopia

1.13.2. Specific objectives

1. To determine species composition, abundance and behaviour of malaria vectors, and their contribution to indoor and outdoor malaria transmission in western Kenya
2. To develop and evaluate a new and alternative trap for outdoor resting malaria vector surveillance
3. To develop and evaluate new and alternative traps for outdoor host-seeking malaria vector surveillance
4. To determine species composition, abundance and behaviour of malaria vectors, patterns of human exposusure to vector bites, and the magnitude of residual malaria transmission in southwestern Ethiopia

CHAPTER TWO

2. GENERAL MATERIALS AND METHODS

2.1. Description of the study area

The study was conducted in two different eco-epidemiological settings of East Africa, western Kenya and southwestern Ethiopia from September 2015 to December 2018 (Figure 2.1).

2.1.1. Western Kenya

The study was done in Ahero (0.13123°S, 34.93960°E, altitude 1,153-1,184 m above sea level, asl) and Iguhu (0.15657°N; 34.74386°E, altitude 1,430–1,580 m asl) sites (Figure 2.1). Ahero is a lowland plain area located in Nyando Sub-County, Kisumu County. Iguhu site is a highland area characterized by undulating hills and valley bottoms located in Ikolomani Sub-County, Kakamega County. Based on the 2019 demographic census of Kenya, the total number of inhabitants in Ahero and Iguhu sites were 9,668 (2,606 households) and 23,766 (5,658 households), respectively (KNBS, 2019). Both sites are bisected by rivers, with Nyando River and River Yala flowing through Ahero and Iguhu sites, respectively. Each site has one government-owned hospital (Ahero Sub-County hospital and Iguhu Sub-County hospital). In both sites, most houses are mud-walled with roofs made of corrugated iron sheets. The inhabitants mainly depend on subsistence farming, with rice and maize being the main cultivated crops in Ahero and Iguhu sites, respectively. The sites have a bimodal pattern of rainfall, with the long rainy season from April to June, which triggers the peak malaria transmission and the short rains from October to November with minor transmission (Munyekenye et al., 2005). *Plasmodium falciparum* is the predominant malaria parasite species in the area and is transmitted by *An. gambiae, An. arabiensis* and *An. funestus* (Zhou et al., 2011, Githeko et al., 2006, Ototo et al., 2015).

2.1.2. Southwestern Ethiopia

The study was carried out in Bulbul *kebele* (7.70285°N; 37.09592°E, altitude 1,694-1,724 m asl), which is located in Kersa district, Oromia Region at about 320 km southwest of Addis Ababa. Bulbul *kebele* is bisected by Gilgel-Gibe River, a major tributary of the larger Gibe River in southwest Ethiopia. Bulbul had about 1,251 households with about 6,003 inhabitants (data from the *Kebele* administration office, 2018). Most of the residents were resettled in this area in 2001

as their original residential area, Tiro Afeta, was submerged by Gilgel-Gibe I hydroelectric dam. The majority of the houses were mud-walled with roofs made of corrugated iron sheets. The inhabitants mostly rely on subsistence farming, with Maize and *Teff* being the main cultivated crops. Bulbul *kebele* had one health center and one health post. As in most parts of Ethiopia, malaria transmission is seasonal in Bulbul area. The transmission peaks from September to October, following the major rains from June to September. Minor transmission occurs in April and May, following the short rains of February to March. *Plasmodium falciparum* and *P. vivax* are the two predominant malaria parasite species in the area and are transmitted by *An. arabiensis* (Yewhalaw et al., 2009).

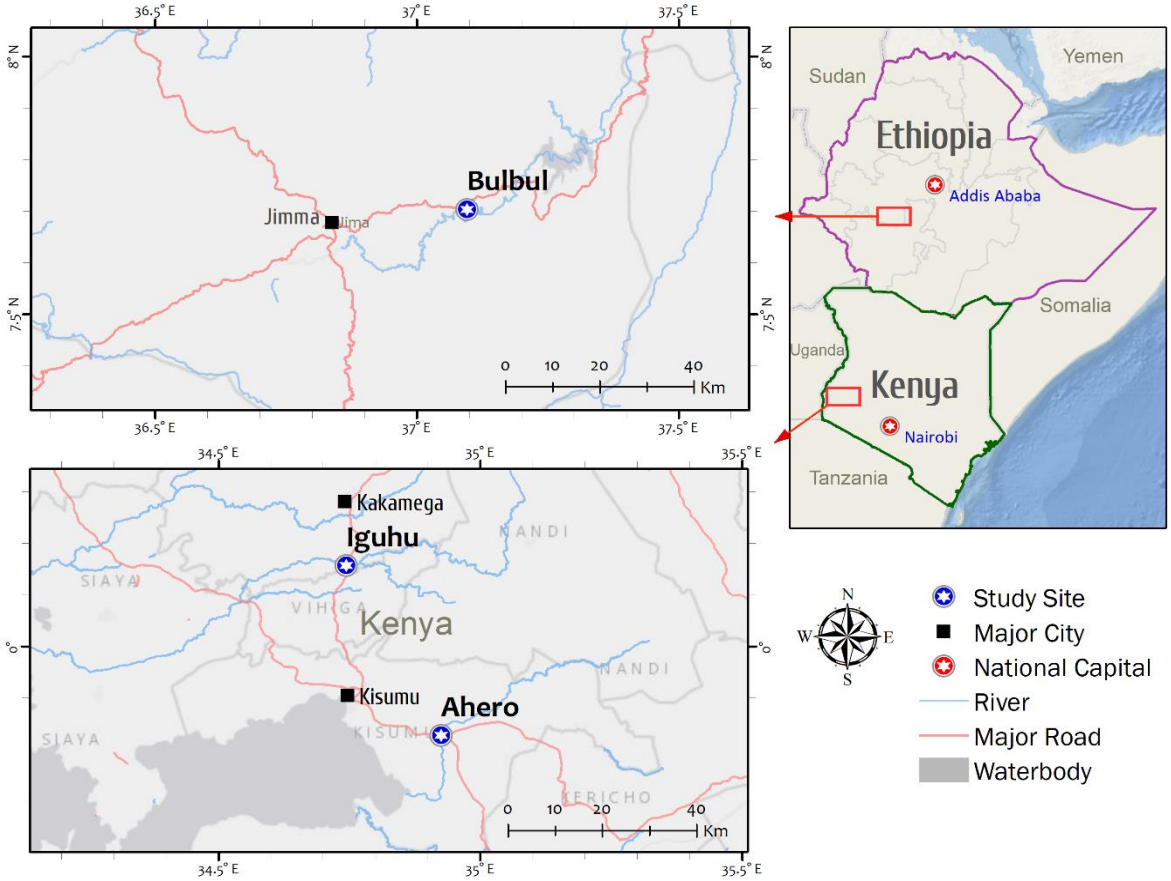

Figure 2.1 Map of the study sites in Ethiopia & Kenya

2.2. Vector surveillance in Kenya

2.2.1. Mosquito collections

Adult *Anopheles* mosquito collections were carried out monthly in Ahero and Iguhu sites during the short rainy season (September to November) in 2015 and dry season (February to April) in 2016 using CDC light traps (indoor and outdoor), PSCs (indoor) and pit shelters (outdoor). Details of the trapping procedures are described in Chapter 3. Along with the mosquito collections, data on ITN ownership and utilization by the households, and the numbers of potential hosts available in the study area including human, bovine, goat, dog and chicken were collected using a questionnaire.

2.3. Development and evaluation of a new trap for outdoor resting malaria vector surveillance

We developed a new durable, safe and affordable trap, hereafter called sticky pot**,** for outdoor resting malaria vector surveillance (Chapter 4). The sticky pots are sticky variants of clay pots that have been used previously to collect outdoor resting *Anopheles* mosquitoes (Odiere et al., 2007). In a sticky pot, the internal surface of the clay pot is covered with waterproof black papers coated with Tangle-Trap sticky substance. The addition of this sticky substance allows for mosquitoes that rest within the pot to be continually trapped for surveillance, rather than only observing the fraction of mosquitoes that happen to be resting at the time of collection in a standard clay pot. Sticky pots were made using locally available clay pots, so they are low cost.

The performance of the sticky pots was evaluated in western Kenya from September 2015 to April 2016 by comparing with pit shelters, clay pots, window exit traps, prokopack aspirator and CDC light traps. Description of each trapping method, experimental design used for comparing the traps, and procedures of mosquito collection are described in detail in Chapter 4.

2.4. Development and evaluation of traps for outdoor host-seeking malaria vector surveillance

We developed two novel, exposure-free traps, hereafter referred to as human-odour-baited CDC light trap (HBLT) and human-baited double net trap (HDNT) for outdoor host-seeking malaria vector surveillance. The HBLT consists of a CDC light trap baited with human-odour pumped

from an ordinary sleeping room (Chapter 5, Plate 5.1a). Human-odour is pumped from the sleeping room to outdoor mosquito catching using a polyvinyl chloride (PVC) pipe. Mosquitoes attracted to the human-odour are collected by setting a CDC light trap (John W. Hock Ltd, Gainesville, FL., USA) near the outer end of the pipe.

The HDNT is a variant of previously designed double net trap (Tangena et al., 2015), with an integrated CDC light trap (Chapter 5, Plate 5.1b). It consists of two box nets (inner and outer nets) with a roof made of canvas. The inner net fully protects a human volunteer who rests on a mattress. The outer net is hung over the inner net and raised 30 cm off the ground. Mosquitoes attracted to the human-bait are collected by setting a CDC light trap between the two nets.

The performance of the HBLT and HDNT in sampling outdoor host-seeking African malaria vectors was evaluated in western Kenya from November 2015 to July 2017 and in southwestern Ethiopia from January to December 2018. Three consecutive experiments, each based on Latin square design, were conducted during the evaluation of these traps. A detailed description of each trapping method and the experimental designs are presented in Chapter 5.

2.5. Vector surveillance in Ethiopia

2.5.1. Mosquito collections

Adult *Anopheles* mosquito collections were carried out monthly in Bulbul site from January to December 2018 using HLC, CDC light trap, HBLT, HDNT and PSC. Mosquito collections using HLC and CDC light trap were carried out both indoors and outdoors, while the HBLT and HDNT were set outdoors and paired with indoor CDC light traps. Details of the trapping procedures are described in Chapters 5 and 6.

2.5.2. Human behaviour survey

Human behaviour data were collected using a semistructured questionnaire. Residents of the study area were asked about the time they went indoors, when they retired to bed, when they woke up in the morning, when they left their houses for outdoor activities and the main activities that keep them outdoors. Moreover, data on the ownership and utilization of ITNs by the households, and the numbers of potential vertebrate hosts available in the study area were collected using the questionnaire.

2.6. Mosquito sample processing

All collected mosquitoes were killed by chloroform and identified morphologically to genus and species using taxonomic keys (Gillies and Coetzee, 1987). Culicine and male *Anopheles* mosquitoes were counted and discarded after recording. Female *Anopheles* mosquitoes were further classified as unfed, bloo-fed, half-gravid and gravid based on their physiological status, and kept individually in labelled 1.5 ml Eppendorf tubes containing silica gel desiccant (Plate 2.1). Samples were stored at − 20 °C freezer at Climate and Human Health Research Laboratory of Kenya Medical Research Institute (KEMRI) or Jimma University Tropical and Infectious Diseases Research Center (TIDRC) Laboratory until used for further processing.

Plate 2.1. Sorting mosquito samples in the field after collection, western Kenya

2.6.1. Molecular identification of vector species complexes

From each study site and each trapping method, sub-samples of *An. gambiae s.l.* and *An. funestus* group were randomly selected for sibling species identification by PCR. Deoxyribonucleic acid (DNA) extractions from the legs and wings of the mosquitoes were carried out by using ethanol precipitation method (Collins et al., 1987) at KEMRI/CDC Entomology Laboratory, and Qiagen DNeasy Kit (Qiagen Inc. Maryland, USA) at Jimma University TIDRC Lab (Appendix 4.1).

Sibling species of the *An. gambiae s.l.* were identified based on species-specific nucleotide sequences found in the ribosomal DNA (rDNA) intergenic spacers, a method previously developed to identify *An. gambiae s.l.* species complex (Scott et al., 1993). For *An. gambiae s.l.* samples collected from western Kenya, oligonucleotide primers specific to *An. gambiae*, *An. arabiensis*, *An. quadriannulatus*, and *An. merus* were used to run multiplex PCR, whereas as for *An. gambiae s.l.* samples collected from southwestern Ethiopia, primers specific to *An.*

arabiensis, *An. gambiae* and *An. amharicus* (previously *An. quadriannulatus* species B) were used. The PCR reactions were conducted in a final volume of 20 µl consisting of 0.25 µM of each primer, Dream Taq PCR master mix (ThermoFisher Scientific, USA, containing DreamTaq DNA Polymerase, DreamTaq Green buffer, $MgCl_2$, and dNTPs) and 2 µl of DNA extract. The samples were amplified in a T100^{TM} Thermal Cycler (Bio-Rad, USA), with cycling conditions of 95°C for 5 minutes followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for 30 seconds and final extension at 72°C for ten minutes. The PCR products were loaded in 1.5% agarose gel premixed with ethidium bromide (2 µg/ml) stain. A marker of 100 bp ladder was run on each gel for species identification. Following gel electrophoresis, the PCR products were visualized under a gel documentation system (Plate 2.2, Appendix 4.1).

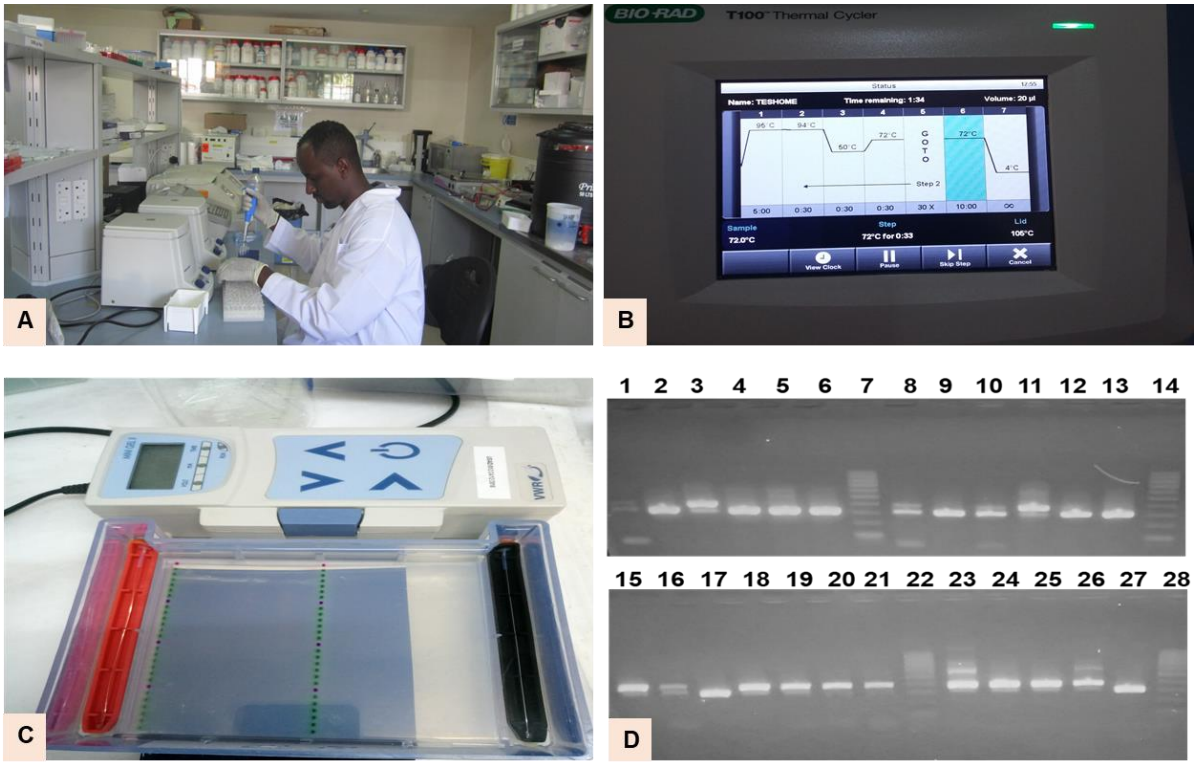

Plate 2.2. Molecular identification of *Anopheles gambae* complex

A) Preparation of PCR mix, B) DNA amplification by PCR thermal cycler C) Gel electrophoresis D) Amplified PCR products as observed under gel documentation system: Lane 1- a negative control, 2 and 3- positive controls for *An. arabiensis* (315 bp) and *An. gambiae* (390 bp), respectively, Lanes 4,5,6,8,9,10,12,13,17 and 28- *An. arabiensis* samples from western Kenya, 11, 15, 16, 18, 19, 20, 21, 23-26 – *An. gambiae* samples from western Kenya, Lanes 7, 14, 22 and 28 – 100 bp ladder (photo credit: Degefa T at KEMRI).

The sibling species of the *An. funestus* group were identified based on species-specific primers in the internal transcribed spacer 2 (ITS2) region on the rDNA, a method previously developed to identify *An. funestus, An. vaneedeni, An. rivulorum, An. leesoni* and *An. parensis* (Koekemoer et al., 2002). Each PCR run was conducted in a final volume of 25 µl consisting of 0.5 µM of each primer, Dream Taq PCR master mix and 3 µl of DNA extract. The samples were amplified in T100^{TM} Thermal Cycler, with cycling conditions of 95°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, extension at 72°C for 40 sec and final extension at 72°C for 10 min. The PCR products were loaded in 1.5% agarose gel. After gel electrophoresis, the PCR products were visualized under gel documentation system (Appendix 4.1).

2.6.2. Detection of blood meal sources

The blood meal sources of blood-fed *Anopheles* mosquitoes were analyzed by a direct ELISA using human, bovine, goat, chicken and dog antibodies (Beier et al., 1988). The direct ELISA begins by incubating blood-meal samples directly in microtiter plate wells. It uses a host-specific antibody-enzyme conjugate to detect homologous IgG in the blood-meal samples and specific substrate to produce a color reaction. Positive controls (venous blood sample collected from human by Medical Laboratory technologist and from other vertebrate hosts by Veterinary technician) were included for each host during the assay. Laboratory reared unfed *An. gambiae s.l.* were used as negative controls. Detailed procedures are described in Appendix 4.2.

2.6.3. Detection of sporozoite infections

Dried head and thorax of the preserved *Anopheles* mosquito specimens were carefully separated from the abdomen and tested for *P. falciparum* and *P. vivax* circum-sporozoite proteins (CSPs) using a sandwich ELISA (Beier et al., 1987, Wirtz et al., 1987). The sandwich ELISA begins with adsorption of capture monoclonal antibody (mAb) to wells of microtiter plate. After the capture mAb has bound to the plate, the well contents are aspirated and the remaining sites are blocked with blocking buffer (BB). Mosquitoes to be tested are ground in BB containing IGEPAL CA-630 and an aliquot is tested. If CSP is present, it will form an antigen-antibody complex with the capture mAb. After incubation for 2 hrs at room temperature, the mosquito triturate is aspirated and the wells are washed. Peroxidase-labeled mAb is then added, completing the formation of the sandwitch. After 1 hr the well contents are aspirated, the wells

are washed again and peroxidase substrate solution is added. As the peroxidase enzyme reacts with the substrate, a dark green product is formed. The intensity of the color is directly proportional to the amount of the CSP antigen present in the test sample. The results are read visually or at 405-411nm using an ELISA plate reader 30 and/or 60 minutes after the substrate has been added (Plate 2.3). Detailed procedures are explained under Appendix 4.3.

Plate 2.3. Enzymelinked immunosorbent assay (ELISA) for detection of *Plasmodium* CSPs

A. Preparation of mosquito triturate for loading in to ELISA plate wells (at KEMRI), B. Sample result of the sporozoite ELISA with the green color indicating *P. falciparum* CSP positive specimens (at KEMRI), C. Sporozoite ELISA indicating washing step at TIDRC, D. Reading ELISA results using ELISA reader machine at TIDRC (photo credit: Degefa T)

2.7. Data analysis

All collected mosquitoes were given individual sample code, and the sample codes were entered into an excel sheet together with all associated information including mosquito species name, physiological status, site of collection, date of collection, method of collection, location (indoor vs outdoor), time of collection and name of the collectors. All laboratory results including PCR, blood-meal ELISA and sporozoite ELISA results were entered into the excel sheet and linked with the mosquito code numbers which were initially assigned to individual mosquito sample during the field collection. Descriptive analyses were done by directly using the excel data. For

advanced statistical analysis, the excel data were exported to either Statistica 8.0 (StatSoft, Tulsa, USA), statistical package for social science version 20.0 (SPSS, Chicago, IL, USA), or R v.3.3 (R Core Team) softaware packages.

The densities of *Anopheles* mosquitoes were calculated as the number of mosquitoes caught per trap per night for all collection methods or as the number of mosquitoes caught per house per day for PSC. The differences in *Anopheles* mosquito density among the different trapping methods, and between indoor and outdoor locations were compared using a generalized linear model (GLM) based on a negative binomial distribution. Depending on the design and specific objectives of the study, mosquito sampling season, months and/or collection days were treated as covariates in the model during the analysis. Gini-Simpson's diversity index (Simpson, 1949, Peet, 1974, Magurran, 2013, Grundmann et al., 2001) was used to determine and compare mosquito species diversity caught by each trapping method. Details of these statistical analyses and additional statistics used were elucidated in Chapter 3-6. The magnitude of human exposure to malaria vector bites occurring indoors and outdoors at various times of the night was determined for both LLIN users and non-users based on both human and vector behaviour data (Chapter 6).

Human blood index (HBI) was calculated as the proportion of *Anopheles* mosquitoes that fed on humans over the total *Anopheles* tested for blood meal origin (Garrett-Jones, 1964). Blood-meal indices of other non-human vertebrate hosts (bovine, goat, dog and chicken) were also calculated in a similar way. Forage ratio (FR), a measure of host preference by mosquitoes, was determined as the proportion of engorged *Anopheles* mosquitoes which have fed on a given host divided by the abundance of that particular host in the study area (Hess et al., 1968, Manly et al., 2007). A host was considered to have been preferred if the lower 95% confidence limit for the FR estimate was greater than one and inferred to have been avoided if the upper 95% confidence limit of the FR estimate was less than one. Mosquito species for which the 95% confidence interval of the FR included one was considered as opportunistic feeder.

The sporozoite rate was estimated as the proportion of mosquitoes positive for *P. falciparum* and/or *P. vivax* CSPs over the total number tested. Annual EIRs for mosquitoes collected by HLC were determined as HBR × sporozoite rate × 365 (WHO, 2013c). The annual EIRs for mosquitoes collected by CDC light traps were calculated using the formula, 1.605 × (no. CSP

positive ELISA results from CDC light traps/no. mosquitoes tested) × (no. mosquitoes collected from CDC light traps/no. trap-nights) × 365 (Lines et al., 1991, Drakeley et al., 2003). The annual EIR of *Anopheles* mosquitoes collected by PSCs was determined as: (no. fed mosquitoes caught by PSC/no. human occupants who spent the night in the sprayed house) × (no. mosquitoes fed on human/no. mosquitoes tested for human blood meal) × (PSC based sporozoite rate) × 365 (WHO, 2013c).

2.8. Ethical consideration

Ethical approval for the study was obtained from the Ethical Review Board of Kenya Medical Research Institute (Protocol No. KEMRI/SERU/3005 and KEMRI/SERU/CGHR/0057/3363) and Jimma University Institutional Review Board (Ref No. IHRPGD/2075/18). Permission was sought from the chief of each study site. Written informed consent was obtained from all household heads and volunteer data collectors.

CHAPTER THREE

3. INDOOR AND OUTDOOR MALARIA VECTOR SURVEILLANCE IN WESTERN KENYA: IMPLICATIONS FOR BETTER UNDERSTANDING OF RESIDUAL TRANSMISSION (Adopted from Degefa et al., 2017)

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Indoor and outdoor malaria vector surveillance in western Kenya: implications for better understanding of residual transmission

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3.1. Abstract

Background: The widespread use of indoor-based malaria vector control interventions has been shown to alter the behaviour of vectors in Africa. There is an increasing concern that such changes could sustain residual transmission. This study was conducted to assess vector species composition, feeding behaviour and their contribution to indoor and outdoor malaria transmission in western Kenya.

Methods: *Anopheles* mosquito collections were carried out from September 2015 to April 2016 in Ahero and Iguhu sites, western Kenya using CDC light traps (indoor & outdoor), pyrethrum spray catches (PSCs) (indoor) and pit shelters (outdoor). Species within *Anopheles gambiae s.l.* and *Anopheles funestus* group were identified using polymerase chain reaction (PCR). Enzyme-

linked immunosorbent assay (ELISA) was used to determine mosquito blood-meal sources and sporozoite infections.

Results: A total of 10,864 female *Anopheles* mosquitoes comprising *An. gambiae s.l.* (71.4%), *An. funestus* group (12.3%), *Anopheles coustani* (9.2%) and *Anopheles pharoensis* (7.1%) were collected. The majority (61.8%) of the anopheline mosquitoes were collected outdoors. PCR results (n = 581) revealed that 98.9% *An. arabiensis* and 1.1% *An. gambiae s.s.* constituted *An. gambiae s.l.* in Ahero while this was 87% *An. gambiae s.s.* and 13% *An. arabiensis* in Iguhu. Of the 108 *An. funestus* group analysed by PCR, 98.1% belonged to *An. funestus s.s.* and 1.9% to *Anopheles leesoni*. The human blood index (HBI) and bovine blood index (BBI) of *An. arabiensis* was 2.5% and 73.1%, respectively. *Anopheles gambiae s.s.* had HBI and BBI of 50% and 28%, respectively. The HBI and BBI of *An. funestus* was 60% and 22.3%, respectively. Forage ratio estimate revealed that *An. arabiensis* preferred to feed on cattle, *An. gambiae s.s.* showed preference for both human and cattle, while *An. funestus* preferred human over other hosts. In Ahero, the sporozoite rates for *An. arabiensis* and *An. funestus* were 0.16% and 1.8%, respectively, whereas in Iguhu, the sporozoite rates for *An. gambiae s.s.* and *An. funestus* were 2.3% and 2.4%, respectively. In Ahero, the estimated indoor and outdoor entomological inoculation rate (EIR) was 108.6 infective bites/person/year (79.0 from *An. funestus* and 29.6 from *An. arabiensis*) and 43.5 infective bites/person/year (27.9 from *An. arabiensis* and 15.6 from *An. funestus*), respectively. In Iguhu, the estimated indoor and outdoor EIR was 24.5 infective bites/person/year (18.8 from *An. gambiae s.s.* and 5.7 from *An. funestus*) and 5.5 infective bites/person/year (all from *An. gambiae s.s.)*, respectively.

Conclusion: *Anopheles gambiae s.s.* showed an increasing tendency to feed on cattle. *Anopheles arabiensis* was highly zoophagic, whereas *An. funestus* showed anthropophagic behaviour. While the majority of malaria transmission occurred indoors, the magnitude of outdoor transmission was considerably high. Additional control tools that complement the existing interventions are required to control residual transmission.

3.2. Introduction

Malaria is a serious vector-borne disease affecting hundreds of millions of people in Africa. In the past decade, a substantial reduction in malaria incidence has been observed in Africa, including Kenya, due to the scale-up of interventions. Vector control is one of the key elements in achieving the remarkable decline of malaria, with the scale-up of insecticide-treated nets (ITNs) and expansion of indoor residual spray (IRS) contributing significantly (WHO, 2015b, Shargie et al., 2010, Bhattarai et al., 2007, Otten et al., 2009). The proportion of households owning at least one ITN in sub-Saharan Africa is estimated to have risen from 3% in 2000 to 67% in 2015 (WHO, 2015b). In western Kenya, the ITN ownership rose from 12.8% in 2004 to over 80% in 2015 (Zhou et al., 2011, Ototo et al., 2015, Ndenga et al., 2016).

Despite the progress made in scaling-up of the interventions, malaria transmission continues to occur. Several factors are responsible for this transmission, including the spread of insecticide resistance (Zhou et al., 2011, Ochomo et al., 2013), shift in vector species composition (Bayoh et al., 2010, Mwangangi et al., 2013a, Russell et al., 2010, Derua et al., 2012) and increasing vector behavioural change towards more zoophagic, exophagic and/or exophilic tendencies following the widespread use of ITNs and IRS (Russell et al., 2011, Durnez and Coosemans, 2013).

Recent reports from East Africa showed strong evidence for shifts in *Anopheles gambiae sensu lato (s.l.)* sibling species composition from predominantly endophagic *An. gambiae sensu stricto (s.s.)* to predominantly exophagic *Anopheles arabiensis* following the scale-up of ITNs (Bayoh et al., 2010, Russell et al., 2010, Derua et al., 2012, Russell et al., 2011, Mutuku et al., 2011). In the lowlands of western Kenya, the proportion of *An. gambiae s.s.* declined from about 85% in 1998 to 1% in 2009 following massive distribution of ITNs, whereas *An. arabiensis* population showed proportionate increment (Bayoh et al., 2010). While malaria transmission by *An. gambiae s.s.* declined significantly, residual transmission continued to occur by *An. arabiensis*. Similarly, the proportion of *An. arabiensis* in the highlands of western Kenya has been increasing gradually (Zhou et al., 2011).

Vector behavioural modifications including changes in host-preference, biting locations (indoor or outdoor) and resting behaviours have been reported following the long-term use of ITNs. For instance, ITN use was associated with shift in host preference of *An. gambiae s.s.* from human to

cattle in Burkina Faso (Lefèvre et al., 2009). The long-term use of ITN increased the outdoor feeding proportion of *An. gambiae s.s.* in Bioko Island (Reddy et al., 2011, Meyers et al., 2016) and *Anopheles funestus* in Tanzania (Russell et al., 2011). However, these changes are not universal. A recent study in Asembo district of western Kenya showed that the majority of biting by *An. arabiensis*, *An. gambiae s.s.* and *An. funestus* occurred indoors despite high ITN coverage in the area (Bayoh et al., 2014).

Malaria is mesoendemic and holoendemic in the highland and lowland areas of western Kenya, respectively (Githeko et al., 2012). The transmission is maintained by *An. gambiae s.s.*, *An. funestus* and *An. arabiensis*. *Anopheles gambiae s.s* and *An. funestus* are considered as highly endophagic and anthropophagic, while *An. arabiensis* is considered as zoophagic and endophilic. However, most of the studies on their feeding and resting behaviour were conducted before the scale-up of vector control interventions (Githeko et al., 1994b, Githeko et al., 1996, Shililu et al., 1998). It is possible that the anthropophagic and endophilic individuals could shift to zoophagic and exophilic tendencies or be reduced to leave zoophagic and exophilic sibling species following the scale-up of ITNs as has been observed elsewhere.

In view of the increasing concern about residual malaria transmission in Africa, there is a pressing need to enhance our understanding about vector behaviours to evaluate the likely success of the current vector control tools. The main aim of this study was to assess vector species composition, feeding behaviour and their contribution to indoor and outdoor malaria transmission in western Kenya.

3.3. Methods

3.3.1. Study sites

The study was conducted in lowland and highland settings of western Kenya. Two sites were selected (Figure 3.1): Ahero (0°.11'S, 34°.55'E, altitude 1162m) in Kisumu County and Iguhu (0°.17'N; 34°.74'E, altitude 1,430–1,580 m a.s.l) in Kakamega county. Iguhu site is highland characterized by valleys and depressions surrounded by densely populated hills whereas Ahero is lowland plain area. The sites have bimodal pattern of rainfall, with long rainy season from April to June, which triggers peak malaria transmission period and short rainy season from October to November with minimal transmission (Munyekenye et al., 2005). The hot and dry season is from

January to March and this marks the lowest transmission (Zhou et al., 2011). *Plasmodium falciparum* is the predominant malaria species in the area and is transmitted by *An. gambiae s.s.*, *An. arabiensis* and *An. funestus* (Zhou et al., 2011, Githeko et al., 2006).

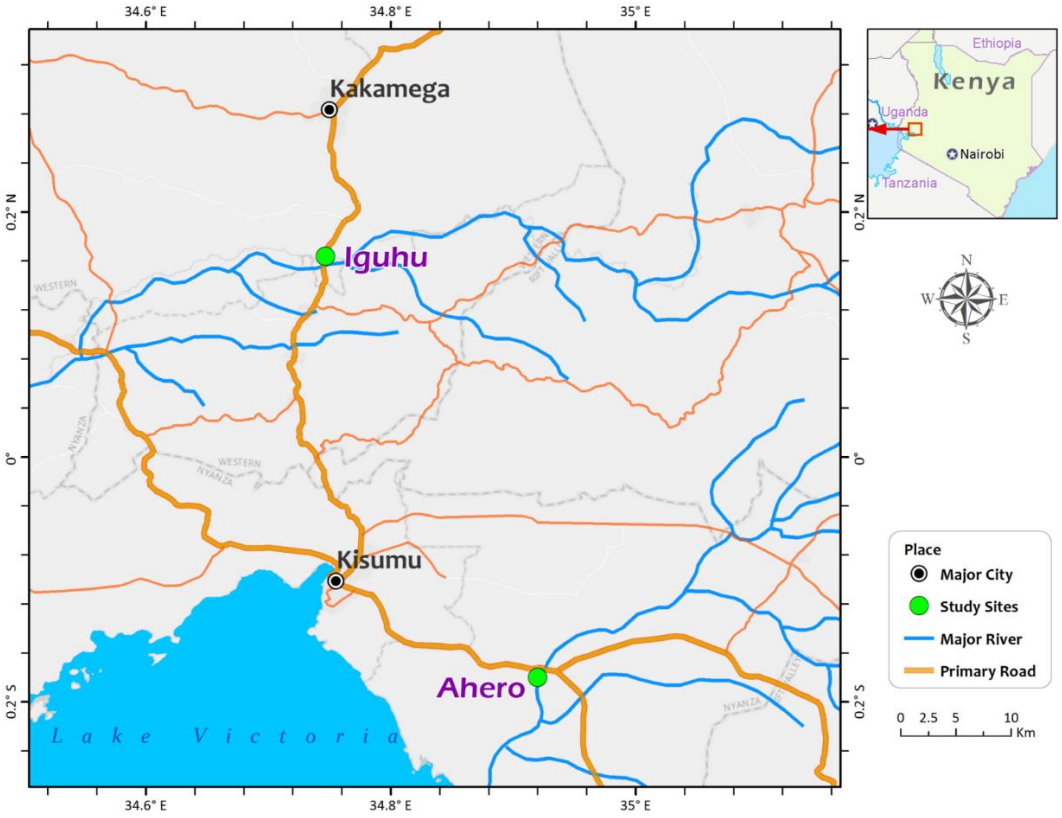

Figure 3.1. Map of the study sites in Kenya

3.3.2. Mosquito collections

Adult mosquito collections were carried out monthly during the short rainy season (September to November) in 2015 and dry season (February to April) in 2016. Indoor and outdoor host-seeking mosquitoes were collected using Centers for Disease Control and Prevention (CDC) light traps (John W. Hock Ltd, Gainesville, FL., USA). For indoor host-seeking mosquito collection, CDC light traps were set inside houses near the bed at a height of 1.5 metre from 18:00 to 06:00 hr in twenty randomly selected houses per month in each study site. For the outdoor host-seeking mosquito sampling, CDC light traps were set outdoor in the vicinity (within 2 metre) of sentinel houses. The same houses were used for mosquito collections each month.

Indoor resting mosquitoes were sampled using pyrethrum spray catches (PSCs) from another twenty randomly selected houses from 06:00 to 09:00 hr following standard protocol (WHO, 1995). Outdoor resting mosquitoes were collected monthly in the mornings (06:00 to 09:00 hr.) from twenty artificial outdoor pit shelters constructed according to the method of Muirhead-Thomson (Muirhead-Thomson, 1958), in the compound of 20 selected houses in each study site. The collections were repeated using the same pit shelters each month.

Along with mosquito collection, data on the numbers of potential hosts in the study area including human, bovine, goat, dog and chicken were collected using questionnaire surveys. All collected mosquitoes were identified morphologically to species using keys (Gillies and Coetzee, 1987). Female *Anopheles* mosquitoes were further classified as unfed, blood fed, half-gravid and gravid. Each mosquito was kept in a labelled 1.5 ml Eppendorf tube containing silica gel desiccant and cotton wool. Samples were stored at -20^{o}C refrigerator at Climate and Human Health Research Laboratory of Kenya Medical Research Institute until used for further processing.

3.3.3. Identification of *Anopheles* species complexes

Members of *An. gambiae s.l.* and *An. funestus* group were identified to species by polymerase chain reaction (PCR), following the protocols developed by Scott *et al.* for *An. gambiae s.l.* (Scott et al., 1993) and Koekemoer *et al.* for *An. funestus* group (Koekemoer et al., 2002).

3.3.4. Detection of blood meal sources

The blood meal sources of freshly fed *Anopheles* mosquitoes were analyzed by a direct enzyme-linked immunosorbent assay (ELISA) (Beier et al., 1988) using human, bovine, goat, chicken and dog antibodies. Positive controls were included for each host during the assay. Laboratory reared unfed *An. gambiae* was used as negative control.

3.3.5. Sporozoite ELISA

Dried head and thorax of the preserved *Anopheles* mosquito specimens were carefully separated from the abdomen and tested for *P. falciparum* circumsporozoite proteins (CSPs) using sandwich ELISA method (Beier et al., 1987, Wirtz et al., 1987).

3.3.6. Data analysis

The density of adult anopheline mosquitoes was calculated as the number of female mosquitoes per trap/night for each collection method. Analysis of variance (ANOVA) was used to compare malaria vector density between indoor and outdoor locations. χ^2-test was employed to test the difference in vector species composition between indoor and outdoor.

Human blood index (HBI) was calculated as the proportion of *Anopheles* mosquitoes that fed on human over the total *Anopheles* tested for blood meal origins (Garrett-Jones, 1964). Bovine, goat, dog and chicken blood indices were also calculated in similar way. Mixed blood meals were included in the calculation of blood meal indices (Pappa et al., 2011). The forage ratio (FR), a measure of host preference by mosquitoes, was determined as the percent of engorged *Anopheles* mosquitoes which have fed on a given host (human, bovine, goat, dog or chicken) divided by the percent which it comprises in the total population of hosts available in the study area (Hess et al., 1968). The FR w_i for species i was calculated as:

$$w_i = \frac{o_i}{p_i}$$

where w_i is the FR for mosquito species i, o_i is the proportion of host species i in the blood meals, and p_i is the proportion of host species i available in the environment.

Statistical significance of the FR estimate for each host was based on overlap of the 95% confidence interval (CI) of the estimate with the value one (Manly et al., 2007). A host was considered to have been preferred if the lower 95% confidence limit for the FR estimate was greater than one. A host was inferred to have been avoided if the upper 95% confidence limit for the FR estimate was less than one. A host for which the 95% CI for its FR included one was considered to have been feed on opportunistically (Manly et al., 2007).

The sporozoite rate was estimated as the proportion of mosquitoes positive for *P. falciparum* CSP over the total number tested. Annual entomological inoculation rate (EIR) was calculated from mosquito collections by CDC light traps using the formula, 1.605 × (no. CSP-positive ELISA results from CDC light traps/no. mosquitoes tested) × (no. mosquitoes collected from CDC light traps/no. trap-nights) × 365 (Lines et al., 1991, Drakeley et al., 2003). The multiplication factor 1.605 is a conversion factor for CDC light trap catches vs. man biting

catches (Lines et al., 1991). The annual EIR of *Anopheles* mosquitoes collected by PSCs was determined as: (no. fed mosquitoes caught by PSC/no. human occupants who spent the night in the sprayed house) × (no. mosquitoes fed on human /no. mosquitoes tested for human blood meal) × (PSC based sporozoite rate) x 365 (WHO, 2003).

The annual EIR for *Anopheles* mosquitoes collected from pit shelters was also estimated as (no. fed mosquitoes caught in the pit shelters/no. human occupants who spent the night in a house nearest to the pit shelter) × (no. human fed mosquitoes/no. mosquitoes tested for human blood meal) × (sporozoite rate from pit shelters) x 365. This formula was employed based on the assumption that all *Anopheles* mosquitoes collected from pit shelters have got their human blood meals from occupants of the nearest house, either indoor or outdoor.

Data were analyzed using STATISTICA 8.0 (StatSoft, Tulsa, USA) and SPSS version 20.0 (SPSS, Chicago, IL, USA) software packages. $P < 0.05$ was considered statistically significant during the analysis.

3.4. Results

3.4.1. Mosquito species composition and abundance

A total of 10,864 female *Anopheles* mosquitoes belonging to four species were collected during the study period (Table 3.1). *Anopheles gambiae s.l.* was the predominant species accounting for 71.4% of the total captures, followed by *An. funestus* group (12.3%), *Anopheles coustani* complex (9.2%) and *Anopheles pharoensis* (7.1%). In addition, 3,263 male anopheline mosquitoes and 5,206 *Culex* species (males and females together) were collected over the study period. There was a significant difference in anopheline mosquito species co-occurrence between the study sites ($F_{1, 952} = 423.02$, $p < 0.0001$). There was also significant difference in anopheline mosquito species co-occurrence between indoor and outdoor locations ($F_{1, 956} = 29.44$, $p < 0.0001$). The majority (61.8%) of the anopheline mosquitoes were collected outdoors.

Table 3.1. Summary of female *Anopheles* mosquitoes collected from indoor and outdoor in lowland (Ahero) and highland (Iguhu) settings of western Kenya (n=120 trap-nights for each trap)

Study sites and *Anopheles* spp	**Indoor**		**Outdoor**		**Total**
	Light trap	**PSC**	**Light trap**	**Pit shelter**	
Ahero					
An. gambiae s.l.	1592	1009	1636	3262	7,499
An. funestus group	628	204	270	142	1,244
An. coustani	321	2	652	15	990
An. pharoensis	78	0	688	0	766
Iguhu					
An. gambiae s.l.	108	51	56	41	256
An. funestus group	49	30	13	4	96
An. coustani	3	0	10	0	13
Total	**2,779**	**1,296**	**3,325**	**3,464**	**10,864**

3.4.2. Indoor and outdoor *Anopheles* mosquito density

Figure 3.2 shows the mean indoor and outdoor density of host-seeking and resting female *Anopheles* mosquitoes. In Ahero, the mean outdoor resting density of *An. gambiae s.l.* was significantly higher than indoor resting density (t_{238} = 8.45, p < 0.0001), whereas the difference in mean indoor and outdoor resting density of *An. funestus* group was not significant (p > 0.05). The mean outdoor host-seeking density of *An. gambiae s.l.* was also higher than indoor resting density, although the difference was not statistically significant (t_{238} = 0.14, p = 0.889). The mean indoor host-seeking density of *An. funestus* group was significantly higher than outdoor host-seeking density of *An. funestus* group (t_{238} = 2.37, p = 0.019). Significantly higher outdoor host-seeking density than indoor was observed for *An. coustani* (t_{238} = 2.589, p = 0.01) and *An. pharoensis* (t_{238} = 4.923, p < 0.0001).

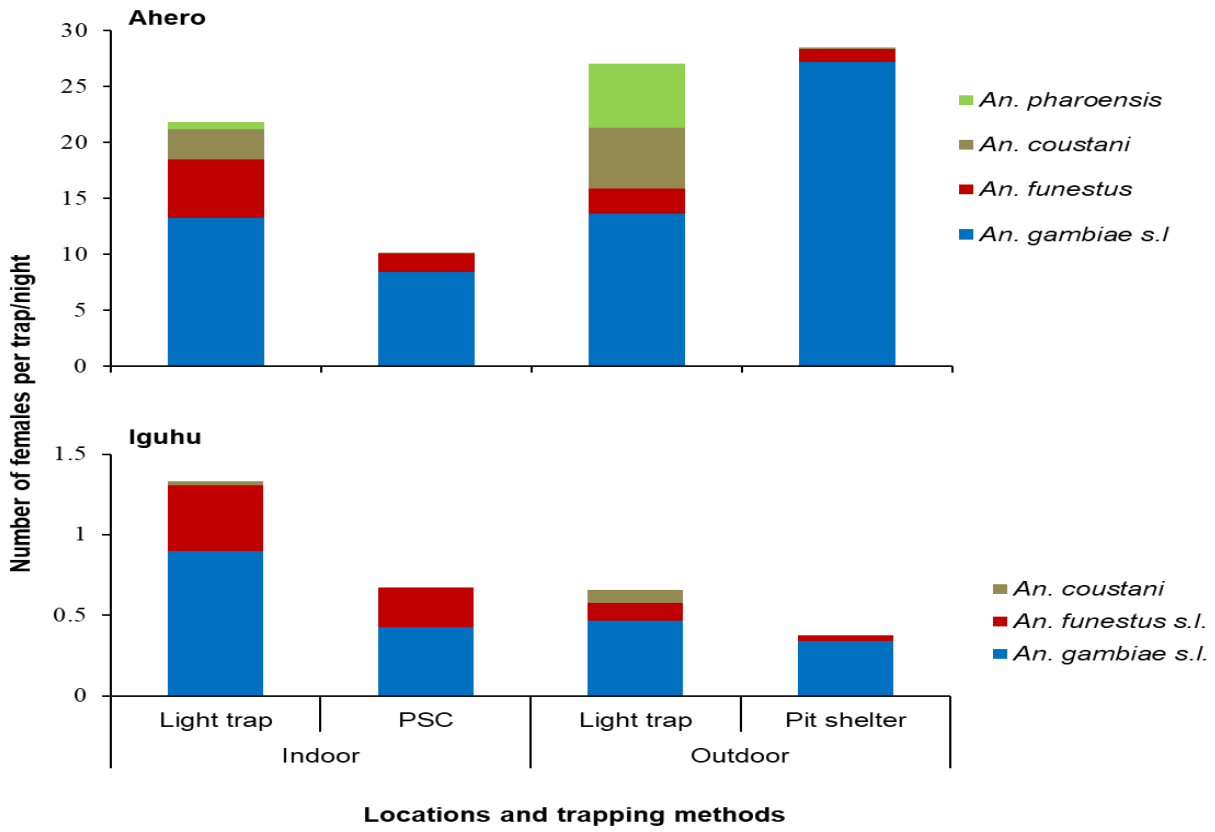

Figure 3.2. Indoor and outdoor host-seeking and resting density of female *Anopheles* mosquitoes collected from Ahero and Iguhu, western Kenya

In Iguhu, the host-seeking densities of *An. gambiae s.l.* and *An. funestus* group were significantly higher indoor than outdoor (*An. gambiae s.l.*, t_{238} = 2.12, p = 0.034; *An. funestus* group, t_{238} = 3.09, p = 0.002). The difference in mean indoor and outdoor resting density of *An. gambiae s.l.* was not significant (t_{238} = 0.97, p = 0.335), while the mean indoor resting density of *An. funestus* group was significantly higher (t_{238} = 3.23, p = 0.001) than outdoor resting density of *An. funestus* group.

3.4.3. Composition of *Anopheles gambiae* and *Anopheles funestus* sibling species

A total of 750 specimens (628 *An. gambiae s.l.* and 122 *An. funestus* group*)* were analysed for identification of their respective sibling species. Of these, 581 *An. gambiae s.l.* and 108 *An. funestus* group specimens were successfully amplified and identified to species by PCR. Figure 3.3 shows member species of the *An. gambiae s.l.* In Ahero, of the assayed *An. gambiae s.l.* specimens, *An. arabiensis* and *An. gambiae s.s* accounted for 98.9% and 1.1%, respectively. In

contrast in Iguhu, *An. gambiae s.s.* and *An. arabiensis* constituted 87% and 13%, respectively of the assayed *An. gambiae s.l.* specimens. Overall, there was significant difference between indoor and outdoor locations in terms of the *An. gambiae s.l.* species composition (χ^2 = 26.443, df = 1, p < 0.0001). The proportion of *An. arabiensis* was higher outdoors than indoors. Of the 108 *An. funestus* group confirmed by PCR, *An. funestus s.s.* (hereafter *An. funestus*) and *Anopheles leesoni* accounted for 98.1% and 1.9%, respectively. All of the PCR confirmed *An. leesoni* speceimens were from outdoor CDC light traps. The member species of the *An. funestus* group did not vary between the study sites.

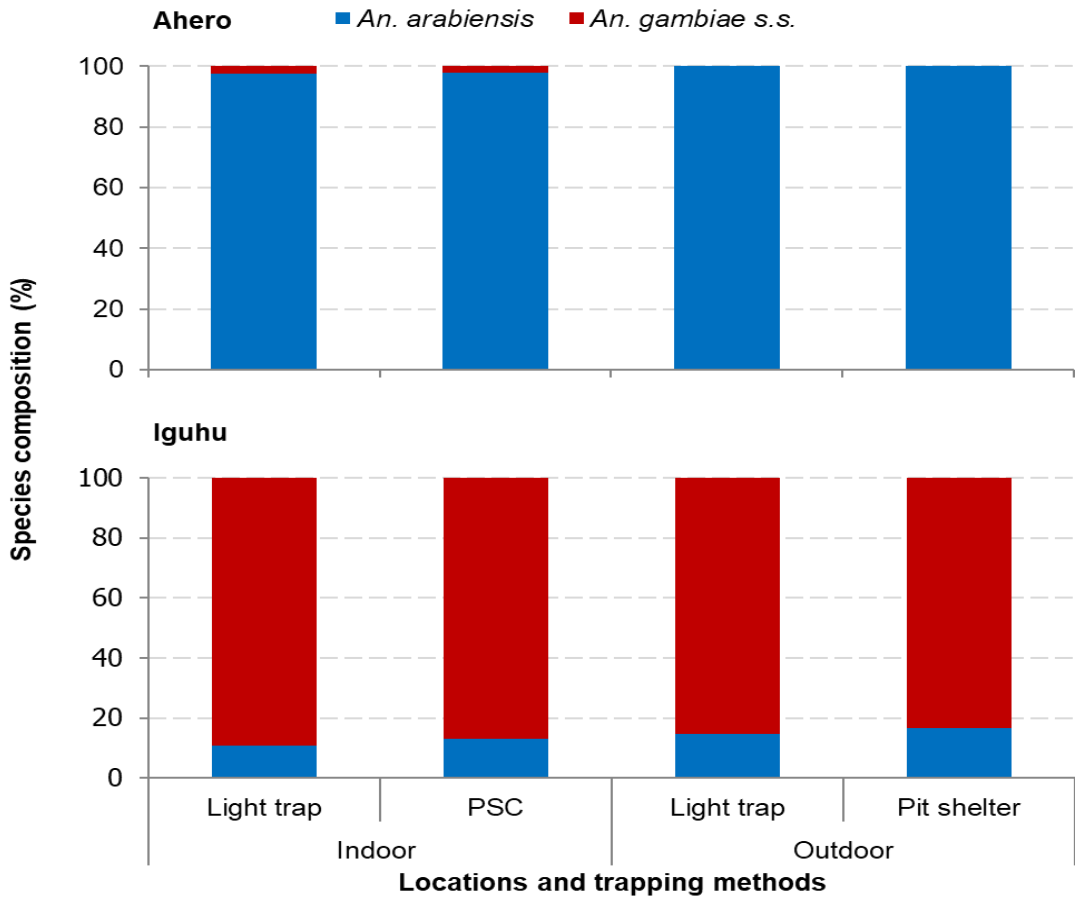

Figure 3.3. Composition of *Anopheles gambiae* sibling species in Ahero and Iguhu, western Kenya

3.4.4. Physiological status

In both indoor and outdoor collections, the majority (> 70%) of the host-seeking anophelines were unfed. About 55% of the indoor resting and 39% of the outdoor resting *An. arabiensis* were blood fed. One third of the indoor resting and 31.7% of the outdoor resting *An. gambiae s.s.* were blood fed. About half of the indoor resting *An. funestus* were blood fed, while this was 11.6% for the outdoor resting *An. funestus.*

3.4.5. Blood meal indices

Table 3.2 shows the host blood indices of *An. arabiensis* and *An. funestus* in Ahero. The HBI of *An. arabiensis* from indoor CDC light traps and PSCs was 8.2% and 1.2%, respectively, whereas the HBI of *An. arabiensis* from outdoor CDC traps and pit shelters was 3.4% and 0.7%, respectively. The overall HBI of *An. arabiensis* was 2.5%. The HBI of *An. funestus* from indoor CDC light traps and PSCs was 72.7% and 63.6%, respectively, while the HBI of *An. funestus* from both outdoor CDC light traps and pit shelters was 50%. In Ahero, the overall HBI for *An. funestus* was 62%.

In contrast, the Bovine blood index (BBI) of *An. arabiensis* from indoor CDC light traps, PSCs, outdoor CDC light traps and pit shelters was 62.3%, 66.7%, 50.8%, and 85.6%, respectively. Overall, the BBI of *An. arabiensis* was 73.1%. The BBI of *An. funestus* from PSCs, outdoor CDC light traps and pit shelters was 27.3%, 22.7% and 41.7%, respectively. None of the *An. funestus* from indoor CDC light traps was positive for bovine blood meal. In Ahero, the overall BBI of *An. funestus* was 25.4%. Blood meal indices for other vertebrate hosts (goat, dog and chicken) were low (< 4%).

Table 3.2. Blood meal origins of *An. arabiensis* and *An. funestus* from indoor and outdoor collections in Ahero, western Kenya

Blood-meal Origins	*An. arabiensis*				*An. funestus*			
	Indoor		Outdoor		Indoor		Outdoor	
	Light trap	PSC	Light trap	Pit shelter	Light trap	PSC	Light trap	Pit shelter
Number tested	122	165	59	298	11	44	4	12
Human	7 (5.7)	1 (0.6)	2 (3.4)	2 (0.7)	8 (72.7)	23 (52.3)	2 (50.0)	6 (50.0)
Bovine	74 (60.7)	108 (65.5)	30 (50.8)	251 (84.2)	0	10 (22.7)	1 (25.0)	5 (41.7)
Goat	5 (4.1)	5 (3.0)	1 (1.7)	4 (1.3)	0	0	0	0
Dog	1 (0.8)	5 (3.0)	1 (1.7)	5 (1.7)	0	0	0	0
Chicken	2 (1.6)	0	0	1 (0.3)	0	0	0	0
Human+Bovine	1 (0.8)	1 (0.6)	0	0	0	2 (4.6)	0	0
Human+Dog	2(1.6)	0	0	0	0	3 (6.8)	0	0
Bovine+Dog	1 (0.8)	1 (0.6)	0	4 (1.3)	0	0	0	0
Goat+Dog	0	1 (0.6)	0	0	0	0	0	0
Dog+Chicken	0	0	0	1 (0.3)	0	0	0	0
Unknown	29(23.8)	43 (26.1)	25 (42.4)	30 (10.1)	3 (27.3)	6 (13.6)	1 (25.0)	1 (8.3)
HBI	8.2	1.2	3.4	0.7	72.7	63.6	50.0	50.0

Note: HBI = Human blood index, PSC: pyrethrum spray catches, HBI was calculated as the number of mosquito positive for human (including mixed blood meal) divided by the total number tested.

Table 3.3 shows the host blood indices of *An. gambiae s.s.* and *An. funestus* in Iguhu. The HBI of *An. gambiae s.s.* from indoor CDC light traps and PSCs was 70.0% and 76.5%, respectively, whereas the HBI of *An. gambiae s.s.* from outdoor CDC light traps and pit shelters was 20.0% and 23.1% respectively. The overall HBI of *An. gambiae s.s.* was 50.0%. The HBI of *An. funestus* from indoor CDC light traps and PSCs was 53.8% and 61.1%, respectively. In outdoor CDC light traps, very small number of fed *An. funestus* was caught, which yielded a HBI of 50%. Hence, in Iguhu, the overall HBI of *An. funestus* was 55.9%.

The BBI of *An. gambiae s.s.* from PSCs, outdoor CDC light traps and pit shelters was 23.5%, 40.0%, and 46.1%, respectively. None of the tested *An. gambiae s.s.* from indoor CDC light traps was positive for bovine blood meal. The overall BBI of *An. gambiae s.s.* was 28%. The BBI of *An. funestus* from indoor CDC light traps, PSCs and outdoor CDC light traps was 15.4%, 16.7%, and 50%, respectively. In Iguhu, the overall BBI of *An. funestus* was 17.6%.

3.4.6. Feeding preference of malaria vectors

The overall blood meal indices and host preferences of *Anopheles* mosquitoes are shown in Table 3.4. Regardless of higher proportion of humans compared to domestic animals in Ahero, *An. arabiensis* showed a strong preference to feed on bovine (Forage ratio, FR = 3.9, 95% CI: 3.7-4.9). *Anopheles gambiae s.s.* showed preference to both human (FR = 1.8, 95% CI: 1.3-2.3) and bovine (FR = 2.3, 95% CI: 1.3-3.3). *Anopheles funestus* showed a preference to human in both Ahero (FR = 2.2, 95% CI: 1.8-2.6) and Iguhu (FR = 2.0, 95% CI: 1.6-2.4).

Table 3.3. Blood meal origins of *An. gambiae s.s.* and *An. funestus* from indoor and outdoor collections in Iguhu, western Kenya

Blood-meal Origins	*An. gambiae* s.s.				*An. funestus*			
	Indoor		Outdoor		Indoor		Outdoor	
	Light trap	PSC	Light trap	Pit shelter	Light trap	PSC	Light trap	Pit shelter
Number tested	10	17	10	13	13	18	2	1
Human	7 (70)	11 (64.7)	2 (20)	3 (23.1)	7 (53.8)	11 (61.1)	1 (50.0)	0
Bovine	0	3 (17.6)	4 (40)	6 (46.1)	2 (15.4)	3 (16.7)	1 (50.0)	0
Goat	0	0	0	0	1 (7.7)	0	0	0
Dog	0	0	0	1 (7.7)	1 (7.7)	0	0	1 (100)
Human+Bovine	0	1 (5.9)	0	0	0	0	0	0
Human+Dog	0	1 (5.9)	0	0	0	0	0	0
Unknown	3 (30)	1 (5.9)	4 (40)	3 (23.1)	2 (15.4)	4 (22.2)	0	0
HBI	70.0	76.5	20	23.1	53.8	61.1	50.0	0

Note: HBI = Human blood index, PSC = pyrethrum spray catches, HBI was calculated as the number of mosquito positive for human (including mixed blood meal) divided by the total number tested.

Table 3.4. Overall blood meal indices and host-preferences of malaria vectors from indoor and outdoor collections in Ahero and Iguhu, western Kenya

Site and species	Parameters	Human	Bovine	Goat	Dog	Chicken
Ahero						
Host abundance in the area (%)		27.8	18.8	4.0	6.0	43.4
An. arabensis	Blood index	2.5	73.1	2.5	3.4	0.6
	FR (95% CI)	0.09 (0.05-0.13)	3.9 (3.7-4.1)*	0.6 (0.3-0.9)	0.5 (0.3-0.7)	0.01(-0.03-0.05)
An. funestus	Blood index	62.0	25.4	0	4.2	0
	FR (95% CI)	2.2 (1.8-2.6)*	1.4 (0.9-1.9)	0	0.7 (-0.1-1.5)	0
Iguhu						
Host abundance in the area (%)		27.5	12.4	2.4	2.5	55.2
An. gambiae s.s.	Blood index	50	28	0	2.0	0
	FR (95% CI)	1.8 (1.3-2.3)*	2.3 (1.3-2.3)*	0	0.8 (-0.7-2.3)	0
An. funestus	Blood index	55.9	17.6	2.9	2.9	0
	FR (95% CI)	2.0 (1.6-2.4)*	1.4 (0.4-2.4)	1.2 (-1.2-3.6)	1.2 (-1.1-3.5)	0

Key*:* FR = Forage ratio, * indicates the preferred host

3.4.7. Sporozoite rates

Overall, 2,608 *Anopheles* mosquitoes comprising *An. arabiensis* (n = 1,280), *An. gambiae s.s.* (n = 214), *An. funestus* (n = 629), *An. coustani* (n = 255) and *An. pharoensis* (n = 230) were tested for *P. falciparum* CSP. Of these, 20 specimens (2 *An. arabiensis*, 5 *An. gambiae s.s*., 12 *An. funestus* and 1 *An. coustani*) were positive for CSP.

Table 3.5 shows the sporozoite rates of *Anopheles* mosquitoes collected from indoors and outdoors. In Ahero, the sporozoite rate of *An. arabiensis* from indoor and outdoor CDC light traps was 0.38% and 0.35%, respectively. However, none of the *An. arabiensis* tested from PSCs and pit shelters were positive. The overall sporozoite rate of *An. arabiensis* was 0.16%. The sporozoite rate of *An. funestus* from indoor CDC light traps and PSCs was 2.6% and 2.0%, respectively, while this was 1.2% from both outdoor CDC light traps and pit shelters. Hence, in Ahero, the overall spozoite rate of *An. funestus* was 1.8%. Moreover, one *An. coustani* specimen from outdoor CDC light trap was positive for CSP.

In Iguhu, the sporozoite rate of *An. gambiae s.s.* from indoor CDC light traps was 3.6%, but none of the *An. gambiae s.s.* tested from PSCs was positive. In contrast, the sporozoite rate of *An. gambiae s.s.* from outdoor CDC light traps and pit shelters was 2.0% and 2.9%, respectively. Overall, the sporozoite rate of *An. gambiae s.s.* was 2.3%. The sporozoite rate of *An. funestus* from indoor CDC light traps and PSCs was 2.4% and 4%, respectively. No CSP was detected in *An. funestus* collected from outdoor CDC light traps and pit shelters. Thus, in Iguhu, the overall sporozoite rate of *An. funestus* was 2.4%.

Table 3.5. Sporozoite rates of *Anopheles* mosquitoes from indoor and outdoor collections in Ahero and Iguhu, western Kenya

Study site and *Anopheles* sp	**Parameters**	**Indoor**		**Outdoor**		**Total**
		Light trap	PSC	Light trap	Pit shelter	
Ahero						
An. arabiensis	No tested	263	264	286	447	1260
	Pf +ve (%)	1 (0.38)	0	1 (0.35)	0	2 (0.16)
An. funestus	No tested	194	100	169	84	547
	Pf +ve (%)	5 (2.6)	2 (2.0)	2 (1.2)	1 (1.2)	10 (1.8)
An. coustani	No tested	50	0	200	0	250
	Pf +ve (%)	0	0	1 (0.5)	0	1 (0.4)
An. pharoensis	No tested	25	0	205	0	230
	Pf +ve (%)	0	0	0	0	0
Iguhu						
An. gambiae s.s.	No tested	84	46	50	34	214
	Pf +ve (%)	3 (3.6)	0	1 (2.0)	1 (2.9)	5 (2.3)
An. funestus	No tested	42	25	13	2	82
	Pf +ve (%)	1 (2.4)	1 (4.0)	0	0	2 (2.4)
An. arabiensis	No tested	8	5	2	5	20
	Pf +ve (%)	0	0	0	0	0
An. coustani	No tested	1	0	4	0	5
	Pf +ve (%)	0	0	0	0	0

Key: *Pf: Plasmodium falciparum, Pf*+ve: number *P. falciparum* CSP positive (rate in percent)

3.4.8. Entomological inoculation rates (EIRs)

The EIRs of *Anopheles* mosquitoes are shown in Table 3.6. In Ahero, the estimated *P. falciparum* EIR of *An. arabiensis* from indoor and outdoor CDC light traps was 29.6 and 27.9 infective bites/person/year (ib/p/year), respectively, whereas the EIR of *An. funestus* from indoor and outdoor CDC light traps was 79.0 and 15.6 ib/p/year, respectively. The overall indoor and outdoor EIR was 108.6 and 43.5 ib/p/year, respectively. About 48% of the total infective bites

by *An. arabiensis* and 16.5% by *An. funestus* occurred outdoor. The EIR of *An. arabiensis* and *An. funestus* from PSCs was 0.03 and 0.92 ib/p/year, respectively.

Table 3.6. Entomological inoculation rates (EIRs) of malaria vectors from indoor and outdoor collections in Ahero and Iguhu, western Kenya

Site and species	Parameters	Indoor		Outdoor	
		Light trap	PSC	Light trap	Pit shelter
Ahero					
An. arabiensis	SR	0.38	0.	0.35	0
	EIR	29.6	0	27.9	0
An. funestus	SR	2.6	2.0	1.2	1.2
	EIR	79.0	0.92	15.6	0.05
An. coustani	SR	0	0	0.5	0
	EIR	0	0	16.8	0
Iguhu					
An. gambiae s.s.	SR	3.6	0	2.0	2.9
	EIR	18.8	0	5.5	0.17
An. funestus	SR	2.4	4.0	0	0
	EIR	5.7	0.82	0	0
An. arabiensis	SR	0	0	0	0
	EIR	0	0	0	0

Note: SR = sporozoite rate in percent, EIR = Annual entomological inoculation rate measured as the number of infective bites/ person/year, PSC: pyrethrum spray catch

In Iguhu, the estimated *P. falciparum* EIR of *An. gambiae s.s.* from indoor and outdoor CDC light traps was 18.8 and 5.5 ib/p/year, respectively, whereas the EIR of *An. funestus* from indoor and outdoor CDC light traps was 5.7 and 0 ib/p/year, respectively. The overall indoor and outdoor EIR was 24.5 and 5.5 ib/p/year, respectively. About 22.6% of the total infective bites by *An. gambiae s.s.* occurred outdoor. The EIR of *An. gambiae s.s.* and *An. funestus* from PSCs was 0 and 0.82 ib/p/year, respectively.

3.5. Discussion

This study showed that *An. arabiensis* was the most abundant species in Ahero (lowland), whereas *An. gambiae s.s.* was the most abundant species in Iguhu (highland) sites of western Kenya. *An. funestus* was the second most abundant species in both sites, which is consistent with previous studies (Zhou et al., 2011, Ototo et al., 2015).

Anopheles arabiensis showed increased exophagic tendency in the study area when compared with the findings of studies conducted before the scale up of vector control interventions (Githeko et al., 1996, Githeko et al., 1994a). For instance, studies by Githeko *et al*. in 1990s, when ITN coverage was negligeable, showed that *An. arabiensis* was two times more likely to bite indoors than outdoors (Githeko et al., 1996). In the present study, the outdoor biting density of *An. arabiensis* was higher than indoor. The increased outdoor host-seeking tendency of *An. arabiensis* in this study compared to the previous reports might be due to the scale-up of ITNs. Bayoh *et al*. also noted that *An. arabiensis* was more likely to bite outdoors in western Kenya when compared with data collected before the scale-up of ITNs (Bayoh et al., 2014). Moreover, *An. arabiensis* showed highly exophilic behaviour in this study, with significantly higher outdoor resting density than indoor resting density.

The proportion of *An. arabiensis* has been increasing in western Kenya highlands. Until 2002, *An. gambiae s.s.* was the only member of *An. gambiae s.l.* complex reported in western Kenya highlands > 1400m a.s.l. (Githeko et al., 2006, Minakawa et al., 2002). The proportion of *An. arabiensis* was reported to be 0.8% in 2003 (Ndenga et al., 2006) and reached 9.2% in 2010 (Zhou et al., 2011). In this study, the proportion of adult *An. arabiensis* has increased to 13%. A recent study reported a higher proportion of *An. arabiensis* (38.2%) in larval population (Kweka et al., 2015). The continued proportional increase in *An. arabiensis* population might be due to the increased ITN coverage (Bayoh et al., 2010, Mwangangi et al., 2013a) and/or the zoophilic and exophagic/exophilic behaviour of this species or due to species shift. Other factors such as climatic and environmental change, which resulted in increased temperature or availability of more habitats in the area, might have also contributed as this was found to favour *An. arabiensis* (Afrane et al., 2007). Such shift in vector species composition could undermine the efficacy of ITNs as the interventions do not target zoophilic and exophilic vector species which avoids the lethal effect of ITNs and sustain residual malaria transmission (Okumu et al., 2013a).

Anopheles gambiae s.s. showed endophagic behaviour, with higher indoor host-seeking density than outdoor. This is in agreement with the earlier reports by Githeko *et al*. (Githeko et al., 1996). Recent studies in western Kenya have also showed that *An. gambiae s.s.* was more likely to seek hosts indoor than outdoor (Bayoh et al., 2014, Cooke et al., 2015). In contrast, studies in Bioko Island, Equatorial Guinea showed that *An. gambiae s.s.* seek hosts outdoor than indoor (Overgaard et al., 2012a). This difference might be due to the variation in molecular forms of *An. gambiae s.s.* (S and M/ *Anopheles coluzzii*) from Kenya and Equatorial Guinea (Lehmann et al., 2003) although the variability in host-seeking behaviour between the two molecular forms is not yet explicitly described.

It is unusual that *An. gambiae s.s.* showed similar feeding preference to human and bovine. Two decades ago, the HBI of indoor resting *An. gambiae s.s.* in western Kenya and other parts of the country was 96-97%, an indication that they had fed exclusively on humans (Githeko et al., 1994b, Shililu et al., 1998, Mwangangi et al., 2003). In this study, the overall HBI of *An. gambiae s.s.* was only 50.0% although predominantly from indoor collection. Compared to the earlier studies conducted in western Kenya before ITNs were used in large scale (Githeko et al., 1994b, Shililu et al., 1998), the HBI of indoor resting *An. gambiae s.s.* has significantly dropped by 20% and the drop was entirely replaced by BBI. For outdoor resting *An. gambiae s.s*., the BBI reached up to 46%. Similar reduction in HBI and increment in BBI has also been reported recently (Ndenga et al., 2016, Mutuku et al., 2011). This suggests an increasing tendency of *An. gambiae s.s*. to feed on bovine following the increased ITN coverage in the western Kenya highlands.

Anopheles funestus s.s. was the predominant species among *An. funestus* group in the study area. Similar findings were reported in Tanzania (Derua et al., 2015). Kweka *et al*. (Kweka et al., 2013) also found that *An. funestus s.s.* was the predominant sibling species in larvae population in western Kenya. However, there was significant difference in terms of the relative proportion of *An. funestus s.s.* between adult and larvae population. In this study, *An. funestus s.s.* accounted for 98.1% of the adult *An. funestus* group. In contrast, Kweka *et al.* found only 32.9% *An. funestus s.s.* in larvae population. This difference could be due to the presence of other zoophilic and exophilic sibling species of *An. funestus* group in the larvae that do not bite or rest indoor or around human dwellings.

Anopheles funestus showed anthropophagic behaviour in both study sites, feeding predominantly on human. The anthropophagic behaviour of *An. funestus* was frequently observed in Kenya (Githeko et al., 1994b, Mwangangi et al., 2003) and elsewhere in Africa (Tanga et al., 2011, Das et al., 2015, Mzilahowa et al., 2012, Dadzie et al., 2013). Nevertheless, they also fed on bovine, with higher BBI than the previous reports (Githeko et al., 1994b, Mwangangi et al., 2003, Tanga et al., 2011, Das et al., 2015, Mzilahowa et al., 2012, Dadzie et al., 2013).

The secondary vectors, *An. pharoensis* and *An. coustani* showed exophagic behaviour, with significantly higher outdoor host-seeking density than indoor. Other studies in Kenya (Githeko et al., 1994a, Mwangangi et al., 2013b) and elsewhere in Africa (Taye et al., 2016, Antonio-Nkondjio et al., 2006, Nepomichene et al., 2015) reported similar phenomenon for these species. It is worth mentioning that both *An. pharoensis* and *An. coustani* were very rare in both indoor resting collections and pit shelters despite their preponderance in CDC light traps. Hence, further studies are required to find out the potential resting places of *An. pharoensis* and *An. coustani.*

The EIR data showed that the majority of malaria transmission by *An. gambiae s.s.* and *An. funestus* occurred indoors, while *An. arabiensis* contributed almost equally to both outdoor and indoor transmission. The higher indoor EIRs despite high ITN coverage could be attributed to inconsistent ITN use (Atieli et al., 2011), increasing insecticide resistance among vectors (Zhou et al., 2011, Ochomo et al., 2013), and shifts in malaria vector biting times from mid-night to early evening and morning when people are still indoor but unprotected by ITNs (Cooke et al., 2015, Wamae et al., 2015). However, the magnitude of the outdoor EIRs was also considerably high compared to previous reports (Bayoh et al., 2014). The ongoing shifts in vector species composition and changes in vector behaviours might have contributed to the high outdoor EIRs.

In addition to the primary vectors, a single specimen of *An. coustani* from outdoor CDC light trap was found to be positive for *P. falciparum* CSP based on ELISA, although not yet confirmed by PCR. Studies are increasingly reporting the importance of the secondary vectors in residual malaria transmission (Mwangangi et al., 2013b, Nepomichene et al., 2015, Stevenson et al., 2012, Laurent et al., 2016, Stevenson et al., 2016). Several studies have demonstrated the susceptibility of *An. coustani* to *P. falciparum* infection (Mwangangi et al., 2013b, Antonio-Nkondjio et al., 2006, Nepomichene et al., 2015, Degefa et al., 2015). Although ELISA technique is not specific enough to incriminate zoophagic mosquitoes as a vector (Wirtz et al.,

1987), a recent study in Madagascar confirmed the presence of *Plasmodium* CSP in *An. coustani* by both ELISA and PCR (Nepomichene et al., 2015), suggesting that this species could play a role in outdoor malaria transmission.

3.6. Conclusion

Anopheles arabiensis was highly exophilic and zoophagic. *Anopheles gambiae s.s.* showed high tendency to feed on bovine while *An. funestus* showed anthropophagic behaviour. While most of malaria transmission occurred indoors, the magnitude of outdoor transmission was considerably high. Additional control tools that complement the existing interventions are required to control residual transmission. Further studies are required to comprehend the role of secondary vectors in malaria transmission.

CHAPTER FOUR

4. EVALUATION OF THE PERFORMANCE OF NEW STICKY POTS FOR OUTDOOR RESTING MALARIA VECTOR SURVEILLANCE IN WESTERN KENYA (Adopted from Degefa et al., 2019)

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Evaluation of the performance of new sticky pots for outdoor resting malaria vector surveillance in western Kenya

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4.1. Abstract

Background: Surveillance of outdoor resting malaria vector population is crucial to monitor possible changes in vector resting and feeding behaviour following the widespread use of indoor-based vector control interventions. However, it is seldom included in routine vector surveillance system in Africa due to lack of well standardized and efficient traps. This study was conducted to evaluate the performance of sticky pots for outdoor resting malaria vector surveillance in western Kenya.

Methods: Mosquito collections were conducted from September 2015 to April 2016 in Ahero and Iguhu sites, western Kenya using sticky pots, pit shelters, clay pots, exit traps, prokopack aspirator and CDC light traps (outdoor and indoor). Species within *Anopheles gambiae s.l.* were identified using polymerase chain reaction (PCR). Enzyme-linked immunosorbent assay (ELISA) was used to determine blood meal sources of malaria vectors.

Results: A total of 23,772 mosquitoes were collected, of which 13,054 were female anophelines comprising *An. gambiae s.l.* (72.9%), *An. funestus* (13.2%), *An. coustani* (8.0%) and *An.*

pharoensis (5.9%). Based on PCR assay (n = 672), 98.6% *An. arabiensis* and 1.4% *An. gambiae s.s.* constituted *An. gambiae s.l.* in Ahero, while this was 87.2% *An. gambiae s.s.* and 12.8% *An. arabiensis* in Iguhu. The sticky pots and pit shelters showed similar performance with regard to the relative abundance and host blood meal indices of *An. gambiae s.l.* and *An. funestsus*. In terms of density per trap, a pit shelter caught on average 4.02 (95% CI: 3.06-5.27) times as many *An. gambiae s.l.* as a sticky pot, while a sticky pot captured 1.60 (95% CI: 1.19-2.12) times as many *An. gambiae s.l.* as a clay pot. Exit traps yielded significantly lower number of *An. gambiae s.l.* than all other traps in Ahero, but higher number of *An. gambiae s.l.* compared to the other outdoor traps in Iguhu. Indoor CDC light traps captured significantly higher number of *An. funestus* than the other traps.

Conclusions: The sticky pots could be a useful and complementary tool for outdoor resting malaria vector surveillance, in settings where using pit shelters is not feasible and less productive. The lower vector density in the sticky pots compared to pit shelters suggests the need to deploy sticky pots in batches (i.e. 4 sticky pots per compound) if comparable results to those that would have been estimated with pit shelters is needed. This study also highlighted the need to concurrently undertake indoor and outdoor vector surveillance to better understand residual malaria transmission.

4.2. Introduction

Surveillance of adult malaria vectors is a prerequisite to determine vector density, species composition, behaviour and sporozoite infection rates for surveillance driven control and to evaluate the impact of control interventions. The surveillance tools and procedures usually differ depending on the type entomological indices to be measured, such as vector biting behaviour, blood meal sources, resting habits or malaria transmission intensity (Service, 1977). The vector species may occur as indoor host-seeking, indoor resting, outdoor host-seeking and outdoor resting fractions, each requiring different surveillance tools and approaches (WHO, 1975).

In most African countries, malaria vector surveillance activities rely mainly on sampling host-seeking and indoor resting mosquitoes. The most commonly used methods for sampling host-seeking vectors are human landing catches (HLC) and Center for Disease Control and Prevention (CDC) light traps (WHO, 2003). Indoor resting vectors are often sampled by pyrethrum spray catches (PSCs) and indoor aspiration using Prokopack aspirator (Vazquez-Prokopec et al., 2009) or Backpack aspirator (Clark et al., 1994). Yet outdoor resting vector sampling is seldom included in the routine vector surveillance system due to lack of well standardized and efficient traps.

However, data from outdoor resting collections is also crucial to monitor possible changes in vector resting and feeding behaviour following the widespread use of indoor-based vector control interventions (WHO, 2013c). This is particularly important in Africa where there is an increasing shift in vector species composition from anthropohagic, endophilic vectors to zoophagic, exophilic sibling species following the wide scale use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS) (Bayoh et al., 2010, Russell et al., 2011, Kitau et al., 2012, Mwangangi et al., 2013a, Derua et al., 2012). Such shifts in vector resting behaviour may also occur within vector species, as evidenced by an increased exophilic tendency in *An. gambiae s.s.* under the influence of insecticide use in houses in western Kenya (Githeko et al., 1996). Such behavioural shift could pose a problem on control efforts as the current interventions (ITNs and IRS) do not target outdoor and early indoor biting vectors which eventually rest outdoors to escape from contact with insecticide-treated surfaces and sustain residual malaria transmission (Durnez and Coosemans, 2013).

Traditionally, mechanical aspiration of mosquitoes from their natural resting sites such as vegetation, cracks on stone walls, holes in rocks and crevices in the ground or artificial pit-shelters has been used as a method for sampling outdoor resting malaria vectors (WHO, 1995, Service, 1993a). Pit shelters have the advantage of providing concentrated sites for collections and representative samples that can be used for quantitative work (WHO, 2013c). However, sampling inside pits is difficult to standardize. It is also difficult to maintain pit shelters especially during the rainy season as the pits could be saturated with water. Moreover, dangerous animals such as snakes may also be encountered in the pits, causing a risk to mosquito collectors. Last but not the least, pits cannot be moved and cannot be deployed in large numbers, which limits its deployment as a general routine surveillance tool.

Recently, alternative sampling tools such as clay pots and resting boxes have also been developed for similar purpose (Odiere et al., 2007, Kweka et al., 2009, Pombi et al., 2014). The advantage of these tools is that they are small and portable so that they could be deployed in large numbers and in different settings. Although clay pots have been shown to have good performance when used in batches (i.e. six pots per compound) (Odiere et al., 2007), retrieving mosquitoes resting within the pots needs active aspiration by collectors which may lead to collection bias due to variation in skill among collectors. Moreover, mosquitoes could escape at any time before collection when the pots are disturbed by animals or children playing in the area. Hence, there is a need to develop and standardize tool for outdoor resting malaria vector surveillance.

The aim of this study was thus to evaluate new sticky pots for outdoor resting malaria vector surveillance. The trapping efficiency of the sticky pots was compared with pit shelters, clay pots, window exit traps and prokopack aspirator in western Kenya. Moreover, Centers for Disease Control and Prevention (CDC) light traps were employed in this study to assess whether mosquito species composition and diversity in the outdoor resting collections (by sticky pots, pit shelters and clay pots) are similar with that of host-seeking vector collections.

4.3. Methods

4.3.1. Study sites

The study was conducted in Ahero (0°.11'S, 34°.55'E, altitude 1162m) and Iguhu (0°.17'N; 34°.74'E, altitude 1,430–1,580 m a.s.l) sites in western Kenya (mmm). Ahero is a lowland plain area located in Kisumu County, while Iguhu is highland with flat-bottomed valleys in Kakamega County. The sites have bimodal pattern of rainfall, with the long rainy season from April to June, which triggers the peak malaria transmission period and the short rainy season from October to November with minimal transmission (Munyekenye et al., 2005). The hot and dry season is from January to March (Zhou et al., 2011). *Plasmodium falciparum* is the predominant malaria species in the area and is transmitted by *Anopheles gambiae sensu stricto (s.s), An. arabiensis* and *An. funestus* (Zhou et al., 2011, Githeko et al., 2006, Ototo et al., 2015).

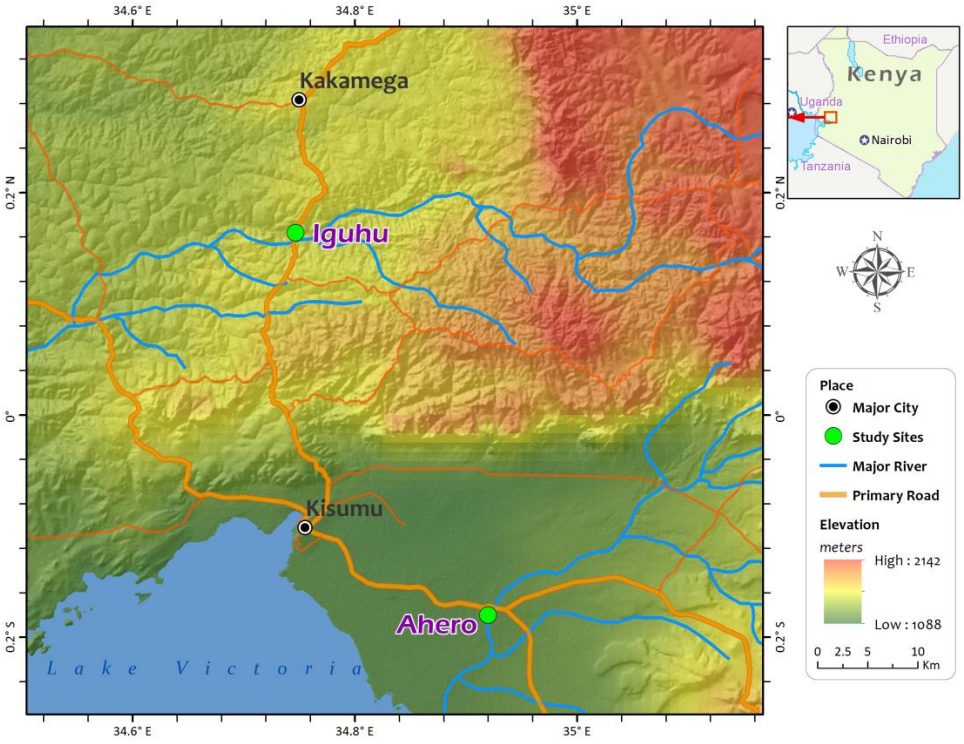

Figure 4.1. Map of the study sites in Kenya

4.3.2. Description of trapping methods

4.3.2.1. Pit shelters

A rectangular pit was dug in to ground (1.5 m in depth, 1.2 m in length and 1 m in width) within 20 m from each selected house (Plate 4.1a). In each of the four vertical sides, about 50-60 cm and 90-100 cm from the bottom of the pit, two little cavities were dug in to a depth of about 30 cm. The main pits were then shaded by artificial framework thatched with locally available reeds. Resting mosquitoes were sampled from 06:00 to 09:00 hr inside the eight cavities by using hand-held mouth aspirators and intensive visual search.

4.3.2.2. Sticky pots

Sticky pots are sticky variants of clay pots that have been used previously to collect outdoor resting *Anopheles* mosquitoes (Odiere et al., 2007). Each sticky pot has an opening of 20 cm width, a round bottom, and a maximum width of 45 cm. The internal surface of the pots was covered with waterproof black papers coated with Tangle-Trap sticky substance (Plate 4.1b). This modification was done based on the assumption that covering the internal wall of clay pots with waterproof sticky paper would trap every mosquito that rests within the pot, not only the fractions present at the time of collection. The sticky pots were placed outdoors from 18:00 to 06:00 hr to trap resting mosquitoes. Trapped mosquitoes were collected from the sticky pots using forceps from 06:00 to 09:00 hr in the morning following each sampling night.

4.3.2.3. Clay pots

Pots similar to sticky pots but without sticky substance were used (Plate 4.1c). The pots were placed outdoors from 18:00 to 06:00 hr. Mosquitoes were collected from the pots once in the morning from 06:00 to 09:00 hr as follows. White mesh from a mosquito cage was carefully placed over the mouth of the pot and secured as described by Odiere et al. (Odiere et al., 2007). The collector then lifted the pot and agitated mosquitoes inside the pot, causing them to fly and move into the cage. The mesh was then removed, and any remaining mosquitoes in the pot were retrieved using an aspirator and transferred to a labeled paper cap. Mosquitoes were finally collected from the cage using aspirator and transferred to the paper cup, completing the collection.

4.3.2.4. Window exit trap

Exit traps are rectangular boxes made of a wooden frame covered with netting material, with a slit-shaped rectangular tilted wire opening at one side as a mosquito entrance and a sealable cotton sleeve aspirator inlet on the other side. The trap was set on a window of each of the selected houses every evening at 18:00 hr (Plate 4.1d). Mosquitoes were retrieved from the trap using hand-held aspirator through a sealable sleeve in the morning from 06:00 to 09:00 hr.

4.3.2.5. Prokopack aspirator

The prokopack aspirator (John W. Hock) was developed by Vasquez-Prokopec *et al.* in 2009 for sampling indoor resting mosquitoes (Vazquez-Prokopec et al., 2009). The aspirator is powered by a 12V battery. Indoor resting mosquito collection using prokopack aspirator from selected houses was performed every morning concurrently with that of outdoor sampling. Mosquitoes resting on the walls and the area under the roof of the houses or ceilings were systematically aspirated by using progressive downward and upward movements along the wall surfaces of the room.

Plate 4.1. Vector sampling tools used for outdoor and/or indoor resting/host-seeking malaria vector surveillance in Ahero and Iguhu sites, western Kenya

a) Pit shelter, b) sticky pot, c) clay pot, d) exit trap, e) outdoor CDC light trap, f) indoor CDC light trap (Pictures captured in the field).

4.3.2.6. CDC miniature light traps

CDC miniature light traps (John W. Hock Ltd, Gainesville, FL., USA) were set inside selected houses near an occupied bed net at a height of 1.5 m from 18:00 to 06:00 hr in the night to collect indoor host-seeking mosquitoes. For the outdoor host-seeking mosquito sampling, CDC light trap was also set in the vicinity (within 2 m) of sentinel houses from 18:00 to 06:00 hr (Plate 4.1e).

4.3.3. Experimental design

Each study site was classified into ten clusters. A cluster was defined as group of houses closely located on a similar topography. Two houses, approximately 50 m apart, were randomly selected from each cluster, hence a total of 20 houses were selected per site. In each cluster, the two houses were numbered as H1 and H2. One of the two houses was then used for the following combination of trapping methods: one sticky pot and one clay pot placed outdoor at about 5 m from the house, an exit trap set on window, sampling from a pit shelter located within 20 m from the house and indoor aspiration was carried out using prokopack aspirator. The second house was used for setting CDC light traps (one indoor and one outdoor). In each cluster, the trapping methods were swapped between the two houses for two consecutive days every month. Mosquito collections were conducted during the short rainy season (September to November) in 2015 and dry season (February to April) in 2016. A total of 120 trap-nights were done for each trapping method in each study site.

4.3.4. Sample processing

All collected mosquitoes were identified morphologically to species or species complexes using keys (Gillies and Coetzee, 1987). Female *Anopheles* mosquitoes were further classified as unfed, freshly fed, half-gravid and gravid. Each female *Anopheles* mosquito was then kept in a labelled 1.5 ml Eppendorf tube with cotton wool over silica gel desiccant. Samples were stored at -20 °C freezer at Climate and Human Health Research Laboratory of Kenya Medical Research Institute (KEMRI) until used for further processing.

4.3.5. Molecular identification of vector species complexes

Members of *An. gambiae sensu lato* (*s.l.*) and *An. funestus* group were identified to species by polymerase chain reaction (PCR), following the protocols developed by Scott et al. for *An.*

gambiae s.l. (Scott et al., 1993) and Koekemoer et al. for *An. funestus* group (Koekemoer et al., 2002), respectively.

4.3.6. Detection of blood meal sources

The blood meal sources of blood fed *Anopheles* mosquitoes were analyzed by a direct enzyme-linked immunosorbent assay (ELISA) using human, bovine, goat, chicken and dog antibodies (Beier et al., 1988). Positive controls were included for each host during the assay. Laboratory reared unfed *An. gambiae* was used as negative control.

4.3.7. Data analysis

The relative abundance of anopheline mosquitoes collected by each trap was determined as the percent composition of each anopheline species relative to the total number of anophelines captured. χ^2-test was used to compare the difference in *Anopheles* mosquito species composition among the trapping methods. The difference in *Anopheles* mosquito density among the different trapping methods was compared using a generalized linear model (GLM) based on negative binomial distribution. Sampling season was treated as covariate in the model. Estimated marginal mean (EMM) density of *Anopheles* mosquitoes was determined for each trap using negative binomial regression by adjusting for season. Pairwise comparison of different traps in terms of the EMM of *Anopheles* mosquitoes was also performed using the negative binomial regression model.

Gini-Simpson's diversity index (1-D) (Simpson, 1949, Peet, 1974, Magurran, 2013) was applied to evaluate mosquito species diversity for each trap. To determine the statistical significance of difference in species diversity among the traps, 95% confidence intervals (CI) were calculated (Grundmann et al., 2001). The Simpson's index of evenness (E) was calculated to obtain a measure of the relative abundance of the different species in the sample (Simpson, 1949, Kwak and Peterson, 2007).

Human blood index (HBI) was calculated as the number of *Anopheles* mosquitoes that fed on human over the total number of *Anopheles* tested for blood meal origins multiplied by a hundred (Garrett-Jones, 1964). Bovine blood index (BBI) and blood meal indices of other hosts (goat, dog and chicken) were also determined in a similar way. Mixed blood meals were included in the

calculation of blood meal indices (Pappa et al., 2011). χ^2-test was used to compare host blood meal indices of malaria vectors between different trapping methods.

Data were analyzed using R 3.3 (R Core Team) and SPSS version 20.0 (SPSS, Chicago, IL, USA) software packages. $p < 0.05$ was considered statistically significant during the analysis.

4.4. Results

4.4.1. Mosquito species composition and abundance

A total of 23,772 mosquitoes were collected during the study period (Table 4.1): 5,847 (24.6%) from pit shelters, 1,627 (6.8%) by sticky pots, 1,249 (5.3%) by clay pots, 6,311 (26.6%) by outdoor CDC light traps, 1,400 (5.9%) by exit traps, 2,715 (11.4%) from indoor by prokopack aspirator and 4,623 (19.4%) by indoor CDC light traps. The majority (74.9%) of the collected mosquitoes were anophelines, while the remaining 25.1% were *Culex* species. Most (89.3%) of the mosquitoes were collected from Ahero site. Of the 17,807 anopheline mosquitoes collected, 73.3% (n =13,054) were female anophelines. *Anopheles gambiae s.l.* was the predominant species accounting for 72.9% of the total female *Anopheles* mosquitoes collected, followed by *An. funestus* group (13.2%), *An. coustani* (8.0%) and *An. pharoensis* (5.9%).

Table 4.1. Summary of mosquitoes collected by different trapping methods in Ahero and Iguhu sites, western Kenya (n = 120 trap-nights per site for each trap)

Site and species	Sex	Outdoor					Indoor		Total
		Pit shelter	Sticky pot	Clay pot	Light trap	Exit trap	Prokopack	Light trap	
Ahero									
An. gambiae s.l.	Female	3,262	706	510	1,636	336	1,031	1,592	9,073
	Male	1,876	634	501	210	168	551	178	4,118
An. funestus group	Female	142	28	16	270	380	135	628	1,599
	Male	72	24	18	26	35	108	7	290
An. coustani	Female	15	2	0	652	41	3	321	1,034
	Male	1	0	0	8	1	0	4	14
An. pharoensis	Female	0	0	0	688	1	0	78	767
	Male	0	1	0	42	0	0	2	45
Culex species	Female	88	51	30	2,044	90	59	1,064	3,426
	Male	79	32	38	463	16	27	214	869
Iguhu									
An. gambiae s.l.	Female	41	9	7	56	159	57	108	437
	Male	86	37	34	4	29	37	7	234
An. funestus group	Female	4	3	2	13	17	42	49	130
	Male	19	1	1	0	11	15	3	50
An. coustani	Female	0	0	0	10	0	1	3	14
	Male	1	1	0	0	0	0	0	2
Culex species	Female	101	53	44	70	53	399	142	862
	Male	60	45	48	119	63	250	223	808
Total		5,847	1,627	1,249	6,311	1,400	2,715	4,623	23,772

Figure 4.2 shows the relative abundance of *Anopheles* mosquitoes collected by different trapping methods. The relative abundance of *Anopheles* species collected by the sticky pots was similar with that of pit shelters (χ^2 = 0.429, df = 2, p = 0.807) and clay pots (χ^2 =3.21, df = 2, p = 0.201), *An. gambiae s.l.* being the most predominant species accounting for 95.9%, 95.4% and 96.5% of the anophelines collected by the sticky pots, pit shelters and clay pots, respectively. However, there was significant difference between outdoor and indoor located traps i.e. pit shelters versus prokopack (χ^2 = 139, df = 2, p < 0.001) and outdoor CDC light traps versus indoor CDC light traps (χ^2 = 720, df = 3, p < 0.001). For instance, the proportion of *An. funestus* group was 15.2% by prokopack aspirator, while it was 3.9%, 4.3%, and 3.4% by sticky pots, pit shelters and clay pots, respectively. Similarly, *An. funestus* group accounted for 23.1% of the anopheline species collected by indoor CDC light traps, while it was 8.5% by outdoor CDC light traps.

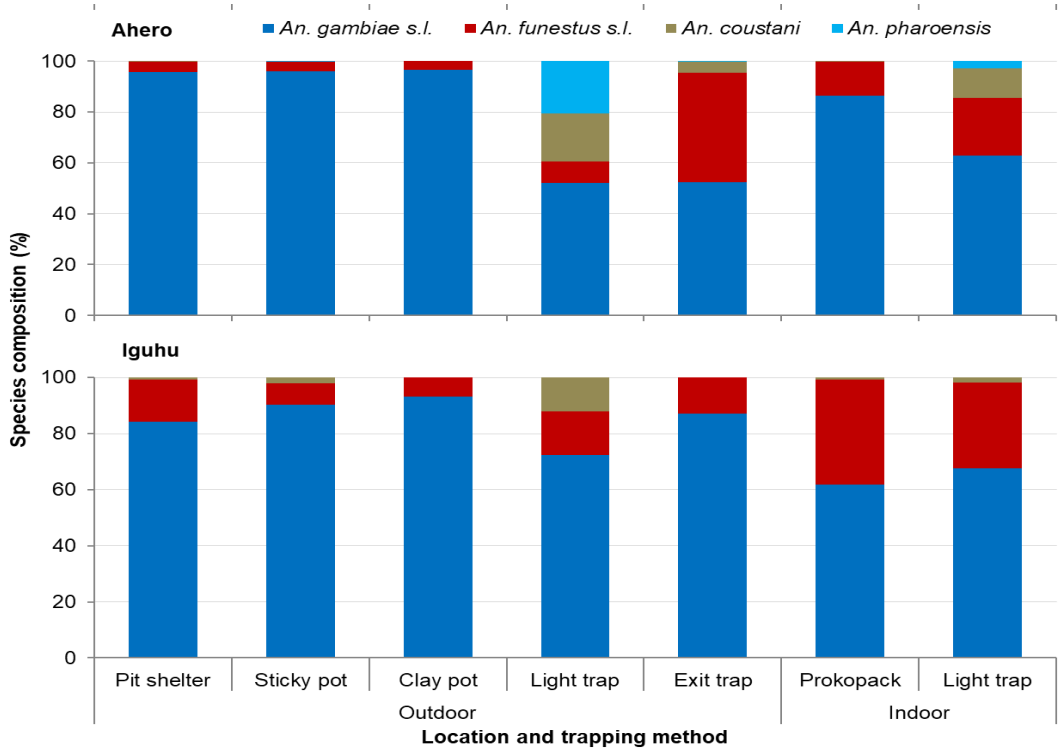

Figure 4.2. The relative abundance of female *Anopheles* mosquitoes collected by different trapping methods in Ahero and Iguhu sites, western Kenya

4.4.2. Species diversity

Mosquito species diversity was significantly higher from sticky pots (Simpson diversity index = 0.26±0.03) than pit shelters (0.18±0.02), but in both traps mosquito species diversity was lower

as compared to outdoor CDC light traps (0.70±0.01), exit traps (0.63±0.01), prokopack aspirator (0.53±0.02) and indoor CDC light traps (0.68±0.01) (Table 4.2). There was no significant difference in mosquito species diversity between collections from sticky pots and clay pots. Outdoor CDC light traps collected mosquitoes of different species more evenly (Simpson's evenness index = 0.87) than the other traps, while the species evenness of mosquitoes collected in pit shelters (evenness index = 0.25) and sticky pots (evenness index = 0.32) were relatively lower compared to other traps.

Table 4.2. Comparison of mosquito species diversity among different trapping methods, western Kenya

Place of collection	**Trapping method**	**Species richness**	**Simpson's diversity index, 1-D (95% CI)**	**Simpson's evenness, E**
Outdoor	Pit shelter	4	0.18 (0.17-0.20)[a]	0.25
	Sticky pot	5	0.26 (0.23-0.29)[b]	0.32
	Clay pot	3	0.27 (0.24-0.30)[b]	0.37
	Light trap	5	0.70 (0.69-0.71)[d]	0.87
	Exit trap	5	0.63 (0.62-0.64)[c]	0.79
Indoor	Prokopack	4	0.53 (0.52-0.55)[e]	0.71
	Light trap	5	0.68 (0.67-0.69)[f]	0.85

4.4.3. Mosquito density

The density of female *Anopheles* mosquitoes varied among different traps (Table 4.3 and 4.4). In Ahero, pit shelters yielded significantly higher number of *An. gambiae s.l.* (EMM density per pit = 24.26, 95% CI: 19.79-28.73) than all other traps ($p < 0.05$). After adjusting for season, a pit shelter caught on average 4.02 (95% CI: 3.06-5.27) and 6.37 (95% CI: 4.83-8.41) times as many *An. gambiae s.l.* per day as a sticky pot and clay pot, respectively. Similarly, pit shelters yielded 2.95 (95% CI: 2.26-3.87), 10.21 (7.67-13.60), 3.19 (2.44-4.16) and 2.96 (95% CI: 2.26-3.87) times density of *An. gambiae s.l.* compared to outdoor CDC light traps, exit traps, prokopack aspirator and indoor CDC light traps, respectively. The mean density of *An. gambiae s.l.* was significantly higher in sticky pots than clay pots and exit traps ($p < 0.05$). A sticky pot caught 1.60 (95% CI: 1.19-2.12) and 2.54 (95% CI: 1.89-3.42) times as many *An. gambiae s.l.* as a clay

pot and an exit trap, respectively. The difference in mean *An. gambiae s.l.* between indoor and outdoor CDC light traps was not significant ($p = 0.986$).

In Iguhu on the other hand, the mean density of *An. gambiae s.l.* was significantly higher from exit traps than all other traps except indoor CDC light traps. The mean density of *An. gambiae s.l.* was significantly higher from pit shelters as compared to sticky pots and clay pots, whereas the difference in mean density of *An. gambiae s.l.* between pit shelters and prokopack aspirator was not significant ($p = 0.20$). The mean density of *An. gambiae s.l.* was significantly higher from indoor CDC light traps than outdoor CDC light traps (Table 4.3).

The mean density of *An. funestus* group was significantly higher from indoor CDC light traps than the other traps in both sites. In Ahero, pit shelters captured higher density of *An. funestus* group than sticky pots and clay pots, whereas in Iguhu the mean density of *An. funestus* group did not vary significantly among the three traps ($p > 0.05$) (Table 4.3).

Table 4.3. Estimated marginal mean density for female *An. gambiae s.l.* and *An. funestus* group in Ahero and Iguhu sites, western Kenya

Site and species	Outdoor					Indoor	
	Pit shelter	**Sticky pot**	**Clay pot**	**Light trap**	**Exit trap**	**Prokopack**	**Light trap**
Ahero							
An. gambiae s.l.	24.26 (19.79-28.73)[a]	6.03 (4.82-7.25)[b]	3.81 (3.02-4.59)[c]	8.21 (6.63-9.80)[c]	2.38 (1.85-2.89)[d]	7.62 (6.14-9.09)[b,c]	8.19 (6.61-9.77)[c]
An. funestus group	0.79 (0.58-1.00)[a]	0.16 (0.09-0.23)[b]	0.09 (0.04-0.14)[b]	1.77 (1.36-2.19)[c]	1.86 (1.44-2.28)[c]	0.74 (0.54-0.94)[a]	4.59 (3.64-5.54)[d]
Iguhu							
An. gambiae s.l.	0.33 (0.21-0.45)[a]	0.07 (0.02-0.12)[b]	0.05 (0.01-0.10)[b]	0.46 (0.31-0.61)[a]	1.20 (0.91-1.49)[c]	0.45 (0.31-0.59)[a]	0.91 (0.67-1.15)[c]
An. funestus group	0.03 (0.001-0.06)[a]	0.02 (0.00-0.05)[a]	0.02 (0.00-0.04)[a]	0.11 (0.04-0.17)[b]	0.14 (0.07-0.21)[b]	0.33 (0.21-0.45)[c]	0.40 (0.26-0.53)[c]

Key: For each study site, across each row, the different letters indicate that the estimated marginal mean density varied significantly ($p < 0.05$). The estimated marginal means were determined using negative binomial regression model by adjusting for season.

Table 4.4. Estimates of a negative binomial regression for comparison of vector density between pit shelter and other trapping methods in western Kenya

Species and place of collection	Trapping method	Ahero		Iguhu	
		Exponentiated estimate (OR)	p-value	Exponentiated estimate (OR)	p-value
An. gambiae s.l.					
Outdoor	Pit shelter	1.0*		1.0*	
	Sticky pot	0.25 (0.20-0.33)	0.000	0.22 (0.10-0.47)	0.000
	Clay pot	0.16 (0.12-0.20)	0.000	0.17 (0.07-0.39)	0.000
	Light trap	0.34 (0.26-0.44)	0.000	1.40 (0.86-2.27)	0.173
	Exit trap	0.10 (0.07-0.13)	0.000	3.65 (2.37-5.61)	0.000
Indoor	Prokopack	0.31 (0.24-0.41)	0.000	1.37 (0.85-2.21)	0.199
	Light trap	0.34 (0.26-0.44)	0.000	2.76 (1.77-4.30)	0.000
An. funestus group					
Outdoor	Pit shelter	1.0*		1.0*	
	Sticky pot	0.20 (0.122-0.33)	0.000	0.75 (0.17-3.35)	0.716
	Clay pot	0.12 (0.07-0.21)	0.000	0.50 (0.09-2.80)	0.433
	Light trap	2.25 (1.58-3.21)	0.000	3.27 (1.04-10.33)	0.044
	Exit trap	2.36 (1.68-3.32)	0.000	4.37 (1.43-13.40)	0.010
Indoor	Prokopack	0.94 (0.64-1.36)	0.726	10.37 (3.60-29.88)	0.000
	Light trap	5.83 (4.14-8.20)	0.000	12.33 (4.3-35.30)	0.000

*Reference value, OR-odds ratio

4.4.4. Composition of *An. gambiae* and *An. funestus* species complexes

A total of 872 specimens (738 *An. gambiae s.l.*) and 134 *An. funestus* group) from different traps were analysed for identification of sibling species. Of these, 672 *An. gambiae s.l.* and 110 *An. funestus* group specimens were successfully amplified and identified to species using species specific PCR. Figure 4.3 shows member species of *An. gambiae s.l.*. In Ahero, of the *An. gambia*e *s.l.* specimens assayed, *An. arabiensis* and *An. gambiae s.s.* accounted for 98.6% and 1.4%, respectively. The proportion of *An. arabiensis* was 100.0% from pit shelters, sticky pots, clay pots and outdoor CDC light traps, while it was 92.9%, 96.5% and 97.4% in exit traps, prokopack aspirator and indoor CDC light traps, respectively. In Iguhu, of the *An. gambiae s.l.*

specimens assayed, *An. arabiensis* and *An. gambiae s.s.* accounted for 12.8% and 87.2%, respectively. Overall, *An. gambiae* sibling species composition did not vary significantly between pit shelters and sticky pots (χ^2 = 0.018, df = 1, p = 0.894), pit shelters and clay pots (χ^2 = 0.122, df = 1, p = 0.727), however there was significant difference in species composition between collections from pit shelters and other traps (p < 0.001). Of the amplified *An. funestus* group specimens, *Anopheles funestus s.s.* (hereafter *An. funestus*) and *An. leesoni* accounted for 98.2% and 1.8%, respectively. The sibling species composition of the *An. funsetus* group did not vary significantly among the different traps (χ^2 = 5.69, df = 6, p = 0.459).

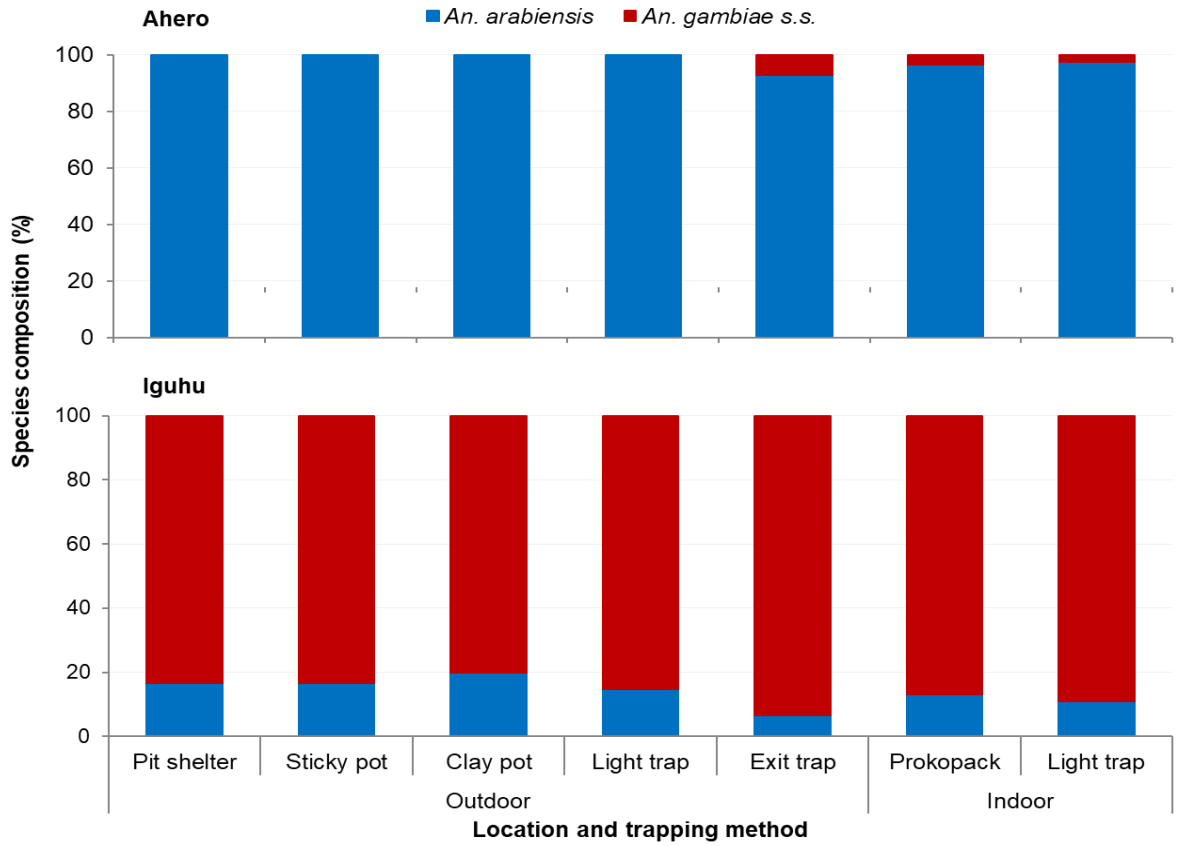

Figure 4.3. Composition of *An. gambiae* sibling species in Ahero and Iguhu sites, western Kenya

4.4.5. Physiologic status

Figure 4.4 shows physiological status of *An. gambiae s.l.* and *An. funestus*. The physiological status of *An. gambiae s.l.* collected by different traps was significantly different (χ^2 = 3510, df = 18, p = <0.001). Pit shelters, sticky pots, clay pots and prokopack aspirator yielded relatively

higher proportion of blood fed *An. gambiae s.l.*, whereas exit traps and CDC light traps captured mostly unfed *An. gambiae s.l.*. Similarly, the physiological status of *An. funestus* varied significantly among the different traps (χ^2 = 694, df = 18, p .001). Prokopack aspirator yielded higher proportion of blood fed *An. funestus*, and relatively fewer unfed *An. funestus* than the other traps. Most of the *An. funestus* collected by exit traps (90%) and CDC light traps (> 94%) were unfed.

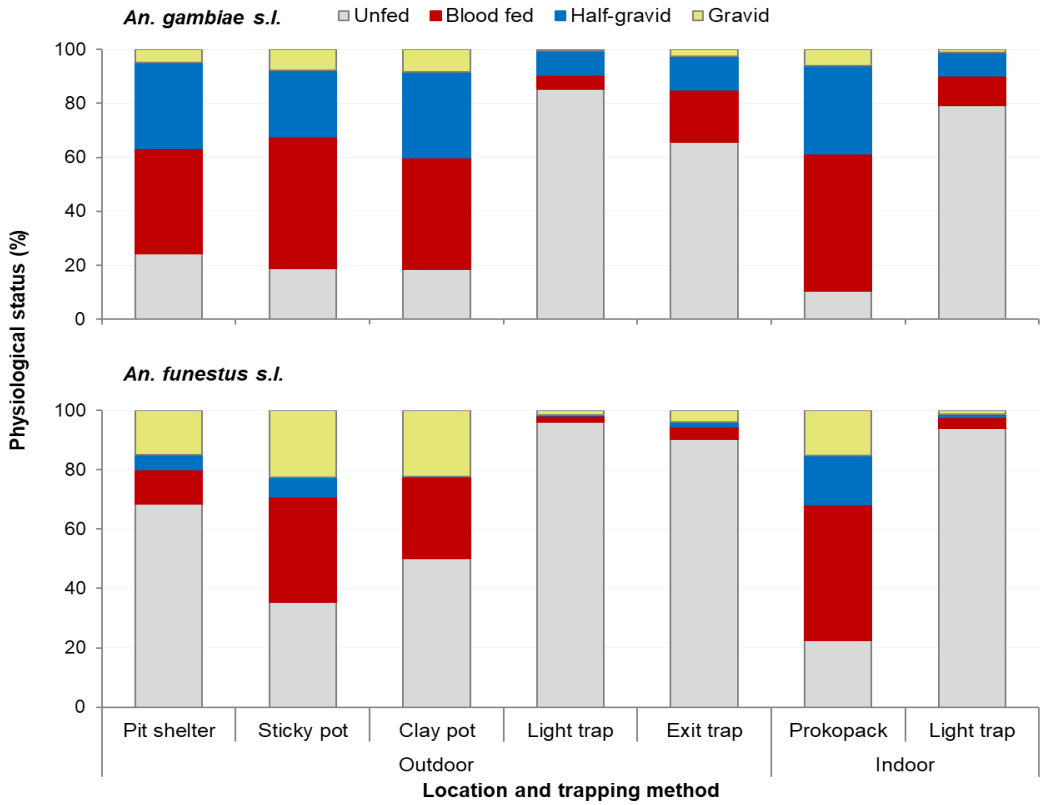

Figure 4.4. Physiological status of *An. gambiae s.l.* and *An. funestus* group collected by different trapping methods, western Kenya

4.4.6. Blood meal sources

Table 4.5 shows the host blood meal indices of malaria vectors collected by different traps. In Ahero, the overall HBI and BBI of *An. arabiensis* was 2.2% and 75.7%, respectively. There was no significant difference between pit shelters and sticky pots in terms of the host blood meal indices of *An. arabiensis* (χ^2 = 0.492, df = 2, p = 0.782). Similarly, blood meal indices of *An. arabinesis* did not vary significantly between pit shelters, clay pots and exit traps (p > 0.05).

However, there was significant difference between pit shelters and outdoor CDC light traps (χ^2 = 33.2, df = 2, p < 0.001), pit shelters and prokopack (χ^2 =14.6, df = 2, p = 0.001), and pit shelters and indoor CDC light traps (χ^2 =35.6, df = 2, p < 0.001) in terms of the blood meal indices of *An. arabiensis*.

In Iguhu, the overall HBI and BBI of *An. gambiae s.s.* was 45.7 and 28.6%, respectively. There was no significant difference between pit shelters and sticky pots in terms of the host blood meal indices of *An. gambiae s.s.* (χ^2 = 0.049, df = 2, p = 0.976). Likewise, the blood meal indices of *An. gambiae s.s.* did not vary significantly between pit shelters, clay pots, outdoor CDC light traps and exit traps (p > 0.05). However, the blood meal indices of *An. gambiae s.s.* varied significantly between pit shelters and prokopack (χ^2 = 7.195, df = 2, p = 0.027) as well as between pit shelters and indoor CDC light traps (χ^2 = 7.48, df = 2, p = 0.024). The HBI of *An. gambiae s.s.* from indoor CDC light traps (70.0%) and prokopack (75.0%) was relatively higher than the HBI of *An. gambiae s.s.* from outdoor traps i.e. pit shelters (23.1%), sticky pots (25.0%), clay pots (33.3%), outdoor CDC light traps (20.0) and exit traps (42.9%). On the other hand, the BBI of *An. gambiae s.s.* from outdoor traps was higher than the BBI of *An. gambiae s.s.* from in the indoor traps (Table 4.5).

The overall HBI and BBI of *An. funestus* was 58.0 and 23.5%, respectively. The host blood meal indices of *An. funestus* did not vary significantly among different traps (χ^2 = 13.24, df = 12, p = 0.352). Blood meal indices of other hosts (goat, dog and chicken) were low for all anopheline species in all traps.

Table 4.5. Blood meal indices of malaria vector species collected by different trapping methods in western Kenya

Species	Blood-meal indices	Outdoor					Indoor		Total
		Pit shelter	Sticky pot	Clay pot	Light trap	Exit trap	Prokopack	Light trap	
An. arabiensis	Number tested	298	66	47	59	30	100	122	722
	HBI	0.7	1.5	0	3.4	3.3	1.0	8.2	2.2
	BBI	85.6	84.8	83	50.8	73.3	68.0	62.3	75.7
	GBI	1.3	1.5	2.1	1.7	0	7.0	4.1	2.6
	DBI	3.4	3.1	2.1	1.7	0	2.0	3.3	2.8
	CBI	0.7	0	0	0	0	6.0	1.6	1.4
	Unknown	10.1	10.6	12.8	42.4	23.3	18.0	23.8	17.0
An. gambiae s.s.	Number tested	13	4	3	10	14	16	10	70
	HBI	23.1	25	33.3	20	42.9	75.0	70	45.7
	BBI	46.2	50	66.7	40	14.3	25.0	0	28.6
	GBI	0	0	0	0	0	0	0	0
	DBI	7.7	0	0	0	0	6.3	0	2.9
	CBI	0	0	0	0	0	0	0	0
	Unknown	23.1	25	0	40	42.9	0	30	24.3
An. funestus	Number tested	13	10	3	6	7	56	24	119
	HBI	46.2	50	33.3	50	57.1	62.5	62.5	58.0
	BBI	38.5	50	66.7	33.3	14.3	19.6	8.3	23.5
	GBI	0	0	0	0	0	1.8	4.2	1.7
	DBI	7.7	0	0	0	0	1.8	4.2	2.5
	CBI	0	0	0	0	0	0	0	0
	Unknown	7.7	0	0	16.7	28.6	17.9	20.8	16.0

Key: HBI-human blood index, BBI-bovine blood index, GBI-goat blood index, DBI-dog blood index, CBI-chicken blood index, HBI was calculated as the proportion (%) of mosquitoes positive for human (including mixed blood-meals) out of the total number of mosquitoes tested. Blood meal indices of other hosts were determined in a similar way.

4.5. Discussion

The results of this study showed that the new sticky pots performed consistently with pit shelters with regard to the relative abundance of anopheline species captured. In both traps, *An. gambiae s.l.* was the most abundant anopheline species with remarkably similar proportion followed by *An. funestus* group, indicating that the sticky pots could be a useful alternative tool for outdoor resting malaria vector surveillance, substituting pit shelters. Although pit shelters have been considered as a productive tool for sampling outdoor resting mosquito vectors (WHO, 1975, WHO, 2013c), digging pits is not practical in many situations especially during a rainy season since the pits could be filled with water, causing a risk to children and livestock wandering in the area (WHO, 1975).

However, the mean density of anophelines per trap was significantly lower in the stick pots compared to pit shelters. This variation could be due to the differences in the size of the two traps. A pit shelter had eight cavities for mosquito collection with a total volume (~12,000cm^3/cavity) roughly equivalent to the volume of five sticky pots (~20,000cm^3/pot). Previous studies have also reported similar findings for traps of smaller size relative to pit shelters. For instance, a pit shelter captured 5-8 times as many *An. gambiae s.l.* as a sticky resting box in Burkina Faso (Pombi et al., 2014). Similarly, a study done by Odiere et al. (Odiere et al., 2007), in which six clay pots were pooled for each pit shelter, showed that a clay pot actually yielded lower number of *An. gambiae s.l.* compared to a pit shelter. In this study, a pit shelter caught on average 4 times as many *An. gambiae s.l.* as a sticky pot. This suggests that deploying four sticky pots per compound could replace a pit shelter for sampling outdoor resting *An. gambiae s.l.* A similar relative catching rate was also recorded for *An. funestus*.

The sticky pots performed better than clay pots in terms of the mean number of outdoor resting *An. gambiae s.l.* collected per trap. This shows that coating the internal surface of the sticky pots with sticky paper increased their trapping efficiency as compared to clay pots. Actually, the adhesive feature of the sticky pots offers an additional advantage of allowing passive collection of resting mosquitoes compared to clay pots and pit shelters, both of which need active aspiration of resting mosquitoes (Odiere et al., 2007, WHO, 1975).

Furthermore, the sticky pots have a number of advantages over pit shelters and clay pots. First, sticky pots are standardized trapping method and not biased by the skill of a collector, while

mosquito collection from pits and clay pots relies on the skill of the collector, and a fraction of mosquitoes could escape during the collection process. Second, sticky pots are cheaper compared to pit shelters. The cost of making a sticky pot was less than US$4, whereas that of building a pit shelter was more than US$25 for this study. Third, sticky pots are portable and can be rotated to different sites for use unlike pit shelters which are fixed. Moreover, sticky pots are environmentally safe compared to pit shelters which may raise community concern associated with digging the pits in their compound.

The host blood-meal indices of anopheline mosquitoes collected by the sticky pots were also similar with that of pit shelters, indicating the importance of the sticky pots for monitoring the feeding behaviour of exophilic anopheline mosquitoes in settings where using pit shelters is not feasible. This could address the problem of outdoor vector surveillance tools in an effort to monitor vector feeding behaviour due to a difficulty of locating adults in their highly dispersed outdoor resting sites (Service, 1977, Silver, 2007). The sticky pots have the potential to overcome such challenge.

When we compare all the traps deployed in this study, mosquito species diversity and mean density varied significantly between traps of different location (indoor vs. outdoor). In Ahero, the density of resting *An. arabiensis* was significantly higher in pit shelters than prokopack aspirator, whereas in Iguhu, the density of *An. gambiae s.l.* (87.2% of which were *An. gambiae s.s.*) was higher from prokopack aspirator than pit shelters. The density of host-seeking *An. arabiensis* was relatively higher in outdoor than indoor CDC light traps in Ahero, while the mean density of host-seeking *An. gambiae s.s.* was significantly higher in indoor than outdoor CDC light traps. Such differences could be explained by variations in vector behaviour rather than difference in the catching efficiency between the traps. Population of *An. arabiensis* are exophilic and exophagic, hence more likely to be captured preponderantly outdoor than indoor, whereas *An. gambiae s.s.* is relatively endophilic and endophagic (Githeko et al., 1996, Bayoh et al., 2014, Cooke et al., 2015), thus more likely to be efficiently captured indoor than outdoor.

It is worth mentioning that the density of *An. gambiae s.s.* was significantly higher from exit traps than prokopack aspirator in both sites. A similar finding was recorded for *An. funestus* in Ahero. This implies that a significant number of these species, most of which were unfed, exited houses. This might verify their endophagic behaviour in normal circumstance, but they could be

forced to leave houses before feeding due to high ITN coverage in the study area (Ototo et al., 2015, Ndenga et al., 2016). While ITN is the main intervention to reduce human vector contact, it could also force previously anthropophagic vectors to adapt feeding on non-human hosts, as has been recently reported for *An. gambiae s.s.* (Ndenga et al., 2016, Degefa et al., 2017) or shift their biting time as it has been the case for *An. funestus* (Sougoufara et al., 2014, Moiroux et al., 2012). Such vector behavioural shifts could hamper malaria control as residual transmission may occur even with high coverage of indoor-based vector control interventions (Durnez and Coosemans, 2013). Hence, vector surveillance is crucial to evaluate the effectiveness of control interventions.

It is important to note that the host blood-meal indices of anophilne mosquitoes varied significantly between indoor and outdoor traps even for anophelines of the same species. For instance, the HBI of *An. arabiensis* collected by indoor CDC light traps was two times as high as the HBI of the same species collected by outdoor CDC light traps. The BBI of indoor resting *An. arabiensis* collected by prokopack aspirator was 68.0%, while the BBI of outdoor resting fraction of *An. arabisnsis* collected by pit shelters, sticky pots and clay pots was about 85%. Similarly, the HBI of indoor resting *An. gambiae s.s.* was three times as high as the HBI of outdoor resting fraction of *An. gambiae s.s.*, whereas the BBI of outdoor resting *An. gambiae s.s.* was two times as high as the BBI of indoor resting *An. gambiae s.s.*. Likewise, the HBI of *An. funestus* was relatively higher in indoor collection than outdoor, while its BBI was higher in outdoor collection than indoor. This could be due to the difference in host availability between indoor and outdoor locations which can affect the feeding behaviour of malaria vectors, as reported elsewhere (Killeen et al., 2001, Lefèvre et al., 2009). This highlights the need to sample outdoor resting/host-seeking fractions of vectors concurrently with indoor resting/host-seeking vectors to determine unbiased vector blood meal indices so that changes in vector feeding and resting behaviour can be monitored.

Given that various entomological indices (e.g., vector density, specie composition, host preferences, biting and resting behaviour, and infection rate) need to be monitored in vector surveillance system, no single trapping method can provide a reliable estimate of vector parameters. For a good representation of resting vector population, indoor resting vector surveillance (using prokopack aspirator or PSCs) needs to be complemented with outdoor resting

vector surveillance. The sticky pots are potential tools to be used for routine surveillance of the outdoor resting vectors in areas where using pit shelters is not practical.

The limitation of this study is that a single sticky pot was set in each selected compound despite its smaller size as compared to the size of a pit shelter, and comparison was made on one-to-one basis. This may underestimate the number of *Anopheles* mosquitoes collected by the sticky pots.

4.6. Conclusion

The results of this study revealed that sticky pots could be an alternative tool for outdoor resting malaria vector surveillance, in settings where using pit shelters is not feasible. Unlike pit shelters and clay pots which require active aspiration, the sticky pots have an advantage of collecting resting mosquitoes passively without bias. The lower vector density in the sticky pots compared to pit shelters suggests the need to deploy sticky pots in batches (i.e. 4 sticky pots per compound) if comparable results to those that would have been estimated with pit shelters is needed. This study also highlighted the need to concurrently undertake outdoor resting/host-seeking and indoor resting/host-seeking vector surveillance to better understand residual malaria transmission.

CHAPTER FIVE

5. EVALUATION OF HUMAN-BAITED DOUBLE NET TRAP AND HUMAN-ODOUR-BAITED CDC LIGHT TRAP FOR OUTDOOR HOST-SEEKING MALARIA VECTOR SURVEILLANCE IN KENYA AND ETHIOPIA (Adopted from Degefa et al., 2020)

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Evaluation of human-baited double net trap and human-odour-baited CDC light trap for outdoor host-seeking malaria vector surveillance in Kenya and Ethiopia

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5.1. Abstract

Background: Surveillance of outdoor host-seeking malaria vectors is crucial to monitor changes in vector biting behaviour and evaluate the impact of vector control interventions. Human landing catch (HLC) has been considered the most reliable and gold standard surveillance method to estimate human-biting rates. However, it is labour-intensive, and its use is facing an increasing ethical concern due to potential risk of exposure to infectious mosquito bites. Thus, alternative methods are required. This study was conducted to evaluate the performance of human-odour-baited CDC light trap (HBLT) and human-baited double net trap (HDNT) for outdoor host-seeking malaria vector surveillance in Kenya and Ethiopia.

Methods: The sampling efficiency of HBLT and HDNT was compared with CDC light trap and HLC using Latin Square Design in Ahero and Iguhu sites, western Kenya and Bulbul site, southwestern Ethiopia between November 2015 and December 2018.

Results:

Overall, 16,963 female *Anopheles* mosquitoes comprising *Anopheles gambiae* sensu lato (s.l.), *Anopheles funestus* group, *Anopheles pharoensis*, *Anopheles coustani* and *Anopheles squamosus* were collected. PCR results (n = 552) showed that *Anopheles arabiensis* was the only member of *An. gambiae s.l.* in Ahero and Bulbul, while 15.7% *An. arabiensis* and 84.3% *An. gambiae* sensu stricto (s.s.) constituted *An. gambiae s.l.* in Iguhu. In Ahero, HBLT captured 2.23 times as many *An. arabiensis* and 2.11 times as many *An. funestus* as CDC light trap. In the same site, HDNT yielded 3.43 times more *An. arabiensis* and 3.24 times more *An. funestus* than the HBLT. In Iguhu, the density of *Anopheles* mosquitoes did not vary between the traps ($p > 0.05$). In Bulbul, HBLT caught 2.19 times as many *An. arabiensis* as CDC light trap, while HDNT caught 6.53 times as many *An. arabiensis* as the CDC light trap. The mean density of *An. arabiensis* did not vary between HDNT and HLC ($p = 0.098$), whereas the HLC yielded signifcantly higher density of *An. arabiensis* compared to HBLT and CDC light trap. There was a signifcant density-independent positive correlation between HDNT and HLC ($r= 0.69$).

Conclusion: This study revealed that both HDNT and HBLT caught higher density of malaria vectors than conventional CDC light traps. Moreover, HDNT yielded a similar vector density as HLC, suggesting that it could be an alternative tool to HLC for outdoor host-seeking malaria vector surveillance.

5.2. Introduction

Estimating the entomological inoculation rate (EIR), the number of infectious mosquito bites per person per unit time, is a key metric used to quantify malaria transmission intensity and evaluate the impact of vector control interventions (Beier et al., 1999, Kelly-Hope and McKenzie, 2009). Estimating EIR requires sampling host-seeking *Anopheles* mosquitoes to determine human-biting rate (HBR) and sporozoite infection rate, the two components of the EIR (Beier et al., 1999, Hay et al., 2000). However, developing standardized methods for estimating the HBR that do not expose collectors to infectious mosquito bites has been a major challenge (Service, 1977, Silver, 2007), especially in African settings where a substantial proportion of biting occurs outdoors (Russell et al., 2011, Reddy et al., 2011, Meyers et al., 2016, Durnez and Coosemans, 2013).

The gold standard method to determine the HBR has been the human landing catch (HLC), which can be employed either indoors or outdoors to capture mosquitoes as they land to feed on a human host (Service, 1977, WHO, 2013c, Lima et al., 2014, WHO, 1995). Nevertheless, HLC is a labour-intensive procedure requiring highly trained collectors and extensive supervision to obtain reliable results. Furthermore, there may be considerable differences between biting rates experienced by different collectors as a result of variability in individual attractiveness and skill in catching mosquitoes (Lindsay et al., 1993, Knols et al., 1995, Qiu et al., 2006), thus it might be difficult to standardize the estimates based on biting catches. Lastly but not the least, conducting HLC raises ethical concerns associated with an increased risk of participants' exposure to infectious mosquito bites if an appropriate antimalarial chemoprophylaxis is not taken (WHO, 2013c, Kilama, 2010, Service, 1977). The increasing risk of arboviral infections further compounds its limitations (Simo et al., 2019). Hence, it may not be practical to deploy the HLC for routine malaria vector surveillance.

As an alternative to HLC, Centers for Disease Control and Prevention (CDC) miniature light traps have been widely employed for host-seeking mosquito collections (Lines et al., 1991, Mbogo et al., 1993, Costantini et al., 1998). The CDC light traps have been shown to have a good performance when used indoors (Lines et al., 1991, Magbity et al., 2002, Fornadel et al., 2010b, Davis et al., 1995) and have been used as a proxy to estimate indoor-HBRs in different settings (Drakeley et al., 2003, Mwangangi et al., 2013b, Massebo et al., 2013b). However, it

may not be effective for the surveillance of outdoor biting malaria vectors in the absence of additional attractants that augment its trapping efficiency (Costantini et al., 1998, Mboera, 2005, Kenea et al., 2017).

Consequently, efforts have been made to develop and evaluate alternative odour-baited trapping methods for determining outdoor-HBRs that would be as efficient as the HLC, and cost-effective, exposure-free, and widely deployable. These include double bed-net traps (Tangena et al., 2015, Gao et al., 2018, Le Goff et al., 1997), tent traps (Govella et al., 2009, Govella et al., 2011, Krajacich et al., 2014, Sikulu et al., 2009) and Mbita traps (Mathenge et al., 2004) among others. The double net traps have been shown to have good efficiency when compared to HLC in some settings (Tangena et al., 2015, Gao et al., 2018). However, they have also their own drawbacks. In some studies for instance, two persons are needed to conduct a double net trap i.e. one individual acting as a bait and the other as collector, and such approach is almost as labour intensive as conducting the HLC (Gao et al., 2018). In another circumstance when one person is used both as bait and collector (Tangena et al., 2015), there might be a possibility of exposure to infectious mosquito bites during the collection process. A similar concern related with operator's exposure to mosquito bites has also been reported for the tent traps, despite their promising potential for monitoring host-seeking malaria vectors (Govella et al., 2009). Although the Mbita trap is considered an exposure-free tool, it is less effective compared to both HLC and CDC light traps (Mathenge et al., 2002, Mathenge et al., 2004, Laganier et al., 2003). Hence, there is a need to look for appropriate tools for outdoor host-seeking malaria vector surveillance.

The aim of this study was thus to evaluate the performance of two exposure-free traps i.e. human-odour-baited CDC light trap (HBLT) and human-baited double net trap (HDNT) for outdoor host-seeking malaria vector surveillance. The HBLT consists of a CDC light trap baited with human-odour pumped from ordinary sleeping room, whereas the HDNT is a variant of previously designed double net trap (Tangena et al., 2015). The trapping efficiency of the HBLT and HDNT was compared with conventional (unbaited) CDC light traps and HLC in western Kenya and southwestern Ethiopia.

5.3. Methods

5.3.1. Study sites

The study was conducted in two different eco-epidemiological settings of East Africa, western Kenya and southwestern Ethiopia (Figure 5.1).

Western Kenya: The study was done in Ahero (0.13123°S, 34.93960°E, altitude 1162 m above sea level, asl) and Iguhu (0.15657°N; 34.74386°E, altitude 1,430–1,580 m asl) sites. Ahero is a lowland plain area located in Kisumu County while Iguhu is highland site characterized by undulating hills and valley bottoms located in Kakamega County (Degefa et al., 2017, Degefa et al., 2019). In both sites, most houses are mud-walled with roofs made of corrugated iron sheets. The inhabitants mainly depend on subsistence farming, with rice and maize being the main cultivated crops in Ahero and Iguhu, respectively. The sites have bimodal pattern of rainfall, with the long rainy season from April to June, which triggers the peak malaria transmission and the short rains from October to November with minor transmission (Munyekenye et al., 2005). *Plasmodium falciparum* is the predominant malaria parasite species in the area and transmitted by *Anopheles gambiae s.s.*, *An. arabiensis* and *An. funestus* (Zhou et al., 2011, Githeko et al., 2006, Ototo et al., 2015, Degefa et al., 2017).

Southwestern Ethiopia: The study was carried out in Bulbul *kebele* (7.70285°N; 37.09592°E, altitude 1705 m asl), which is located in Kersa district, Oromia Region at about 320 kms southwest of Addis Ababa. The majority of the houses are mud-walled with roofs made of corrugated iron sheets. The inhabitants mostly rely on subsistence farming. Maize and *Teff* are the main cultivated crops. As in most parts of Ethiopia, malaria transmission is seasonal in Bulbul area. The transmission peaks from September to October, following the major rains from June to September. Minor transmission occurs in April and May, following the short rains of February to March. *Plasmodium falciparum* and *P. vivax* are the two predominant malaria parasite species in the area and are transmitted by *An. arabiensis* (Yewhalaw et al., 2009).

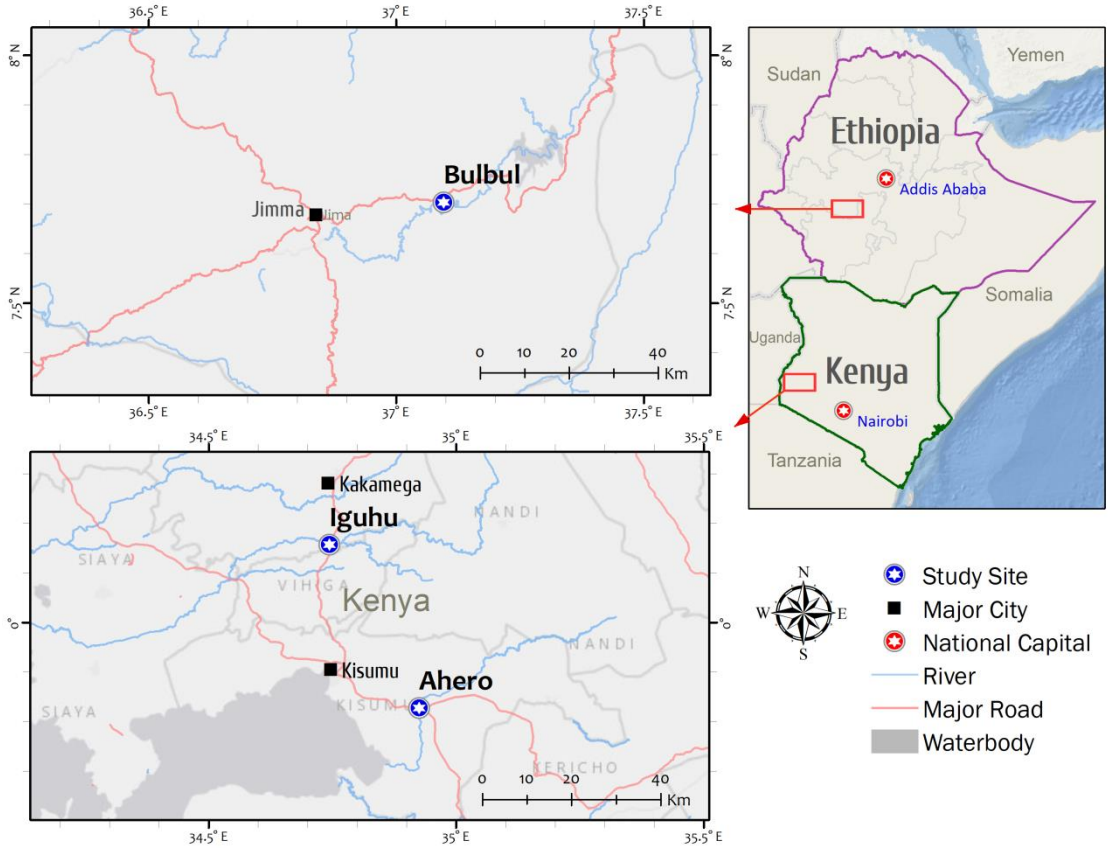

Figure 5.1. Map of the study sites in Kenya and Ethiopia

5.3.2. Description of trapping methods

5.3.2.1. Human-odour baited CDC light trap (HBLT)

The HBLT comprises a polyvinyl chloride (PVC) pipe that moves human odour from indoor (sleeping room) to outdoor mosquito catching station (Plate 5.1a). The inner end of the pipe is wide (4-inch diameter) while its outer segment is narrow (2-inch diameter). A fan was installed into the inner end of the pipe to enhance outflow of the odour. A CDC light trap (John W. Hock Ltd, Gainesville, FL., USA) was set outdoor near the outer end of the pipe to capture mosquitoes attracted to the human odour. The pipe was connected from the sleeping room to the outdoor station through a small hole (2-inch diameter) made on the wall or window of selected houses. The length of the pipe from the wall of the house to its outer end was 2 m. The inner opening of the pipe was covered with untreated net to make sure that the pipe pumps odour only. The inner (wide section) of the pipe was connected with its outer (narrow) section using reducing bush so that the two parts could be easily disconnected when they were not in use. Outdoor host-seeking

mosquito collection using the HBLT was done from 18:00 to 6:00 hr during each collection night.

5.3.2.2. Human-baited double net trap (HDNT)

The HDNT in this study consisted of two box nets (inner and outer nets) with a roof made of canvas. The inner net (97 cm high x 200 cm long x 100 cm wide) fully protects a human volunteer who rests on a mattress. The outer net (100 cm high x 250 cm long x 150 cm wide) is raised 30 cm off the ground. Mosquitoes attracted to the human-bait are collected by setting a CDC light trap between the two nets (Plate 5.1b). The HDNT is an exposure free tool since the lured mosquitoes are captured by the CDC light trap rather than by the person acting as a bait unlike the previously designed bed net traps (Tangena et al., 2015). Outdoor mosquito sampling using the HDNT was conducted from 18:00 to 6:00 hr during each collection night.

5.3.2.3. CDC miniature light traps

Conventional CDC miniature light traps were also set outdoor at about 2 m from selected houses at a height of 1.5 meter from the ground from18:00 to 06:00 hr (Plate 5.1c).

5.3.2.4. Human landing catch (HLC)

The HLC was performed by a male adult volunteer, who acted as both bait and collector (Plate 5.1d). The collector seated outdoor on a chair with the legs exposed from foot to knee and captured mosquitoes as soon as they land on the exposed legs before they commence feeding using a flashlight and mouth aspirator (WHO, 2013c, Service, 1977). There were two collection shifts: one collector worked from 18:00 to 24:00 hr during each collection night, followed by the second collector from 24:00 to 06:00 hr. Each hour's collection was kept separately in labeled paper cups. A supervisor was assigned to coordinate the collection activities and watch volunteers not to fall asleep during the collection nights. All collectors were provided with anti-malaria prophylaxis to avoid a risk of contracting malaria during the collection period. Mosquitoes were identified to species the next morning.

Plate 5.1. Vector sampling tools used for outdoor host-seeking malaria vector surveillance in western Kenya and southwest Ethiopia

a) Human-odour-baited CDC light trap, b) Human-baited double net trap, c) Unbaited CDC light trap, d) Human landing catch

5.3.3. Experimental design

The study consisted of three consecutive experiments. The first experiment was conducted to compare HBLT with unbaited CDC light trap to test a hypothesis that the use of human-odour in HBLT could significantly improve its trapping efciency as compared to the unbaited CDC light trap. In the second experiment, HDNT was compared with the HBLT. In the third experiment, the HBLT, HDNT and CDC light trap were compared with HLC, the gold standard method. Details of the experimental designs are presented as follows:

5.3.3.1. Human-odour-baited and unbaited CDC light traps comparison (Experiment 1)

This experiment was carried out in Ahero and Iguhu sites, western Kenya. Each study site was classified into three clusters. Two houses with corresponding outdoor mosquito catching station, about 2 m from each selected house, were selected from each cluster. The HBLT and unbaited

CDC light trap were assigned to one of the two outdoor catching stations and swapped between the two houses daily in each cluster in both study sites. The experiment was conducted from November 2015 to February 2016. A total of 60 trapping-nights were done for each trap in each study site.

5.3.3.2. Human-odour-baited CDC light trap and human-baited double net trap comparison (Experiment 2)

Experiment 2 was conducted from June to July 2017 in the same study sites as experiment 1, using the same houses in each cluster. The HBLT and HDNT were assigned to one of the two outdoor catching stations and swapped between the two houses daily in each cluster in both study sites. A total of 42 trapping-nights were done for each trapping method in each study site.

5.3.3.3. Comparison of alternative outdoor traps with human landing catch (Experiment 3)

The third experiment was conducted in Bulbul, southwestern Ethiopia. Four representative houses of similar size and design with corresponding outdoor mosquito catching stations were randomly selected. The HBLT, HDNT, CDC light trap and HLC were assigned to one of the four outdoor catching stations. The traps were rotated among the selected houses once monthly using 4x4 Latin Square Design. All traps were set simultaneously from 18:00 to 6:00 hr. A total of 48 trapping-nights were conducted for each trapping method. The experiment was conducted from January to December 2018.

5.3.4. Sample processing

All collected mosquito samples were identified morphologically to species or species complexes using keys (Gillies and Coetzee, 1987). Adult female *Anopheles* mosquitoes were kept individually in labelled 1.5 ml Eppendorf tubes containing silica gel desiccant. Samples were stored at − 20 °C freezer at Climate and Human Health Research Laboratory of Kenya Medical Research Institute (KEMRI) or Jimma University Tropical and Infectious Diseases Research Center (TIDRC) Laboratory until used for further processing.

5.3.5. Molecular identification of vector species complexes

Members of *An. gambiae s.l.* and *An. funestus* group were identified to their respective sibling species by polymerase chain reaction (PCR), following the protocols developed by Scott et al. (Scott et al., 1993) and Koekemoer et al. (Koekemoer et al., 2002) for *An. gambiae s.l.* and *An. funestus* group, respectively.

5.3.6. Detection of sporozoite infections

Dried head and thorax of the preserved *Anopheles* mosquito specimens were carefully separated from the abdomen and tested for *P. falciparum* and *P. vivax* circum-sporozoite proteins (CSPs) using sandwich ELISA method (Beier et al., 1987, Wirtz et al., 1987).

5.3.7. Data analysis

The difference in *Anopheles* mosquito density among different trapping methods was compared using a generalized linear model based on negative binomial distribution. Trap type was fitted as the main factor in the model. Experimental night was treated as a covariate for the first and second experiments, whereas sampling month was also considered as a covariate for the third experiment. The estimated marginal mean (EMM) density of *Anopheles* mosquitoes was determined for each trap using the negative binomial regression by adjusting for experimental night and month. Gini-Simpson's diversity index (1-D) (Simpson, 1949, Peet, 1974, Magurran, 2013) was applied to evaluate mosquito species diversity for each trap. To determine the statistical significance of difference in species diversity among the traps, 95% confidence intervals (CI) were calculated (Grundmann et al., 2001). The Simpson's index of evenness (E) was computed to obtain a measure of the relative abundance of different mosquito species in each setting (Simpson, 1949, Kwak and Peterson, 2007).

Further analysis was conducted for the third experiment to determine whether each of the alternative outdoor trapping methods was correlated with the reference method i.e. HLC. Pearson correlation coefficient for the relationship among log-transformed catches for each *Anopheles* species was determined. To test if the sampling efficiency of each alternative trap (HDNT, HBLT or CDC light trap) relative to the HLC was affected by mosquito density, the ratios of the number of mosquitoes in each alternative trap to the number of mosquitoes in HLC [log(HLC + 1)−log(Alternative trap + 1)] were plotted against the average mosquito abundance, calculated as [log(HLC + 1) + log(Alternative trap + 1)]/2 (Altman and Bland, 1983). Simple linear regression

analysis was done for the relationship between the ratios and their average mosquito abundance (Altman and Bland, 1983). The value of R-square (R^2) derived from the analysis was then interpreted as an estimate of the proportion of deviation from perfect linear correlation due to density-dependence rather than random error, with a high and significant value indicating density-dependence.

The sporozoite rate was estimated as the proportion of mosquitoes positive for *Plasmodium* CSP over the total number tested. Data were analyzed using SPSS version 20.0 (SPSS, Chicago, IL, USA) software package. $P < 0.05$ was considered statistically significant during the analysis.

5.4. Results

5.4.1. Mosquito species composition and abundance

Overall, 30,278 female mosquitoes (25,135 from Ahero, 1,407 from Iguhu and 3,736 from Bulbul) were collected outdoors over the course of 600 trapping-nights. Of these, 16,963 (56.0%) were anophelines, with the remaining 13,315 (44.0%) being *Culex* species. 15,201 of the anophelines were collected from Ahero and Iguhu sites (5,042 by HBLT, 1,128 by CDC light traps and 9,031 by HDNT). *Anopheles gambiae s.l.* was the predominant species accounting for 57.3% of the anophelines collected from Ahero and Iguhu, followed by *An. pharoensis* (22.3%), *An. coustani* (15.5%) and *An. funestus* group (4.9%). In Bulbul site, *An. pharoensis* was the most abundant species, accounting for 41.0% of the collected anophelines, followed by *An. coustani* (30.7%), *An. gambiae s.l.* (27.7%), *An. squamosus* (0.4%) and *An. funestus* group (0.2%).

5.4.2. Composition of vector species complexes

A total of 602 *An. gambiae s.l.* specimens [258 from Ahero, 184 from Iguhu and 160 from Bulbul] and 90 *An. funestus* group (from Ahero and Iguhu) were analysed for identification of sibling species. Of these, 552 *An. gambiae s.l.* and 84 *An. funestus* group specimens were successfully amplified and identified to species by PCR. In Ahero, all of the amplified *An. gambiae s.l.* specimens were confirmed to be *An. arabiensis*. In Iguhu, *An. arabiensis* and *An. gambiae s.s.* accounted for 15.7% and 84.3% of the *An. gambiae s.l.*, respectively. The sibling species composition of *An. gambiae s.l.* did not vary among the different trapping methods ($\chi 2 = 0.086$, $df = 2$, $p = 0.958$). Of the amplified *An. funestus* group specimens, *An. funestus s.s.* and *An.*

leesoni accounted for 90.5% and 9.5%, respectively. Similar to Ahero, *An. arabiensis* was the only identified member species of the *An. gambiae s.l* in Bulbul site from Ethiopia.

5.4.3. Mosquito density and species diversity

5.4.3.1. Human-odour-baited and unbaited CDC light traps comparison (Experiment 1)

Between November 2015 and February 2016, a total of 2,783 female *Anopheles* mosquitoes were collected by HBLT and CDC light trap in Ahero and Iguhu sites. Overall, HBLT yielded 1.43 (95% CI: 1.09-1.86, p = 0.009) times higher density of anophelines than CDC light trap [Table 5.1]. In Ahero, the HBLT caught on average 2.23 (95% CI: 1.49-3.36, p < 0.001) times as many *An. arabiensis* per night as CDC light trap. Similarly, the HBLT captured 2.11 (95% CI: 1.28-3.47, p = 0.003) times higher number of *An. funestus* group per night compared to CDC light traps. There was no significant between the HBLT and CDC light trap in terms of the mean density of An. pharoensis and An. coustani (p > 0.05). In Iguhu site, the density of anophelines was low from both HBLT and CDC light trap [Table 5.1].

The diversity of mosquito species captured was significantly higher for HBLT (Simpson diversity index ± 2SD = 0.63 ± 0.01) than for CDC light trap (0.59 ± 0.02). Moreover, the HBLT collected mosquitoes of different species more homogenously (Simpson evenness, E = 0.79 ± 0.02) than CDC light trap (0.71 ± 0.02).

Table 5.1. Estimates of a negative binomial regression for the comparison of outdoor host-seeking *Anopheles* mosquito density between HBLT and CDC light trap in western Kenya

Site and species	Trap	Number collected	EMM (95%CI)	OR (95% CI)	p value
Ahero					
An. gambiae s.l.	HBLT	332	5.52 (4.19-7.26)	2.23 (1.49-3.36)	<0.001*
	Light trap	149	2.47 (1.83-3.33)	1.0[a]	
An. funestus group	HBLT	99	1.65 (1.20-2.27)	2.11 (1.28-3.47)	0.003*
	Light trap	47	0.78 (0.53-1.15)	1.0[a]	
An. pharoensis	HBLT	554	8.21 (6.27-10.75)	1.28 (0.87-1.87)	0.213
	Light trap	421	6.43 (4.89-8.46)	1.0[a]	
An. coustani	HBLT	641	9.06 (6.93-11.86)	1.16 (0.79-1.71)	0.442
	Light trap	497	7.80 (5.95-10.23)	1.0[a]	
Iguhu					
An. gambiae s.l.	HBLT	15	0.22 (0.12-0.41)	2.10 (0.79-5.57)	0.137
	Light trap	7	0.11 (0.05-0.24)	1.0a	
An. funestus group	HBLT	10	0.16 (0.08-0.31)	1.65 (0.56-4.87)	0.360
	Light trap	6	0.10 (0.04-0.22)	1.0[a]	
An. coustani	HBLT	4	0.07 (0.02-0.18)	4.0 (0.43-36.94)	0.221
	Light trap	1	0.02 (0.002-0.12)	1.0[a]	
Total	HBLT	1,655	12.74 (10.58-15.35)	1.43 (1.09-1.86)	0.009*
	Light trap	1,128	8.92 (7.38-10.78)	1.0[a]	

Note: A total of 60 trap-nights were conducted for each trap in each study site, HBLT: human odour-baited CDC light trap, EMM: estimated marginal mean density, [a] Reference value, OR: odds ratio, CI: confidence interval, *statistically significant

5.4.3.2. Human-odour CDC light trap and human-baited double net trap comparison (Experiment 2)

A total of 12,418 anopheline mosquitoes were collected by HDNT and HBLT in Ahero and Iguhu sites during the second experiment. Overall, HDNT yielded 2.75 (95% CI: 2.01-3.74, $p < 0.001$) times higher density of anophelines compared to HBLT [Table 5.2]. In Ahero, the HDNT caught 3.43 (95% CI: 2.22-5.30, $p < 0.001$) times as many *An. arabiensis* per night as HBLT. Likewise, the HDNT captured 3.24 (95% CI: 1.99-5.25, $p < 0.001$) times as many *An. funestus*

group and 3.55 (95% CI: 2.25-5.61, p < 0.001) times as many *An. coustani* per night as the HBLT. No significant difference was found in the mean density of *An. pharoensis* between the two traps (p = 0.183). In Iguhu site, the mean density of *An. gambiae s.l.* and *An. funestus* group did not vary significantly between the HDNT and HBLT (p > 0.05) [Table 5.2).

Table 5.2. Estimates of a negative binomial regression for the comparison of outdoor host-seeking *Anopheles* mosquito density between HDNT and HBLT in western Kenya

Site and species	**Trap**	**Number collected**	**EMM (95%CI)**	**OR (95% CI)**	**p value**
Ahero					
An. gambiae s.l.	HDNT	6,188	148.83 (109.67-201.97)	3.43 (2.22-5.30)	<0.001*
	HBLT	1,862	43.40 (31.90-59.04)	1.0[a]	
An. funestus group	HDNT	392	9.21 (6.67-12.71)	3.24 (1.99-5.25)	<0.001*
	HBLT	137	2.84 (1.99-4.06)	1.0[a]	
An. pharoensis	HDNT	1,386	32.91 (24.09-44.96)	1.36 (0.87-2.13)	0.183
	HBLT	1,016	24.25 (17.72-33.19)	1.0[a]	
An. coustani	HDNT	895	21.30 (15.59-29.11)	3.55 (2.25-5.61)	<0.001*
	HBLT	252	6.00 (4.32-8.34)	1.0[a]	
Iguhu					
An. gambiae s.l.	HDNT	92	2.17 (1.50-3.13)	1.29 (0.75-2.20)	0.353
	HBLT	70	1.68 (1.14-2.47)	1.0[a]	
An. funestus group	HDNT	34	0.81 (0.52-1.27)	1.42 (0.72-2.79)	0.308
	HBLT	24	0.57 (0.35-0.94)	1.0[a]	
An. pharoensis	HDNT	6	0.13 (0.05-0.32)	1.45 (0.38-5.58)	0.587
	HBLT	4	0.09 (0.03-0.26)	1.0[a]	
An. coustani	HDNT	38	0.86 (0.55-1.34)	1.65 (0.83-3.27)	0.151
	HBLT	22	0.52 (0.31-0.87)	1.0[a]	
Total	HDNT	9,031	108.69 (87.54-134.96)	2.75 (2.01-3.74)	<0.001*
	HBLT	3,387	39.60 (31.84-49.25)	1.0[a]	

Note: A total of 42 trap-nights were conducted for each trap in each study site, HDNT: human odour-baited double net trap, HBLT: human odour-baited CDC light trap, EMM: estimated marginal mean density, OR: odds ratio, CI: confidence interval, [a] Reference value, *statistically significant

The diversity of mosquito species collected did not vary significantly between HDNT (Simpson diversity index = 0.66 ± 0.01) and HBLT (0.64 ± 0.01). Similarly, the species evenness did not vary significantly between the HDNT (E = 0.82 ± 0.01) and HBLT (0.81 ± 0.01).

5.4.3.3. Compparison of alternative outdoor traps with human landing catch (Experiment 3)

A total of 1,762 *Anopheles* mosquitoes were caught outdoors by HDNT, HBLT, CDC light trap and HLC in Bulbul site from January to December 2018. The EMM density of each anopheline species per trap is shown in Table 5.3. On average, the HBLT caught 2.19 (95% CI: 1.18-4.10, p = 0.014) times as many *An. arabiensis* per night as CDC light trap, while the HDNT caught 6.53 (95% CI: 3.64-11.72, p < 0.001) times as many *An. arabiensis* per night as the CDC light trap. The mean density of *An. arabiensis* did not vary between HDNT and HLC (p = 0.098), whereas the HLC caught 4.35 (95% CI: 2.64-7.17, p < 0.001) times as many *An. arabiensis* as HBLT and 9.54 (95% CI: 5.35-17.02, p < 0.001) times as many as CDC light trap.

The mean density of *An. pharoensis* captured by HBLT was 2.04 (95% CI: 1.15-3.61, p = 0.015) times higher compared to CDC light trap, whereas the mean density of the same species collected HDNT was 6.65 (95% CI: 3.87-11.42, p < 0.001) times higher compared to the CDC light trap. No significant difference was found in the mean density of *An. pharoensis* between the HDNT and HLC (p = 0.062), while the HLC collected 4.94 (95% CI: 3.07-7.95, p < 0.001) times as many *An. pharoensis* per night as the HBLT and 10.06 (95% CI: 5.89-17.18, p < 0.001) times as many as the CDC light trap (Table 5.3).

The mean density of *An. coustani* caught by HBLT was 2.11 (95% CI: 1.12–3.99, p = 0.021) times higher compared to CDC light trap, while the mean density of *An. coustani* caught by HDNT was 3.84 (95% CI: 2.10–7.02, p < 0.001) times higher compared to the CDC light trap. The HLC captured 3.61 (95% CI: 2.26–5.76, p < 0.001) times as many *An. coustani* per night as the HDNT, 6.57 (95% CI 3.95–10.90) times as many as the HBLT and 13.88 (95% CI 7.79–24.72, p < 0.001) times as many as the CDC light trap. Very few *An. squamosus* and *An. funestus* group were collected by HLC, HDNT and HBLT, whereas none of this species were collected by the CDC light trap [Table 5.3].

Table 5.3. Estimates of a negative binomial regression for comparison of outdoor host-seeking *Anopheles* mosquito density density between different traps in Bulbul, southwestern Ethiopia

Site and species	Traps	Number collected	EMM (95%CI)	OR (95% CI)	p value
An. gambiae s.l.	HDNT	168	3.32 (2.40-4.59)	0.69 (0.44-1.07)	0.098
	HBLT	55	1.12 (0.76-1.65)	0.23 (0.14-0.38)	<0.001*
	Light trap	25	0.51 (0.31-0.83)	0.11 (0.06-0.19)	<0.001*
	HLC	240	4.85 (3.56-6.63)	1.0[a]	
An. pharoensis	HDNT	243	4.79 (3.51-6.55)	0.66 (0.43-1.02)	0.062
	HBLT	78	1.47 (1.02-2.12)	0.20 (0.13-0.33)	<0.001*
	Light trap	35	0.72 (0.46-1.12)	0.10 (0.06-0.17)	<0.001*
	HLC	366	7.25 (5.35-9.81)	1.0[a]	
An. coustani	HDNT	101	1.83 (1.29-2.61)	0.28 (0.17-0.44)	<0.001*
	HBLT	52	1.01 (0.67-1.51)	0.15 (0.09-0.25)	<0.001*
	Light trap	26	0.48 (0.29-0.78)	0.07 (0.04-0.13)	<0.001*
	HLC	362	6.62 (4.88-18.99)	1.0[a]	
Other anophelines[#]	HDNT	3	0.06 (0.02-0.19)	0.52 (0.12-2.21)	0.372
	HBLT	2	0.04 (0.01-0.16)	0.35 (0.07-1.83)	0.213
	LT-out	0	0	NA	NA
	HLC-out	6	0.12 (0.04-0.27)	1.0[a]	
Total	HDNT	515	10.02 (7.45-13.49)	0.53 (0.35-0.80)	0.003*
	HBLT	187	3.63 (2.63-5.00)	0.19 (0.12-0.29)	<0.001*
	Light trap	86	1.74 (1.21-2.48)	0.09 (0.06-0.15)	<0.001*
	HLC	974	18.99 (14.20-25.40)	1.0[a]	

Note: A total of 48 trap-nights were conducted for each trap in each study site, HDNT: human odour-baited double net trap, HBLT: human odour-baited CDC light trap, HLC: human landing catch, EMM: estimated marginal mean density, OR: odds ratio, CI: confidence interval, [#] Other anophelines include *An. squamosus* and *An. funestus* group, [a] Reference value, *statistically significant

The diversity of mosquito species collected in Bulbul was significantly higher for HDNT (Simpson diversity index = 0.70 ± 0.01) than for HBLT (0.63 ± 0.04), CDC light trap (0.50 ± 0.07) and HLC (0.63 ± 0.02). The diversity of mosquito species collected by HBLT was significantly higher than that of CDC light trap, whereas the HBLT and HLC collected mosquito

of similar species diversity. The HDNT collected mosquitoes of different species more homogeneously (E = 0.85 ± 0.02) than HBLT (E = 0.76 ± 0.05), CDC light trap (E = 0.67 ± 0.09) and HLC (E = 0.75 ± 0.02).

5.4.4. Correlation of the alternative traps with human landing catch

The correlation coefcients of alternative traps with HLC are shown in Table 5.4. There were signifcant positive correlations between HDNT and HLC in terms of the number of *An. arabiensis* (r= 0.691, p= < 0.001) and *An. pharoensis* (0.739, p < 0.001) (r= 0.691, p= < 0.001) captured, and R^2 values did not deviate signifcantly from zero (Figure 5.3; Table 5.4), which means that the relative sampling efciency (RSE) of the HDNT was not dependent on mosquito density for these species. For *An. coustani*, a signifcant positive correlation was found between the HDNT and HLC (r= 0.655, p < 0.001), but the RSE was density-dependent. Signifcant positive correlations were also found between HBLT and HLC for *An. arabiensis* (r= 0.708, p < 0.001), *An. pharoensis* (r= 0.454, p= 0.001) and *An. coustani* (r= 0.664, p= 0.001), but the RSEs were dependent on mosquito density (Figure 5.3; Table 5.4).

Table 5.4. Correlation and density-dependence of the sampling efficiency of alternative outdoor trapping methods relative to human landing catches in Bulbul, Southwestern Ethiopia

Species	**Alternative vs. HLC**	**Correlation coefficient**		**Density-dependence**		
		R	**P-value**	**R-square**	**T**	**P-value**
An. gambiae s.l.	HDNT	0.691	<0.001	0.006	0.284	0.597
	HBLT	0.708	<0.001	0.304	20.135	<0.001
	Light trap	0.469	0.001	0.461	39.408	<0.00
An. pharoensis	HDNT	0.739	<0.001	0.066	3.244	0.078
	HBLT	0.454	0.001	0.140	7.505	0.009
	Light trap	0.199	0.176	0.411	32.042	<0.001
An. coustani	HDNT	0.655	<0.001	0.233	13.973	0.001
	HBLT	0.664	<0.001	0.521	50.020	<0.001
	CDC Light trap	0.569	<0.001	0.657	88.070	<0.001

Note: HLC: human landing catch, HDNT: human-baited double net trap, HBLT: human-odour-baited CDC light trap

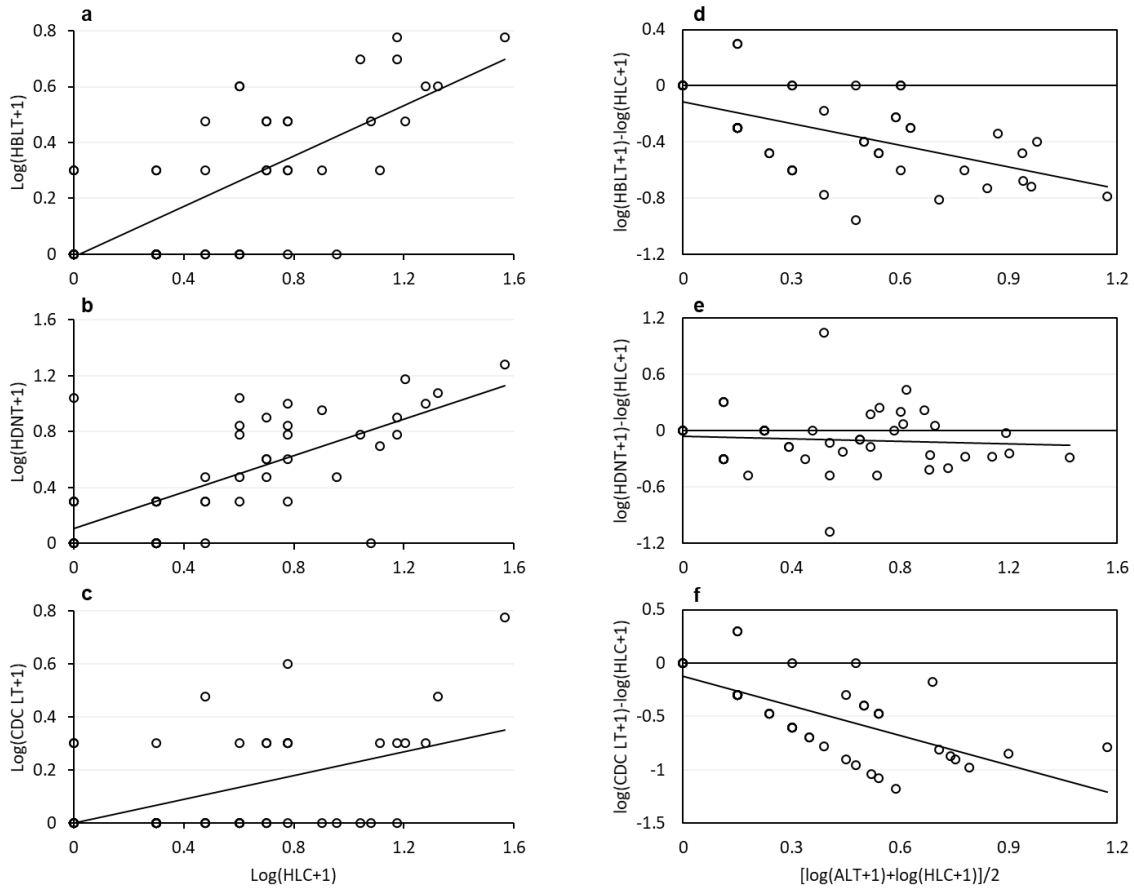

Figure 5.2. Correlation and density-dependence of the alternative outdoor trapping methods relative to human landing catch for catching *An. arabiensis* in Bulbul, southwestern Ethiopia (a, c and e show the correlation of HDNT, HBLT and CDC light traps with HLC, respectively, whereas b, d and f indicate the RSE of the HDNT, HBLT and CDC light traps, respectively).

5.4.5. Sporozoite rate

Overall, 7,344 (43.3% of the total) *Anopheles* mosquitoes (5,273 from Ahero, 309 from Iguhu and 1,762 from Bulbul) were tested for *P. falciparum* and *P. vivax* CSPs. Of these, 27 specimens (17 from Ahero, 4 from Iguhu and 6 from Bulbul) were positive for *Plasmodium* CSPs.

Table 5.5 shows the sporozoite rates of anophelines collected from Ahero and Iguhu sites. In Ahero, the sporozoite rate of *An. arabiensis* was 0.12% from HBLT and 0.16% from HDNT. None of the tested *An. arabiensis* from CDC light trap were positive. In the same study site, the sporozoite rate of *An. funestus* group was 2.1% from HBLT, 2.4% from HDNT and 2.1% from CDC light trap. In Iguhu, the sporozoite rate of *An. gambiae* s.s. was 1.5% from

HBLT and 2.9% from HDNT, while the sporozoite rate of *An. funestus* group from HDNT was 3.0%. No CSP was detected in *An. funestus* group collected by HBLT and CDC light trap. Thus, the overall sporozoite rate of *An. arabiensis*, *An. gambiae* s.s. and *An. funestus* group was 0.14%, 2.1% and 2.2%, respectively. None of the tested *An. pharoensis* and *An. coustani* specimens were positive.

Table 5.5. *Plasmodium falciparum* sporozoite rates of outdoor host-seeking *Anopheles* mosquitoes collected by different trapping methods in western Kenya

Study site and species	**Parameters**	**Experiment 1**		**Experiment 2**		**Total**
		HBLT	Light trap	HBLT	HDNT	
Ahero						
An. arabiensis	No tested	201	149	651	1929	2,930
	Pf +ve (%)	0	0	1 (0.15)	3 (0.16)	4 (0.14)
An. funestus group	No tested	99	47	136	287	570
	Pf +ve (%)	2 (2.0)	1 (2.1)	3 (2.2)	7 (2.4)	13 (2.3)
An. pharoensis	No tested	168	146	305	416	1035
	Pf +ve (%)	0	0	0	0	0
An. coustani	No tested	193	150	125	270	738
	Pf +ve (%)	0	0	0	0	0
Iguhu						
An. gambiae s.s	No tested	12	6	53	69	140
	Pf +ve (%)	0	0	1 (1.9)	2 (2.9)	3 (2.1)
An. funestus group	No tested	9	6	20	33	68
	Pf +ve (%)	0	0	0	1 (3.0)	1 (1.5)
An. arabiensis	No tested	2	1	11	12	26
	Pf +ve (%)	0	0	0	0	0
An. pharoensis	No tested	0	0	4	6	10
	Pf +ve (%)	0	0	0	0	0
An. coustani	No tested	4	1	22	38	65
	Pf +ve (%)	0	0	0	0	0

Note: HBLT: human-odour-baited CDC light trap, HDNT: human-baited double net trap, *Pf* +ve: number of *P. falciparum* positive *Anopheles* mosquitoes (rate in percent)

In Bulbul site, of the assayed anopheline specimens, 6 (2 *An. arabiensis*, 3 *An. pharoensis* and 1 *An. coustani*) were positive for *Plasmodium* CSPs (four specimens for *P. vivax* and two for *P. falciparum*) (Table 5.6). The sporozoite rate of *An. arabiensis* was 0.6% from HDNT and 0.4% from HLC. No CSP was detected in *An. arabiensis* collected by HBLT and CDC light trap. The sporozoite rate of *An. pharoensis* was 1.3% from HBLT, 0.4% from HDNT and 0.3% from HLC.

The sporozoite rate of *An. coustani* from HLC was 0.3%, whereas no CSP was detected in *An. coustani* collected by the other trapping methods. Hence, the overall sporozoite rate of *An. arabiensis*, *An. pharoensis* and *An. coustani* was 0.4%, 0.3% and 0.2%, respectively.

Table 5.6. Sporozoite rates of outdoor host-seeking *Anopheles* mosquitoes collected by different methods in Bulbul, southwestern Ethiopia

Method	**Species**	**No tested**	**Pf n (%)**	**Pv210 n (%)**	**Pv247 n (%)**	**Total n (%)**
HDNT	*An. gambiae s.l*	168	1 (0.6)	0	0	1 (0.6)
	An. pharoensis	243	0	0	1 (0.4)	1 (0.4)
	An. coustani	101	0	0	0	0
	An. squamosus	2	0	0	0	0
	An. funestus group	1	0	0	0	0
HBLT	*An. gambiae s.l*	55	0	0	0	0
	An. pharoensis	78	0	1 (1.3)	0	1 (1.3)
	An. coustani	52	0	0	0	0
	An. squamosus	1	0	0	0	0
	An. funestus group	1	0	0	0	0
Light trap	*An. gambiae s.l*	25	0	0	0	0
	An. pharoensis	35	0	0	0	0
	An. coustani	26	0	0	0	0
HLC	*An. gambiae s.l*	240	1 (0.4)	0	0	0 (0.4)
	An. pharoensis	366	0	1 (0.3)	0	1 (0.3)
	An. coustani	362	0	1 (0.3)	0	1 (0.3)
	An. squamosus	4	0	0	0	0
	An. funestus group	2	0	0	0	0
Overall	*An. gambiae s.l.*	488	2 (0.4)	0	0	2 (0.4)
	An. pharoensis	722	0	2 (0.3)	1 (0.1)	3 (0.4)
	An. coustani	541	0	1 (0.2)	0	1 (0.2)
	An. squamosus	7	0	0	0	0
	An. funestus group	4	0	0	0	0

Note: HDNT: human-baited double net trap, HBLT: human-odour-baited CDC light trap, HLC: human landing catch, *Pf: P. falciparum*, *Pv: P. vivax*, n: number positive (rate in percent)

5.5. Discussion

In this study, the potential of two human-odour baited traps, the HBLT and HDNT, to provide exposure-free alternatives to the HLC for surveillance of outdoor host-seeking African malaria vectors was evaluated. The results showed that both HBLT and HDNT yielded significantly higher anopheline mosquito density compared to the conventional CDC light trap. This suggests that the use of human-bait in HBLT and HDNT significantly enhanced the trapping efficiency both traps. This indicates the usefulness of these tools for outdoor host-seeking vector surveillance.

The HBLT collected about twice as many *An. arabiensis* and *An. funestus* group as unbaited CDC light trap. This indicates that the HBLT could also surpass the trapping efficiency of CO_2-baited CDC light traps that have been compared with unbaited CDC light traps previously (Sriwichai et al., 2015, Hiwat et al., 2011, Chen et al., 2011, Service, 1993a). For instance, CO_2-baited CDC light trap captured 1.39 times as many *Anopheles* mosquitoes as unbaited CDC light trap in Thailand (Sriwichai et al., 2015), whereas in other studies conducted in south-central Ethiopia and Suriname, synthetic CO_2 did not improve the trapping efficiency of CDC light traps (Kenea et al., 2017, Hiwat et al., 2011). The lower relative sampling efficiency of the CO_2-baited CDC light traps in the previous studies might be due to a lower attraction of synthetic CO_2 as compared to natural human odour. It was hypothesized that when synthetic CO_2 is used in traps in isolation from other attractant stimuli produced by hosts, it could be considered as an artificial arrangement, and mosquitoes might not fly directly towards it but rather show an erratic behaviour (Service, 1977). Thus, the HBLT could represent a better outdoor vector surveillance tool than both unbaited and CO_2-baited CDC light traps.

However, the HBLT yielded 4.35 times lower number of *An. arabiensis* compared to HLC, and 4.94 and 6.57 times lower for *An. pharoensis* and *An. coustani*, respectively. Similarly, the HBLT yielded signifcantly lower density of anophelines than HDNT. These variations are probably due to the diference in the location of persons used as bait. Although all traps were set outdoors in this study, a bait for HBLT was located indoor and odour was pumped-out through a pipe, while in the case of HLC and HDNT, human-baits were positioned outdoors on the actual mosquito catching stations. This means that the HBLT lacks thermal cues that may serve as supplementary short-range mosquito attractant (Service, 1993a), unlike the HLC and HDNT. On

the other hand, HLC may also overestimate human-biting rates to some extent since the human-baits are relatively more available to host-seeking mosquitoes than under normal circumstance. Although it is habitual practice in Africa to spend evening and early-morning hours outdoors (Finda et al., 2019, Monroe et al., 2019a, Monroe et al., 2019b), people may not stay undisturbed in one place with legs exposed throughout the night unlike that of HLC.

The HDNT caught 6.53 times as many *An. arabiensis* and 6.65 times as many *An. pharoensis* as CDC light trap in Bulbul while the mean density of both *An. arabiensis* and *An. pharoensis* did not vary signifcantly between the HDNT and HLC, indicating the potential of the HDNT to substitute HLC. In previous studies in Africa, in which human served as both bait and mosquito collector in double net traps, the double net traps yielded signifcantly lower number of anophelines than HLC (Le Goff et al., 1997, Service, 1963). The double net trap collected 7.5 times lower number of anophelines compared to HLC in Cameroon (Le Goff et al., 1997) and about four times lower number of anophelines in Nigeria (Service, 1963). The double net traps might have underestimated the density of *Anopheles* mosquitoes in the previous studies since mosquitoes could escape the double net traps when they were unable to reach the bait (Service, 1977). While the probability of mosquitoes escaping the double net traps could be minimized by conducting hourly collections as described by Tangena et al. (Tangena et al., 2015), such approach may also expose humans to infective mosquito bites when they get out of the inner net to perform mosquito collection. In the present study, the trapping efficiency of the HDNT was enhanced by setting a CDC light trap between the double nets so that mosquitoes could be trapped as soon as they enter the HDNT. The HDNT could also provide a full protection since a person serving as bait in the HDNT does not involve in mosquito collections.

Moreover, the HDNT showed a significant positive correlation with HLC for sampling *An. arabiensis* and other secondary vectors, and its sampling efficiency did not depend on mosquito density. This suggests that the HDNT could represent an efficient alternative tool to HLC for surveillance of outdoor host-seeking malaria vectors. Furthermore, the HDNT yielded higher mosquito species diversity compared to both CDC light trap and HLC. This makes the HDNT a more useful tool for exploring outdoor mosquito species diversity.

The advantage of HDNT and HBLT is that they are not as labour intensive as HLC. In HDNT, a person acting as bait can rest throughout the night. Similarly, HBLT uses odours from human

resting in ordinary sleeping rooms. In the case of HLC (Doolan, 2002) and the previous design of double bed net traps (Tangena et al., 2015, Gao et al., 2018, Service, 1977, Akiyama, 1973), the collectors have to remain active, and collect mosquitoes throughout the night. In addition, mosquito collections using HDNT and HBLT do not rely on the skill of collectors unlike that of HLC which is prone to bias due to interpersonal variation in the skill of the collectors.

Both HBLT and HDNT have limitations. The HBLT uses two batteries, one for a CDC light trap and the other for a pipe, hence may not be feasible in settings where there is no electricity. Using human odour in HBLT requires connecting a pipe from a sleeping room to outdoor mosquito catching station through a hole made on windows or mud-wall of the rooms. Rooms with cement-plastered wall and without window are not appropriate to set HBLT. Hence, further modification is needed to easily dispense human odour. Both HBLT and HDNT were set in the evening and trapped mosquitoes were collected from the traps once in the morning instead of hourly collection, hence we did not compare hourly anopheline mosquito density between these traps and HLC. Further modification using collection bottle rotator that allows automatic hourly collections may be needed to use them for monitoring vector biting times.

5.6. Conclusion

This study revealed that both HBLT and HDNT performed better than the conventional CDC light traps to sample outdoor host-seeking malaria vectors. Moreover, the HDNT yielded a similar vector density as outdoor HLC, suggesting that it could represent an alternative tool to HLC for outdoor biting malaria vector surveillance. The HBLT could be used as an alternative when the HDNT cannot be used especially when there is flood that may affect a person resting under the net.

CHAPTER SIX

6. PATTERNS OF HUMAN EXPOSURE TO EARLY EVENING AND OUTDOOR BITING MOSQUITOES AND RESIDUAL MALARIA TRANSMISSION IN ETHIOPIA (Adopted from Degefa et al., 2021)

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Patterns of human exposure to early evening and outdoor biting mosquitoes and residual malaria transmission in Ethiopia

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6.1. Abstract

Background: Ethiopia has shown a notable progress in reducing malaria burden over the past decade, mainly due to the scaleup of vector control interventions such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Based on the progress, the country has set goals to eliminate malaria by 2030. However, residual malaria transmission due to early evening and outdoor biting vectors could pose a challenge to malaria elimination efforts. This study assessed vector behavior, patterns of human exposure to vector bites and residual malaria transmission in southwestern Ethiopia.

Methods: *Anopheles* mosquitoes were collected monthly from January to December 2018 using Human landing catches (HLCs), human-baited double net traps, CDC light traps and pyrethrum spray catches. Human behaviour data were collected using questionnaire to estimate the magnitude of human exposure to mosquito bites occurring indoors and outdoors at various times of the night. Enzyme-linked immunosorbent assay (ELISA) was used to determine mosquito blood meal sources and sporozoite infections.

Results: A total of 2,038 female *Anopheles* mosquitoes comprising *Anopheles arabiensis* (30.8%), *An. pharoensis* (40.5%), *An. coustani* (28.1%), *An. squamosus* (0.3%) and *An. funestus* group (0.2%) were collected. *Anopheles arabiensis* and *An. pharoensis* were 2.4 and 2.5 times more likely to seek hosts outdoors than indoors, respectively. However, 66% of human exposure to *An. arabiensis* and 39% of exposure to *An. pharoensis* bites occurred indoors for LLIN non-users. For LLIN users, 75% of residual exposure to *Arabiensis* bites occurred outdoors while 23% occurred indoors before bed time. Likewise, 84% of residual exposure to *An. pharoensis* bites occurred outdoors while 15% occurred indoors before people retired to bed. *Anopheles arabiensis* and *An. pharoensis* were 4.1 and 4.8 times more likely to feed on bovine than humans, respectively. Based on the HLC, an estimated indoor and outdoor EIR of *An. arabiensis* was 6.2 and 1.4 infective bites/person/year, respectively, whereas *An. pharoensis* had an estimated outdoor EIR of 3.0 infective bites/person/year.

Conclusion: *Anopheles arabiensis* and *An. pharoensis* showed exophagic and zoophagic behaviour. Human exposure to *An. arabiensis* bites occurred mostly indoors for LLIN non-users, while most of the exposure to both *An. arabiensis* and *An. pharoensis* bites occurred outdoors for LLIN users. Malaria transmission by *An. arabiensis* occurred both indoors and outdoors, whereas *An. pharoensis* contributed exclusively to outdoor transmission. Additional control tools targeting early-evening and outdoor biting malaria vectors are required to complement the current control interventions to control residual transmission and ultimately achieve malaria elimination.

6.2. Introduction

Malaria remains one of the most serious vector-borne diseases, affecting hundreds of millions of people mainly in the sub-Saharan Africa including Ethiopia. Yet unprecedented success has been achieved over the past two decades in reducing the disease burden, averting an estimated 663 million malaria cases in Africa between 2001 and 2015 (WHO, 2015b, Bhatt et al., 2015). Vector control is one of the key elements in achieving the remarkable reduction in malaria, with long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) estimated to have averted 68% and 10% of the cases, respectively (WHO, 2015b, Otten et al., 2009, Bhattarai et al., 2007).

Similarly, morbidity and mortality due to malaria has remarkably declined in Ethiopia over the past decade as a result of large-scale distribution of LLINs and high coverage of IRS, together with nationwide implementation of artemisinin-based combination therapy (ACT) (Otten et al., 2009, Taffese et al., 2018, Shargie et al., 2010, FMoH, 2016). Based these gains, the country has set goals to eliminate malaria by 2030 and the elimination program is being implemented in 239 selected low malaria transmission districts encompassing six different regions (PMI, 2020a). More than 11 million LLINs have been distributed through mass campaigns in 2018 alone to further reduce malaria cases and accelerate the progress towards elimination (WHO, 2019b, PMI, 2019a).

However, malaria transmission continues to occur and still remains a significant public health problem in Ethiopia despite the progress made in scaling up of the control measures (Abraham et al., 2017, Taffese et al., 2018). This transmission could be attributed to several factors including the spread of insecticide resistance (Yewhalaw et al., 2011, Messenger et al., 2017) and preference of malaria vectors to bite outdoors and in the early evening when people are indoors but unprotected by existing tools (Kibret and Wilson, 2016, Kenea et al., 2016, Yohannes and Boelee, 2012). The current indoor-based malaria vector control interventions such as LLINs offer protection from anthropophagic and endophagic vectors, but have little impact on vector species predominantly feeding on animals and humans outdoors (Durnez and Coosemans, 2013).

In Ethiopia, the primary vector of malaria is *An. arabiensis*. This vector species has a peculiar feature in that it can readily feed on humans to sustain intense malaria transmission (Abraham et al., 2017, Massebo et al., 2013b, Animut et al., 2013, Kibret et al., 2014), but often enough on animals to evade the effect of LLINs and IRS, and to maintain residual malaria transmission

(Killeen et al., 2017, Massebo et al., 2015). Such dual feeding preference of *An. arabiensis* could pose a challenge to malaria control and elimination efforts as malaria transmission may continue even with a high coverage of the current vector control interventions (Durnez and Coosemans, 2013, Killeen et al., 2017). Moreover, the feeding behaviour of *An. arabiensis* could vary in different eco-epidemiological settings depending on several factors including host availability (Fettene et al., 2004, Habtewold et al., 2001) and the genetic structure of the vector itself (Lulu et al., 1991, Lulu et al., 1998, Mekuria et al., 1982).

In addition to the vector behavior, human habits and sleeping patterns could also be vital determinants of malaria transmission since exposure to malaria vector bites occurs when unprotected people and vector biting activities overlap in time and space (Monroe et al., 2019a, Finda et al., 2019, Edwards et al., 2019). Addressing the challenge of residual malaria transmission on malaria elimination efforts requires better understanding of both the local vector and human behaviour. Moreover, quantifying the magnitude of human exposure to infectious mosquito bites which occurs indoors and outdoors is crucial to evaluate of the likely success of the current vector control measures (Killeen et al., 2006). However, most vector surveillance activities in Ethiopia focused mainly on vector behaviour with less or no attention to human behaviour that also contributes to residual malaria transmission. The aim of this study was to assess vector behaviour, patterns of human exposure to mosquito bites and residual malaria transmission in southwestern Ethiopia.

6.3. Materials and Methods

6.3.1. Study area

The study was carried out in Bulbul *kebele* (7.70285°N; 37.09592°E, altitude 1705 m asl), which is located in Kersa district, Jimma Zone 320 km southwest of the capital, Addis Ababa (Figure 6.1). The inhabitants mostly rely on subsistence farming, with maize and *teff* being the main cultivated crops in the area. Most houses are mud-walled with roofs made of corrugated iron sheets. Malaria transmission is seasonal in Bulbul area. The transmission peaks from September to October, following the major rains from June to September. Minor transmission occurs in April and May, following the short rains of February to March. *Plasmodium falciparum* and *Plasmodium vivax* are the two predominant malaria parasite species co-occurring in the area and are transmitted mainly by *An. arabiensis* (Yewhalaw et al., 2009).

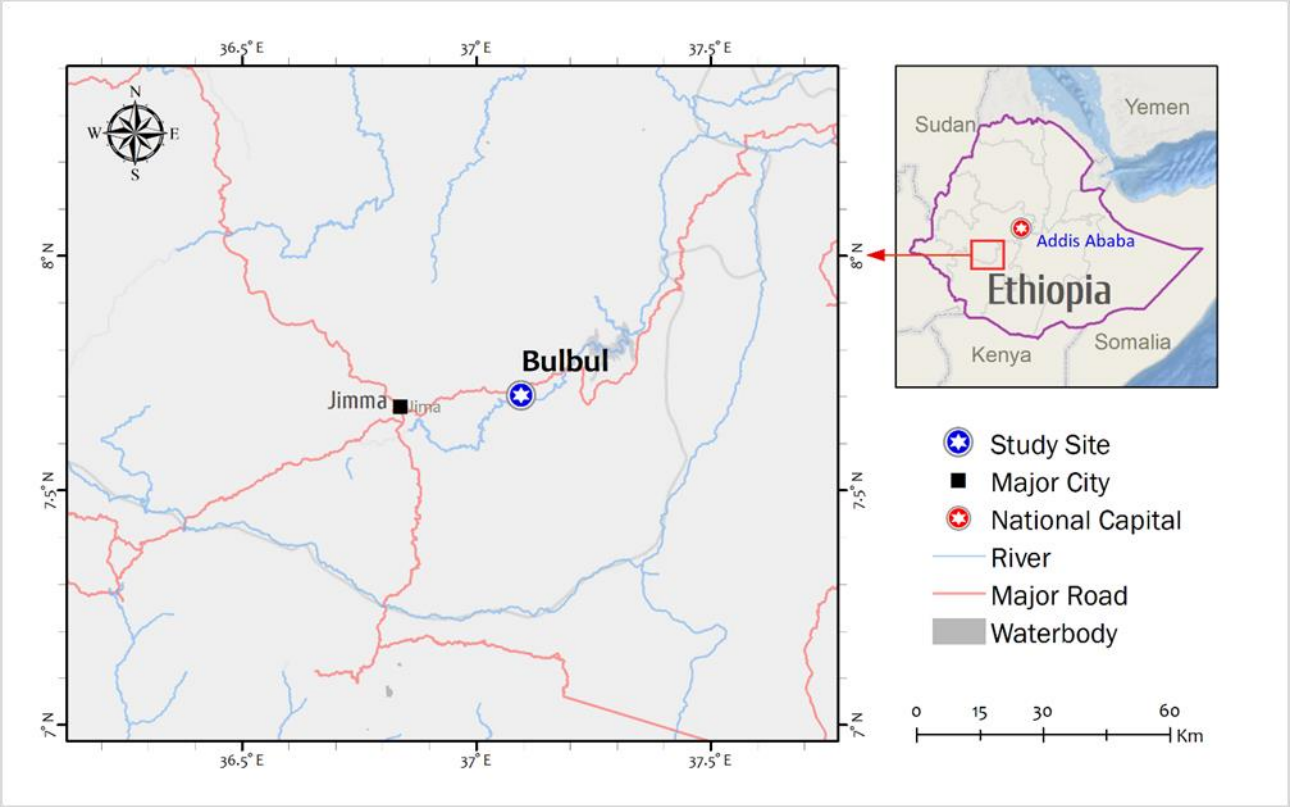

Figure 6.1. Map of the study site in Ethiopia

6.3.2. Mosquito sampling

Adult mosquito collections were carried out monthly from January to December 2018. Host-seeking mosquitoes were collected both indoors and outdoors using human landing catches (HLC), Centers for Disease Control and Prevention (CDC) miniature light traps and human-baited double net traps (HDNT). Indoor resting mosquitoes were collected using pyrethrum spray catches (PSCs).

The HLC was performed in four randomly selected houses per month by adult male volunteers. For each house, two collectors (one indoor and the other outdoor) seated on stools with their legs exposed from foot to knee to capture mosquitoes as soon as they land on the exposed legs, before they commence blood-feeding, using a flashlight and mouth aspirator (Service, 1977, WHO, 2013c). There were two collection shifts: one team worked from 18:00 to 24:00 hr during each collection night, followed by the second team from 24:00 to 06:00 hr. Each hour's collection was kept separately in labelled paper cups. A supervisor was assigned to coordinate the collection activities and watch volunteers not to fall asleep during the collection nights. All collectors were

provided with anti-malaria prophylaxis to avoid a risk of contracting malaria during the collection period. Mosquitoes were identified to species the next morning. The CDC light traps were set indoors beside human-occupied bed nets in other four randomly selected houses monthly and paired with outdoor HDNT. Details of the HDNT are described elsewhere (Degefa et al., 2020). Both traps were set from 8:00 to 6:00 hr during each collection night. The PSC was conducted monthly in twenty randomly selected houses from 06:00 to 09:00 hr following standard protocol (WHO, 1995).

All collected mosquitoes were identified morphologically to species or species complexes using a dichotomous key described by Gillies and De Meillon (Gillies and Coetzee, 1987). Female *Anopheles* mosquitoes were further classified as unfed, freshly fed, half-gravid and gravid. Each mosquito was kept individually in a labelled 1.5 ml Eppendorf tube containing silica gel desiccant. Samples were stored at -20°C freezer at Jimma University Tropical and Infectious Diseases Research Center (TIDRC) Laboratory until used for further processing.

6.3.3. Human behavior survey

Questionnaire survey was conducted in October 2018 in 140 randomly selected households residing in the study area. The residents were asked about the time they usually went indoors, when they retired to bed, when they woke up in the morning and when they left their houses for outdoor activities. Moreover, data on the ownership and utilization of nets by the households, and the numbers of potential vertebrate hosts available in the study area including human, bovine, goat, dog and chicken were collected using the questionnaire survey.

6.3.4. Mosquito sample processing

Anopheles gambiae s.l. specimens were identified to sibling species by polymerase chain reaction (PCR), following the protocol developed by Scott *et al* (Scott et al., 1993). Dried head and thorax of the preserved *Anopheles* mosquito specimens were carefully separated from the abdomen and tested for *Plasmodium* circumsporozoite protein (CSP) using sandwich ELISA (Beier et al., 1987, Wirtz et al., 1987). The blood meal sources of freshly fed *Anopheles* mosquitoes collected by PSC and CDC light trap were assayed by a direct enzyme-linked immunosorbent assay (ELISA) using human, bovine, goat, chicken and dog antibodies (Beier et al., 1988).

6.3.5. Data analysis

The mean density of host-seeking *Anopheles* mosquitoes was compared between indoor and outdoor locations using a generalized linear model based on negative binomial distribution. Season of collection was treated as a covariate in the model. Crude biting rate for each anopheline species was determined as the mean number of *Anopheles* mosquitoes collected by HLC per person per night.

Human exposure to malaria vector bites was calculated based on data from both human and vector behavior. Behaviour-adjusted human biting rate (aHBR) experienced by unprotected individuals at each time of the night (t) was determined based on the proportion of people reported to have stayed indoors (I) multiplied by indoor biting rate (Bi) plus the proportion of people reported to have stayed outdoors (1-I) multiplied by the outdoor biting rate (Bo) (Killeen et al., 2006). The aHBR per night was then calculated by summing hourly biting rates:

$$aHBR = \sum_{t=1}^{12} [B_{i,t} I_t + B_{o,t}(1 - I_t)] \quad (1)$$

where t = 1 represents the time period from 6:00 to 7:00 pm, t = 2 from 7:00 to 8:00 pm, and continue as such up to t = 12 for the time period from 05:00 to 6:00 am.

The mean biting rate experienced by protected individuals (aHBRp) per night was calculated by adjusting the indoor biting rates for the sleeping fraction of the population taking into account the personal protection (ρ) provided by LLINs:

$$aHBR_p = \sum_{t=1}^{12} [B_{i,t}(S_t(1 - \rho) + (I_t - S_t)) + B_{o,t}(1 - I_t)] \quad (2)$$

where S_t represents the proportion of people who reported to have retired to bed for sleeping. Personal protective efficacy of 98.3% (ρ = 0.983) was assumed for LLINs (PermaNet 2.0) based on findings from experimental hut trials conducted elsewhere (Mahande et al., 2018).

The proportion of human exposure to mosquito bites which occur indoors (π_i) for unprotected individuals was calculated from the mean indoor (B_i) and outdoor (B_o) hourly biting rates as follows (Killeen et al., 2006, Seyoum et al., 2012).

$$\pi_i = \sum_{t=1}^{12} [B_{i,t} I_t] \,/\, \sum_{t=1}^{12} [B_{i,t} + B_{o,t}(1 - I_t)] \quad (3)$$

The proportion of human exposure to mosquito bites which occurs during sleeping hours (π_s) for unprotected individuals was determined in a similar way to equation 3, with a numerator calculated as the sum of the products of the mean hourly indoor biting rate ($B_{i,\ t}$) and the proportion of humans reported to have retired to bed (S) for each hour of the night (t):

$$\pi_s = \sum_{t=1}^{12} [\, B_{i,t}\, S_t] \,/\, \sum_{t=1}^{12} [B_{i,t} I_t + B_{o,t}(1 - I_t)] \qquad (4)$$

The parameter π_i is an indicator of the maximum possible personal protection provided by any indoor interventions, whereas π_s is an indicator of maximum personal protection an intervention such as LLIN could provide during sleeping hours. The proportion of mosquito bites directly prevented using LLIN (P*s) was calculated as the product of π_s and the protective efficacy of LLINs (Killeen et al., 2006, Moiroux et al., 2014, Monroe et al., 2019a).

The proportion of residual human exposure to mosquito bites which occur indoors ($\pi_{i,n}$) for LLIN users was calculated by adjusting π_i taking into account the personal protection (ρ) provided by LLIN:

$$\pi_{i,p} = \sum_{t=1}^{12} [B_{i,t}\, (S_t(1-\rho) + (I_t - S_t))] \;/\; \sum_{t=1}^{12} [B_{i,t}\, (S_t(1-\rho) + (I_t - S_t)) + B_{o,t}(1 - I_t)] \qquad (5)$$

Human blood index (HBI) was calculated as the proportion of *Anopheles* mosquitoes that fed on humans over the total *Anopheles* tested for blood meal origin (Garrett-Jones, 1964). Blood-meal indices of other non-human vertebrate hosts were also calculated in a similar way. Host abundance was determined from questionnaire survey data as the number of a particular host divided by the total number of all potential hosts (human, cattle, goat, dog and chicken) multiplied by 100. The forage ratio (FR), a measure of host preference by mosquitoes, was determined as the proportion of engorged *Anopheles* mosquitoes which fed on a given host divided by the abundance (proportion) of that particular host in the study area (Hess et al., 1968, Manly et al., 2007). A host was considered to have been preferred if the lower 95% confidence limit for the FR estimate was greater than one and inferred to have been avoided if the upper 95% confidence limit of the FR estimate was less than one. A host for which the 95% confidence interval for its FR included one was considered to have been by mosquitoes opportunistically.

The sporozoite rate was estimated as the proportion of mosquitoes positive for *P. falciparum* and/or *P. vivax* CSP over the total number tested. Annual entomological inoculation rate (EIR) was determined separately for indoor and outdoor mosquito collections as aHBR x sporozoite infection rate x 365. The overall annual EIR was obtained by summing the indoor and outdoor EIRs.

Data were analysed using SPSS version 20.0 (SPSS, Chicago, IL, USA) software package. $p < 0.05$ was considered statistically significant during the analysis.

6.4. Results

6.4.1. Mosquito species composition and abundance

A total of 2,038 female anopheline mosquitoes comprising *Anopheles gambiae s.l.* (30.8%), *An. pharoensis* (40.5%), *An. coustani* (28.1%), *An. squamosus* (0.3%) and *An. funestus* group (0.2%) were collected by all methods during the study period (Table 6.1). The majority (73.1%) of the anopheline mosquitoes were collected outdoors. A total of 278 *An. gambiae s.l.* specimens were analysed for molecular identification of sibling species. Of these, 252 (90.6%) specimens were successfully amplified by PCR and all were *An. arabiensis*.

Table 6.1. Summary of female *Anopheles* mosquitoes collected from indoor and outdoor in Bulbul, southwestern Ethiopia

Species	**Indoor**			**Outdoor**		**Total**
	HLC	Light trap	PSC	HLC	HDNT	
An. arabiensis	106	72	42	240	168	628
An. pharoensis	170	34	13	366	243	826
An. coustani	89	20	1	362	101	573
An. squamosus	1	0	0	4	2	7
An. funestus group	1	0	0	2	1	4
Total	**367**	**126**	**56**	**974**	**515**	**2,038**

Note: PSC: pyrethrum spray catch, HLC: human landing catch, HDNT: human-baited double net trap

6.4.2. Indoor and outdoor *Anopheles* mosquito density

Table 6.2 shows the results of a negative binomial regression model for the comparison host-seeking *Anopheles* mosquito density between indoor and outdoor location. Based on the gold standard surveillance method (HLC), *An. arabiensis* was 2.41 (95% CI: 1.46-3.98) times more likely to be captured outdoors than indoors, suggesting this species to display exophagic behavior in the study area. Similarly, the density of *An. arabiensis* was 3.74 (95% CI: 2.07-6.76) times higher outdoors than indoors based on the alternative methods (HDNT *vs*. CDC light trap). Likewise, the mean density of *An. pharoensis* and *An. coustani* was significantly higher outdoors than indoors based on both the gold standard and alternative surveillance methods (Table 2).

Table 6.2. Estimates of a negative binomial regression for the comparison of host-seeking *Anopheles* mosquito density between indoor and outdoor location in Bulbul, southwest Ethiopia

Species	**Traps**	**Location**	**EMM (95% CI)**	**OR (95% CI)**	**p value**
Standard method					
An. arabiensis	HLC	Outdoor	3.47 (2.48-4.48)	2.41 (1.46-3.98)	0.001*
	HLC	Indoor	1.44 (0.98-2.12)	1.0[a]	
An. pharoensis	HLC	Outdoor	5.05 (3.65-7.00)	2.48 (1.53-4.00)	<0.0001*
	HLC	Indoor	2.04 (1.42-2.95)	1.0[a]	
An. coustani	HLC	Outdoor	2.0 (1.12-3.60)	3.71 (2.13-6.45)	<0.0001*
	HLC	Indoor	0.54 (0.28-1.04)	1.0[a]	
Alternative methods					
An. arabiensis	HDNT	Outdoor	2.34 (1.61-3.40)	3.74 (2.07-6.76)	<0.0001*
	Light trap	Indoor	0.62 (0.39-1.01)	1.0[a]	
An. pharoensis	HDNT	Outdoor	3.30 (2.32-4.67)	6.61 (3.71-11.77)	<0.0001*
	Light trap	Indoor	0.51 (0.31-0.84)	1.0[a]	
An. coustani	HDNT	Outdoor	0.96 (0.53-1.74)	8.74 (3.97-19.21)	<0.0001*
	Light trap	Indoor	0.11 (0.47-0.26)	1.0[a]	

Note: HLC: human landing catch, HDNT: human-baited double net trap, EMM: estimated marginal mean density, OR: odds ratio, [a] Reference value. EMM was determined using a negative binomial regression model by adjusting for season

6.4.3. Hourly biting activity of *Anopheles* mosquitoes

The crude biting rates of all *Anopheles* species were higher outdoors than indoors throughout the night (Figure 6.2). The mean indoor and outdoor biting rate of *An. arabiensis* was 2.2 and 5.0 bites/person/night (b/p/night), respectively. The indoor and outdoor biting rate of *An. pharoensis* was 3.5 and 7.6 b/p/night, respectively, whereas the indoor and outdoor biting rate of *An. coustani* was 1.9 and 7.5 b/p/night, respectively. The peak biting activity of *An. arabiensis* was recorded in the evening between 9:00 pm and 10:00 pm and then started to decline when people were indoors (Figure 6.2). The peak biting activities of *An. pharoensis* and *An. coustani* were observed early part of the evening between 7:00 pm and 8:00 pm.

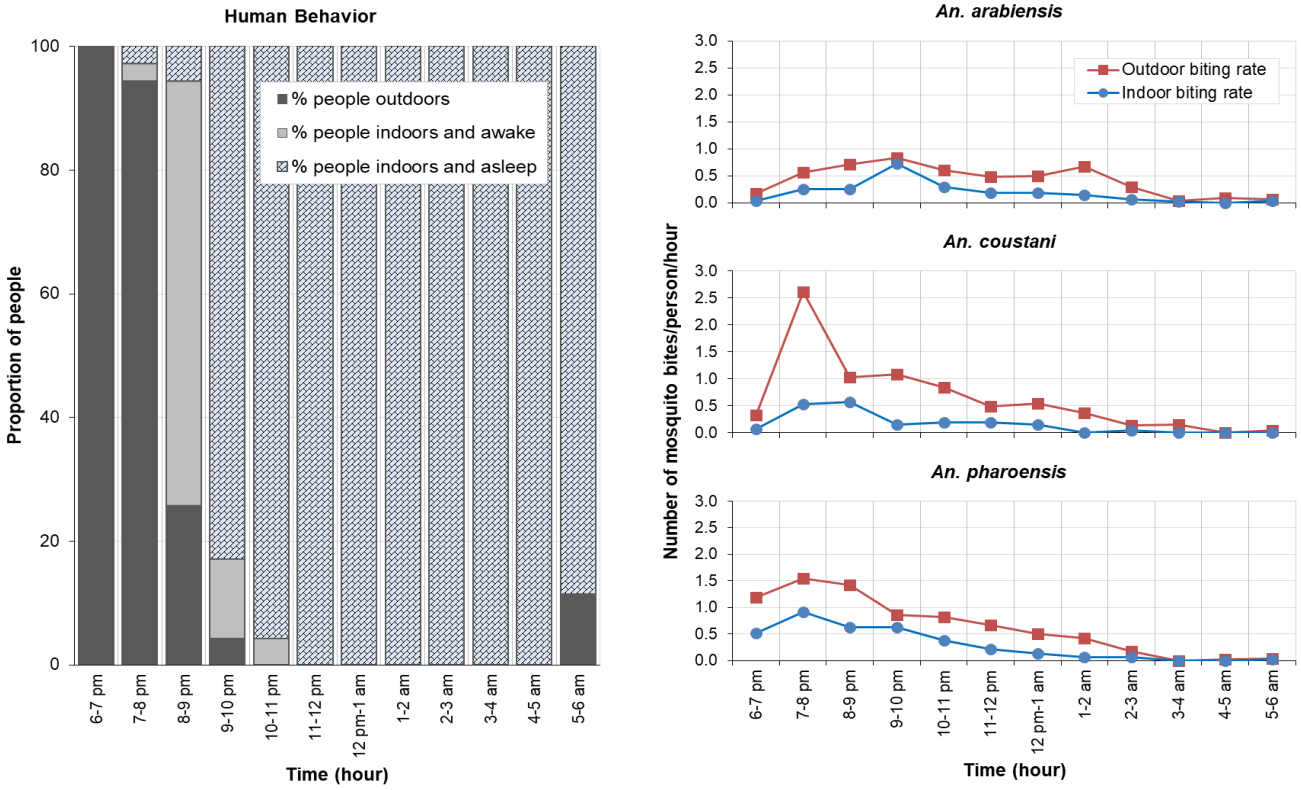

Figure 6.2. Proportion of people outdoors, indoors and awake, and indoors and asleep throughout the night, and the crude biting rates of Anopheles mosquitoes (indoor and outdoor) in Bulbul, southwestern Ethiopia.

6.4.4. Human exposure to mosquito bites

For unprotected individuals (LLIN non-users), an estimated 66% and 56% of human exposure to *An. arabiensis* bites occurred indoors and during sleeping hours, respectively (Figure 6.3). About 39% of exposure to *An. pharoensis* bites and 27% of exposure to *An. coustani* bites occurred

indoors for unprotected individuals. Use of LLIN was estimated to prevent 55.2%, 27.8% and 16.8% of exposure from *An. arabiensis*, *An. pharoensis* and *An. coustani* bites, respectively, which otherwise would occur. For LLIN-users, the majority (75%) of residual human exposure to *An. arabiensis* bites occurred outdoors while 23% occurred indoors before people retired to bed. Likewise, the majority (84%) of residual exposure to *An. pharoensis* bites occurred outdoors while 15% occurred indoors before bed time. Similarly, most of the residual exposure to *An. coustani* occurred outdoors (Figure 6.3).

Results of questionnaire survey showed that 88.5% of the households had at least one LLIN. Over 94% of the study participants reported to have stayed outdoors or between outdoors and indoors until 8:00 pm. About 83% of the respondents reported going to bed by 9:00 pm. The main activities that kept people outdoors include household chore, praying, keeping cattle and social gatherings.

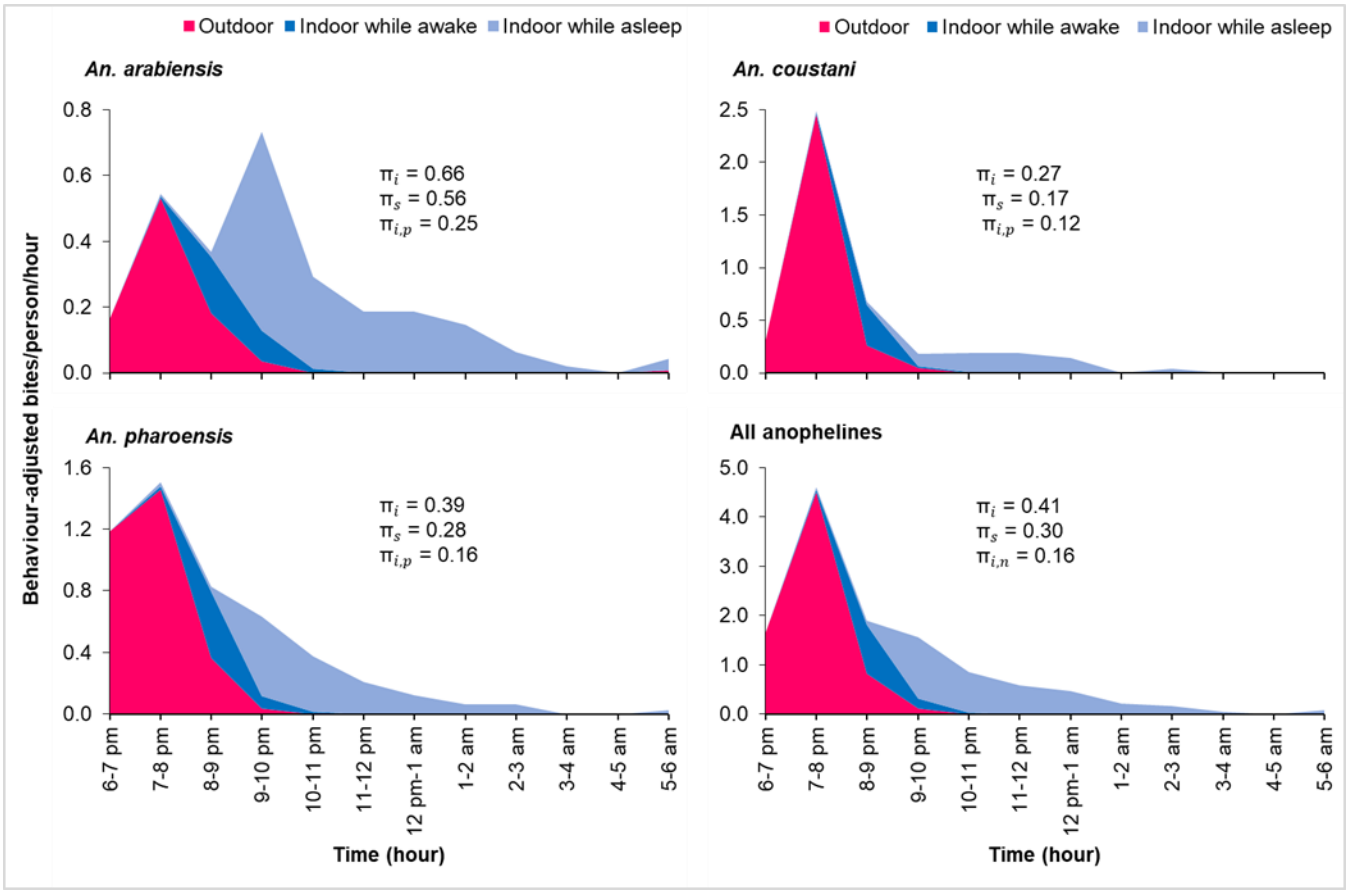

Figure 6.3. Behaviour-adjusted estimates of human exposure to *Anopheles* mosquitoes occurring indoors and outdoors in Bulbul, southwestern Ethiopia

6.4.5. Blood meal origins and feeding preferences

Table 6.3 shows the blood-meal sources of *An. arabiensis* and other anopheline mosquito species. The HBI, bovine blood index (BBI) and goat blood index (GBI) of *An. arabiensis* were 19.2%, 65.4% and 11.5%, respectively. *Anopheles pharoensis* had HBI, BBI and GBI of 16.7, 66.7% and 5.5%, respectively. Very few fed *An. coustani* were caught and all were positive for bovine. None of the tested anopheline specimens were positive for dog, whereas 1.9% of the tested *An. arabiensis* specimens were positive for chicken.

Table 6.3. Blood meal sources of *Anopheles* mosquitoes collected from indoor in Bulbul, southwestern Ethiopia

Blood meal indices	***An. arabiensis***			***An. Pharoensis***			***An. coustani***
	Light trap	**PSC**	**Total**	**Light trap**	**PSC**	**Total**	**Light trap**
No. tested	24	28	52	10	8	18	4
Human	4 (16.7)	4 (14.3)	8 (15.4)	1 (10.0)	1 (12.5)	2 (11.1)	0
Bovine	17 (70.8)	17 (60.7)	34 (65.4)	7 (70.0)	5 (62.5)	12 (66.7)	4 (100.0)
Goat	2 (8.3)	2 (7.1)	4 (7.7)	0	0	0	0
Chicken	0	1 (3.6)	1 (1.9)	0	0	0	0
Dog	0	0	0	0	0	0	0
Human+Goat	1 (4.2)	1 (3.6)	2 (3.8)	1 (10.0)	0	1 (5.5)	0
Unknown	0	3 (10.7)	3 (5.8)	1 (10.0)	2 (25.0)	3 (16.7)	0

Note: PSC: pyrethrum spray catch

Regardless of higher proportion of humans in the study area compared to other vertebrate hosts, *An. arabiensis* and *An. pharoensis* were 4.1 and 4.8 times more likely to feed on bovine than humans (Table 6.4).

Table 6.4. Host preference of Anopheles arabiensis in Bulbul, southwestern Ethiopia

Species	Parameters	Human	Bovine	Goat	Chicken
Host abundance in the area (%)		39.0	32.2	6.8	22.0
An. arabiensis	Blood index	19.2	65.4	11.5	1.9
	FR (95% CI)	0.49 (0.22-0.77)	2.03 (1.63-2.43)*	1.69 (0.42-3.0)	0.09 (0.0-0.26)
An. pharoensis	Blood index	16.7	66.7	5.5	0
	FR (95% CI)	0.43 (0.0-0.87)	2.07 (1.39-2.75)*	0.81 (-0.74-2.37)	0

Note: FR: forage ratio; CI: confidence interval; * indicates the preferred host

6.4.6. Sporozoite rate and Entomological inoculation rate

A total of 2,036 anopheline mosquitoes were tested for *Plasmodium* CSP, of which 6 specimens (*3 An. arabiensis*, 2 *An. pharoensis* and 1 *An. coustani*) were positive (Table 6.5). The sporozoite rate of *An. arabiensis* from indoor and outdoor HLC was 0.9% and 0.4%, respectively, whereas the sporozoite rate of *An. pharoensis* from indoor and outdoor HLC was 0 and 0.3%, respectively. The sporozoite rates of A*n. arabiensis* and *An. pharoensis* from HDNT were 0.6% and 0.4%, respectively. None of the *An. arabiensis* and *An. pharoensis* tested from CDC light trap and PSC were positive. No *Plasmodium* CSP was detected in *An. squamosus* and *An. funestus* group. Based on the HLC, an estimated indoor and outdoor EIR of *An. arabiensis* was 6.2 and 1.4 infective bites/person/year (ib/p/year), respectively, while *An. pharoensis* had an estimated outdoor EIR of 3.0 ib/p/year. HDNT-based EIRs of *An. arabiensis* and *An. pharoensis* were 2.0 and 4.5 ib/p/year, respectively (Table 6.5).

Table 6.5. Indoor and outdoor human biting rates, sporozoite rates and annual entomological inoculation rates (EIRs) of *Anopheles* mosquitoes in Bulbul, southwestern Ethiopia

***Anopheles* species**	**Parameters**	**Indoor**		**Outdoor**		**Total**	
		HLC	Light trap	HLC	HDNT	HLC	ALT
An. arabiensis	No tested	106	70	240	168	346	238
	aHBR	1.8	1.8	0.9	0.9	2.7	2.7
	Pf +ve (%)	1 (0.9)	0	1 (0.4)	1 (0.6)	2 (0.6)	1 (0.4)
	Pf EIR	6.2	0	1.4	2.0	7.6	2.0
	Pv +ve (%)	0	0	0	0	0	0
	Pv EIR	0	0	0	0	0	0
An. pharoensis	No tested	170	34	366	243	536	277
	aHBR	2.0	2.0	3.0	3.0	5.0	5.0
	Pf +ve (%)	0	0	0	0	0	0
	Pf EIR	0	0	0	0	0	0
	Pv +ve (%)	0	0	1 (0.3)	1 (0.4)	1 (0.2)	1 (0.4)
	Pv EIR	0	0	3.0	4.5	3.0	4.5
An. coustani	No tested	89	20	362	101	451	121
	aHBR	1.2	1.2	3.0	3.0	4.2	4.2
	Pf +ve (%)	0	0	0	0	0	0
	Pf EIR	0	0	0	0	0	0
	Pv +ve (%)	0	0	1 (0.3)	0	1 (0.2)	0
	Pv EIR	0	0	3.0	0	3.0	0

Note: HLC: human landing catch, HDNT: human-baited double net trap, ALT: alternative methods, aHBR-behavior-adjusted human biting rate; *Pf: P. falciparum, Pf: P. vivax; EIR:* annual entomological inoculation rates

6.5. Discussion

This study indicated that *An. pharoensis* was the most abundant anopheline species in the study area followed by *An. arabiensis* and *An. coustani*. Previous studies reported that *An. arabiensis* was the predominant species in different malaria endemic settings of southwestern Ethiopia (Taye et al., 2016, Degefa et al., 2015). The higher abundance of *An. pharoensis* over *An. arabiensis* in this study could be attributed to difference in mosquito breeding habitats. The present study area is located in the Omo-Gibe River Basin with abundant aquatic vegetations that might have favoured *An. pharoensis. Anopheles pharoensis* prefers to breed in vegetated swamps unlike *An. arabiensis* which typically breeds in small, sunlit temporary water pools (Kenea et al., 2011).

Anopheles arabiensis exhibited exophagic behaviour, seeking hosts mostly outdoors rather than indoors. Similar findings were also reported from different parts of Ethiopia (Kenea et al., 2016, Taye et al., 2016, Getachew et al., 2019, Kibret and Wilson, 2016). *Anopheles arabiensis* was shown to be preponderantly exophagic even before the scaleup of indoor-based vector control interventions in Ethiopia (Tirados et al., 2006, White, 1974b), suggesting that the exophagic behaviour of this species might be genetically determined (White, 1974b). Moreover, the long-term use of the current vector control interventions (LLINs and IRS) might have further enhanced the proportion of outdoor biting fraction of *An. arabiensis* as observed elsewhere in Africa. For instance in western Kenya, *An. arabiensis* was more likely to bite outdoors (Degefa et al., 2017, Bayoh et al., 2014) when compared with data collected before the scale-up of LLINs (Githeko et al., 1996, Githeko et al., 1994a). Likewise, *An. pharoensis* showed exophagic behaviour in the study area. Similar findings were also reported for this species from different parts of Ethiopia (Kenea et al., 2016, Taye et al., 2006, Taye et al., 2016, Kibret et al., 2014).

In the absence of personal protection by LLINs, the majority of human exposure to *An. arabiensis* bites occurred indoors (π = 66%) despite the outdoor host-seeking preference of this species. This is due to coincidence of humans and the peak biting activities of *An. arabiensis* since most people spend their time indoors when this species is mostly active (Figure 6.2). A similar phenomenon was documented for other malaria vector species in Africa (Sherrard-Smith et al., 2019). For instance, *An. funestus* and *An. quadriannulatus* did not show preference to bite indoors in Zambia, yet a substantial proportion of human contact with both species has been

shown to occur indoors in the absence of LLIN use in the country (Seyoum et al., 2012). This highlights the need to consider human behaviour to determine the actual magnitude of human exposure to mosquito bites which may occur indoors and/or outdoors.

For LLIN non-users, 56% of human exposure to *An. arabiensis* bites occurred at times when using LLINs is feasible, indicating that the maximum possible personal protection that could be provided by LLIN is only 56%. This implies that with only the current indoor-based vector intervention (LLINs), malaria elimination may not be achieved since the remaining exposure to *An. arabiensis* bites could still occur outdoors and/or indoors before people retire to bed. A study conducted in Tanzania also showed that less than half (46%) of all human exposure to *An. arabiensis* bites occurred at times when using ITNs was feasible (Govella et al., 2010). Only 28% of human exposure to *An. pharoensis* bites occurred at times when LLINs would be in use if they were available, indicating that the majority of exposure to *An. pharoensis* also occurs outdoors and before sleeping hours.

For LLIN users, the majority (75%) of residual human exposure to *An. arabiensis* bites occurred outdoors while 23% occurred indoors before people retired to bed. Similarly, most (84%) of the residual exposure to *An. pharoesnsis* bites occurred outdoors, while 15% occurred indoors before sleeping time. The findings suggest that additional control measures which can protect against outdoor exposure or which target immature stages of vectors are required to complement the current indoor-based vector control interventions (LLINs and IRS) to interrupt transmission due to exposure to vector bites occurring outdoors and in the early evening hours.

Anopheles arabiensis showed a preference to feed on bovine to humans. The findings of previous studies conducted in different parts of Ethiopia showed that the feeding behaviour of *An. arabiensis* varied across different geographical locations. The species exhibited zoophagic behaviour in some settings (Hadis et al., 1997, Massebo et al., 2015), anthropophagic in other places (Yohannes et al., 2005, Tirados et al., 2006, Kibret et al., 2017) and anthropozoophilic (opportunistic) tendency in some areas (Habtewold et al., 2001, Getachew et al., 2019). Such interpopulation variations in feeding behaviour might be due to difference in host availability between different settings (Killeen et al., 2001, Habtewold et al., 2001). Interpopulation genetic variation in *An. arabiensis* might have also contributed to the variation in its feeding behaviour between different localities. Subpopulation of *An. arabiensis* with preference to feed on cattle

have been shown to correlate with arrangement of 3Ra chromosomal inversion (Lulu et al., 1998, Main et al., 2016). Such phenomenon could increase the proportion of zoophagic fraction of *An. arabiensis* in settings where the 3Ra inversion is documented (Lulu et al., 1991, Lulu et al., 1998). Similarly, *An. pharoensis* showed zoophagic behavior, preferring to feed on bovine to other potential hosts available in the study area.

The zoophagic behaviour of *An. arabiensis* and *An. pharoensis* can be considered as an opportunity to introduce complementary vector control intervention such as zooprophylaxis to divert host-seeking mosquitoes from humans (Iwashita et al., 2014, Habtewold et al., 2001). Anthropophilic and endophagic malaria vectors can be controlled by LLINs and IRS, whereas those species predominantly feeding on cattle outdoors could sustain residual malaria transmission despite high coverage of indoor-based vector control interventions. Hence, targeting zoophagic vectors is crucial to achieve malaria elimination. Zooprophylaxis can reduce malaria transmission by pulling mosquitoes toward dead-end hosts and by reducing vector density if cattle are treated with insecticides (Bulterys et al., 2009, Chaccour et al., 2018).

The estimated indoor and outdoor EIRs for *An. arabiensis* were 6.2 and 1.4 ib/p/year, respectively, indicating the contribution of *An. arabiensis* to both indoor and outdoor malaria transmission. The occurrence of indoor malaria transmission despite high LLIN coverage in the study area might be attributed to the exposure of people to vector bites in the evening before sleeping hours. Resistance of malaria vectors to insecticides (Yewhalaw et al., 2011, Messenger et al., 2017) might have also contributed to the indoor EIR. In addition, *An. pharoensis* had an estimated outdoor EIR of 3.0 ib/p/year, indicating the contribution of this species to outdoor transmission. Although *An. pharoensis* has been considered as a secondary vector in Ethiopia, a recent study revealed similar tends of susceptibility of this species to *Plasmodium* parasite infection as *An. arabiensis* (Abduselam et al., 2016), indicating that *An. pharoensis* could also play a major role in outdoor malaria transmission. Other recent studies have also documented an increasing role of *An. pharoensis* in malaria transmission in the country (Kibret et al., 2014, Abraham et al., 2017).

On the other hand, the EIRs of *An. arabiensis* reported in this study are lower compared to the EIRs of *An. arabiensis* previously reported from different parts of Ethiopia (Degefa et al., 2015, Massebo et al., 2013b, Kibret et al., 2017, Animut et al., 2013, Abraham et al., 2017) and

elsewhere in Africa (Degefa et al., 2017, Himeidan et al., 2011). This could be attributed to a relatively higher coverage of LLINs in the study area.

The strength of this study is that both vector and human behaviour data were considered in the calculation of human biting rates and EIRs to better understand where and when exposure to mosquito bites and residual malaria transmission occur. Moreover, this study employed both gold standard method i.e. HLC and alternative methods (CDC light traps and HDNT) for vector surveillance to determine vector density, human biting rates and sporozoite rates. The findings of this study suggest that CDC light trap can be paired with HDNT for routine indoor and outdoor malaria vector surveillance as an alternative tool to HLC. The limitation of the study was that the sporozoite infection rates reported in this study were based on ELISA and the positive specimens were not confirmed by PCR. The proportions of human exposure to mosquito bites were estimated assuming no seasonal changes in sleeping habits of people in the study area; hence night to night differences in sleeping time were not tracked in this study.

6.6. Conclusion

Populations of *An. arabiensis* and *An. pharoensis* showed exophagic and zoophagic behaviour. The majority of human exposure to *An. arabiensis* bites occurred indoors for LLIN non-users, while most of the residual exposure to both *An. arabiensis* and *An. pharoensis* bites occurred outdoors for LLIN users. Malaria transmission by *An. arabiensis* occurred both indoors and outdoors, while *An. pharoensis* contributed exclusively to outdoor transmission. Additional control tools targeting outdoor and early evening biting vectors are required to complement the current control interventions to control residual transmission and ultimately achieve malaria elimination. Further studies are required to comprehend the role of *An. coustani* in malaria transmission in Ethiopia.

CHAPTER SEVEN

7. GENERAL DISCUSSION AND CONCLUSIONS

7.1. Discussion of the main findings

The main aim of the study was to develop and evaluate surveillance tools for outdoor resting and outdoor host-seeking malaria vectors, and to determine vector behaviour, patterns of human exposure to vector bites, and residual malaria transmission in Kenya and Ethiopia. The study started in September 2015 with indoor and outdoor malaria vector surveillance in Ahero and Iguhu sites in western Kenya (Chapter 3). A new sticky pot was developed for outdoor resting malaria vector surveillance and its performance was evaluated in western Kenya between September 2015 and April 2016 (Chapter 4). Two other exposure-free tools, the HBLT and HDNT, were developed for outdoor host-seeking malaria vector surveillance and their performance was evaluated in western Kenya and Bulbul *Kebele*, southwestern Ethiopia from November 2015 to December 2018 (Chapter 5). Furthermore, vector behaviour, patterns of human exposure to vecor bites and residual malaria transmission were assessed in Bulbul site from January to December 2018 (Chapter 6). Over the three years study period, a total of 31,862 female *Anopheles* mosquitoes (29,551 from western Kenyan and 2,311 from southwestern Ethiopia) comprising at least seven species were collected.

In western Kenya, *An. arabiensis* and *An. gambiae* were the predominant anopheline species in Ahero and Iguhu sites, respectively followed by *An. funestus* in both sites. *Anopheles arabiensis* exhibited exophagic and zoophagic behaviour, *An. gambiae* showed endophagic behaviour with a preference to feed both on human and cattle, while *An. funestus* exhibited endophagic and anthropophagic behaviour. The overall *P. falciparum* sporozoite rates of *An. arabiensis* and *An. gambiae* were 0.16% and 2.3%, respectively. The sporozoite rate of *An. funestus* was 1.8% in Ahero and 2.4% in Iguhu. The overall EIRs of *An. arabiensis* and *An. gambiae* were 57.5 and 24.3 ib/p/year, respectively. *Anopheles funestus* had overall EIRs of 94.6 and 5.7 ib/p/year in Ahero and Iguhu sites, respectively. About 48% of the infective bites by *An. arabiensis*, 22.6% by *An. gambiae* and 16.5% by *An. funestus* occurred outdoors.

In Bulbul, southwestern Ethiopia, *An. pharoensis* was the predominant anopheline species followed by *An. arabiensis*. Both species exhibited exophagic and zoophagic behaviours. The overall sporozoite rates of *An. arabiensis* and *An. pharoensis* were 0.6% and 0.2%, respectively. Human-behaviour adjusted EIRs of *An. arabiensis* and *An. pharoensis* were 7.6 and 3.0 ib/p/year, respectively. About 18.5% of the infective bites by *An. arabiensis* and all by *An. pharoensis* occurred outdoors in the area.

In addition to the work presented in the previous chapters, a summary of the implications of the study findings for malaria control and elimination efforts, strengths and limitations of the study, key messages that this study conveys for health policy makers and the remaining knowledge gaps which should be prioritized for future research are discussed in the following sections.

7.1.1. Vector behaviour and residual malaria transmission

The success of malaria vector control interventions depends on local vector behaviours and their response to the control measures (Russell et al., 2013). The rationale underpinning the use of the existing frontline malaria vector control interventions (LLINs and IRS) was actually based on the assumption that the most potent vectors of malaria in the world bite predominantly indoors in the middle of the night so that sleeping under a treated net during this period could greatly reduce exposure to malaria transmission, and ultimately lead to malaria elimination (Pates and Curtis, 2005, Gillies and Coetzee, 1987, Huho et al., 2013). However, there is an increasing concern that such assumption may not be applicable in Africa due to atleast two factors pertaining to vector behaviour: 1) The long-term use of the current vector control interventions could change the behaviour of vectors from anthropophagic to anthropozoophagic/zoophagic, endophagic to exophagic, and endophilic to exophilic tendencies (Durnez and Coosemans, 2013, Russell et al., 2011, Sherrard-Smith et al., 2019, Moiroux et al., 2012). Such behavioural change could allow mosquito vectors to escape from contact with insecticide treated surfaces and maintain residual malaria transmission (Durnez and Coosemans, 2013, Sherrard-Smith et al., 2019). 2) The preponderance of robust vector population like *An. arabiensis* with pre-existing behavioural plasticity could attenuate the impact of these interventions in Africa (Durnez and Coosemans, 2013, Perugini et al., 2020). Hence, continuous monitoring of vector species composition and behaviours is important for better understanding and control of residual malaria transmission.

This study documented increased proportion of *An. arabiensis* in the highlands of western Kenya compared to the findings of earlier studies conducted in the area before ITNs were used in large scale. Fifteen years ago, *An. gambiae* was the only member species of *An. gambiae s.l.* in the highlands of western Kenya (Shililu et al., 1998, Minakawa et al., 2002, Githeko et al., 2006, Ndenga et al., 2006). In this study, *An. arabiensis* accounted for over 13% of the *An. gambiae s.l* in the same area. In the lowland site (Ahero) of western Kenya, over 98% of *An. gambiae s.l.* populations were *An. arabiensis* (Chapter 3 and 4). In southwestern Ethiopia, *An. arabiensis* was the only member species of *An. gambiae s.l.* (Chapter 5 and 6). This species exhibited exophogic behaviour with a tendency to bite in the early evening, and zoophagic behaviour with preference to feed on cattle in both western Kenya and southwestern Ethiopia. Such proportional increment in *An. arabiensis* population coupled with its exophagic and zoophagic behaviours could undermine the efficacy of LLINs as the current vector control interventions do not target zoophilic vector species which avoids the lethal effect of insecticide treated surfaces (Okumu et al., 2013a), and hence residual malaria transmission may continue to occur despite the scale-up of the current vector control interventions.

In this study, *Anopheles gambiae* showed endophagic behaviour in the highlands of western Kenya, which is in agreement with the findings of earlier studies conducted in the same area (Githeko et al., 1996, Bayoh et al., 2014). However, it has showed a preference to feed both on humans and bovine, unusual behaviour for this species compared to the findings of earlier studies conducted in western Kenya before ITNs were used in large scale (Githeko et al., 1994b, Shililu et al., 1998, Mwangangi et al., 2003). Two decades ago, the HBI of *An. gambiae* population from western Kenya was greater than 96% while its BBI was less than 5% (Githeko et al., 1994b, Shililu et al., 1998), an indication that this species was strictly anthropophagic. Compared to the earlier reports, the HBI of indoor resting *An. gambiae* population was dropped by 20% in this study while its BBI was increased by a similar proportion. This suggests an increasing tendency of *An. gambiae* to feed on cattle following the increased ITN coverage in the western Kenyan highlands. Such dual host preference of *An. gambiae* could be a challenge to malaria control efforts in the country, as this vector species readily feed on unprotected humans to maintain intense malaria transmission, but can also feed on bovine to perpetuate its existence when humans are not accessible.

In this study, the secondary vectors *An. phariensis* and *An. coustani* exhibited exophagic and zoophagic behaviours. *Anopheles pharoensis* implicated in outdoor malaria transmission in Bulbul. Other studies have also documented an increasing role of *An. pharoensis* in malaria transmission in Ethiopia (Abraham et al., 2017, Kibret et al., 2014). Furthermore, *An. coustani* was found to be positive for *Plasmodum* CSP in both western Kenya and southwestern Ethiopia (Chapter 3 and 6). Several studies have demonstrated the susceptibility of *An. coustani* to *Plasmodium* infection (Mwangangi et al., 2013b, Antonio-Nkondjio et al., 2006, Nepomichene et al., 2015, Degefa et al., 2015). In Madagascar, *An. coustani* was confirmed to play a significant role in malaria transmission (Nepomichene et al., 2015, Goupeyou-Youmsi et al., 2020), indicating that this species could also play a role in outdoor malaria transmission in Africa.

The findings of this study suggest that additional control interventions that can target local vector behaviours are required to control residual malaria transmission and ultimately achieve elimination. The zoophagic behaviour of *An. arabiensis* and other secondary vectors such as *An. pharoensis* can be considered as an opportunity to introduce supplementary vector control intervention based on zooprophylaxis to divert host-seeking mosquitoes from humans (Habtewold et al., 2001, Iwashita et al., 2014). Zooprophylaxis has been shown to reduce malaria transmission by pulling mosquitoes toward dead-end hosts and also reduce vector density when cattle are treated with insecticides (Bulterys et al., 2009, Chaccour et al., 2018). Exophagic vectors can be targeted by introducing other control measures such as ATSB that can lure and kill outdoor host-seeking mosquito vectors (Fiorenzano et al., 2017, Tenywa et al., 2017). Microbial larvicides such as *Bti* and *Bs* (Walker and Lynch, 2007, Derua et al., 2019), and other LSM strategies can also be considered as supplementary interventions to target immature stages of mosquito vectors (WHO, 2013b, Fillinger and Lindsay, 2011).

7.1.2. Human behaviour and residual malaria transmission

Quantifying the magnitude of human exposure to infectious mosquito bites which occurs indoors and outdoors is another crucial parameter to evaluate the likely success of the existing malaria vector control interventions (Edwards et al., 2019, Finda et al., 2019, Monroe et al., 2019a). This study revealed that over 94% of the study participants from southwestern Ethiopia reported to have stayed outdoors or shifted between outdoors and indoors until 8:00 pm. The maximum possible personal protection that could be provided by LLINs against *An. arabiensis* bites was

only 56%. This implies that with only the current indoor-based vector intervention (LLINs), malaria elimination may not be achieved since the remaining exposure to *An. arabiensis* bites could still occur outdoors and/or indoors before people retire to bed.

7.1.3. Efficacy of the new vector surveillance tools

Quantifying the magnitude of outdoor malaria transmission have been difficult in Africa due to lack of well standardized, viable and safe tools for surveillance of outdoor resting and host-seeking malaria vectors (Service, 1977, WHO, 2013c). To address this gap, we developed and evaluated three exposure free tools: a sticky pot for outdoor resting malaria vector surveillance, and HBLT and HDNT for outdoor host-seeking malaria vector surveillance.

The sticky pot is a sticky variant of clay pots which have been used previously to collect outdoor resting *Anopheles* mosquitoes (Odiere et al., 2007). In a sticky pot, the internal surface of the clay pot was covered with waterproof black papers coated with Tangle-Trap sticky substance. The addition of this sticky substance allows for mosquitoes that rest within the pot to be continually trapped for surveillance, rather than only observing the fraction of mosquitoes that happen to be resting at the time of collection in a standard clay pot. In this study, the stick pot caught significantly higher number of *An. arabiensis* compared to clay pot, indicating that covering the internal surface of the pots with sticky paper has increased their trapping efciency. Moreover, the sticky pots have correlated with pit shelters with regard to the relative abandance and blood meal indices of anopheline species. However, the mean density of anophelines per trap was signifcantly lower in the stick pots compared to pit shelters. For instance, a pit shelter caught on average four times as many *An. arabiensis* as a sticky pot. This suggests the need to deploy the sticky pots in batches i.e. four sticky pots per compound to replace a pit shelter for routine surveillance of outdoor resting malaria vectors. Sticky pots can be made using locally available clay pots, so they are low cost.

The HBLT consists of a CDC light trap baited with human-odour pumped from an ordinary sleeping room. The HDNT is a variant of the previously designed double net trap (Tangena et al., 2015), with an integrated CDC light trap. Mosquitoes attracted to the human-bait are collected by setting a CDC light trap between the two nets. These two trapping methods used human odour as an attractant, but they are exposure-free tools since the lured mosquitoes are captured by the

CDC light trap rather than by the person acting as a bait unlike the HLC (WHO, 2013c) and the previous designs of bednet traps (Tangena et al., 2015, Gao et al., 2018). In this study, the HBLT captured two times as many malaria vectors as the regular CDC light trap in both western Kenya and southwestern Ethiopia. The HDNT caught 6 times as many malaria vectors as the CDC light trap. This implies that both the HBLT and HDNT had better efficiency compared to the ordinary CDC light trap. The HDNT yielded a similar vector density as the gold standard HLC with positive correlation between the two traps, suggesting that the HDNT could be a better alternative to HLC for routine surveillance of outdoor host-seeking malaria vectors.

7.1.4. Strengths and limitations of the study

One of the strengths of this study is that it was conducted in three different ecoepidemiological sites in East Africa: lowlands of western Kenya with altitude of about 1162 m asl, highlands of western Kenya with altitude ranging from 1430-1580 m asl, and highlands of southwestern Ethiopia with altitude of over 1,700 m asl. This abetted to understand the variations in vector species composition, abundance, diversity, behaviour and malaria transmission intensity among the different ecoepidiomological settings, and between countries. Similarly, the new vector surveillance tools were evaluated in two different countries; hence their applicability can be broadly generalized for use across East Africa and other African countries with similar eco-epidemiological settings. Moreover, both vector and human behaviour data were included in the calculation of human biting rates and EIRs in Ethiopia (Chapter 6) to better understand where and when human exposure to mosquito vector bites and residual malaria transmission occur.

On the other hand, this study had also some limitations. The HLC, the gold standard method for estimating HBR, was not conducted in western Kenya due to logistic issue. Hence, HBR and EIR calculations for western Kenya were made based on CDC light trap by using a conversion factor between the HLC versus CDC light trap. The sporozoite infection rates reported in this study were based on ELISA and the positive specimens were not confimed by PCR. Although ELISA have been commonly used for detection of *Plasmodium* CSP in mosquitoes (Beier et al., 1987, Wirtz et al., 1987), it may overestimate the sporozoite rate by detecting sporozoites in mosquito haemolymph in addition to the salivary gland (Marie et al., 2013, Hillyer et al., 2007).

7.2. General Conclusions

- *Anopheles gambiae s.l.* was the predominant species in western Kenya followed by *An. funestus* group. *Anopheles gambiae s.l.* composed of 98.9% *An. arabiensis* and 1.1% *An. gambiae* in Ahero, while 87% *An. gambiae* and 13% *An. arabiensis* constituted *An. gambiae s.l.* in Iguhu. *Anopheles funestus* group consisted of 98.1% *An. funestues s.s.* and 1.9% *An. leesoni* in the area.
- The proportion of *An. arabiensis* has increased (to over 13%) in the western Kenya highlands compared to previous reports (< 1%) documented before the scaleup of vector control interventions, while the proportion of *An. gambiae* has declined proportinately
- *Anopheles gambiae*, which was historically known by its strict anthropophagy in western Kenya, has showed an increased tendency to feed on cattle. *Anopheles arabiensis* was highly zoophagic while *An. funestus* showed anthropophaic behaviour in this study
- The majority of malaria transmission by *An. gambiae* and *An. funestus* occurred indoors in western Kenya, while *An. arabiensis* contributed almost equally to both outdoor and indoor transmission.
- The new sticky pot captured significantly higher number of malaria vectors compared to clay pot. Moreover, the sticky pots have showed correlations with pit shelters in terms the relative abundance and host blood indices of malaria vectors, suggesting that the sticky pot could be a useful and complementary tool for outdoor resting malaria vector surveillance, in settings where using pit shelters is not feasible and less productive.
- Both HBLT and HDNT caught significantly higher density of malaria vectors than the conventional CDC light traps. The HDNT yielded a similar vector density as HLC with a strong positive correlation, suggesting that it could be an alternative tool to HLC for routine surveillance of outdoor host-seeking malaria vectors.
- *Anopheles pharoensis* was a predominat species in Bulbul, southwestern Ethiopia followed by *An. arabiensis.* Both *An. arabiensis* and *An. pharoensis* showed exophagic and zoophagic behaviour with a tendency to bite in the early evening
- Human exposure to *An. arabiensis* bites occurred mostly indoors for unprotected individuals (LLIN non-users). About 56% of human exposure to *An. arabiensis* bites

occurred indoors at times when using LLINs is feasible, indicating that the maximum possible personal protection that could be provided by LLIN was only 56%.

- For LLIN users, the majority (75%) of the residual human exposure to *An. arabiensis* bites occurred outdoors.
- Human exposure to *An. pharoensis* bites occurred mainly outdoors for both LLIN users and non-users
- *Anopheles arabiensis* contributed to both indoor and outdoor malaria transmission while *An. pharoensis* contributed exclusively to outdoor transmission

7.3. General Recommendations

For programmatic operation

- The behaviour of local malaria vectors should be monitored regularly in order to plan and implement interventions that can target the behaviour of the local vectors
- The sticky pots could be used as alternative tool for outdoor resting malaria vector surveillance in settings where using pet shelters is not feasilble
- The HDNT could be used as an alternative to HLC for routine surveillance of outdoor host-seeking malaria vector surveillance

For Policy

- Additional control tools targeting early evening and outdoor biting malaria vectors should planned and implemented to complement the current control interventions to control residual transmission and ultimately achieve malaria elimination
- Both human and local malaria vector behaviour should be considered to maximaxize the impact of current vector control measures and to plan supplementary interventions

For research

- Further studies are required to comprehend the role of secondary and suspected vectors in malaria transmission
- Further research is needed to explore an easier means of dispensing human odour for setting the HBLT

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APPENDICES

Appendix 1. CV of the PhD Candidate

Personal Information

- *Name*: Teshome Degefa Demie
- *Sex*: Male
- *Date of Birth*: September 22, 1989
- *Blace of Birth*: Halila, Arsi, Ethiopia
- *Nationality*: Ethiopian
- *Languages*: English, Afan Oromo and Amharic

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Educational Backround

- 2015-Present: PhD student in Tropical and Infectious Diseases, Jimma University, Ethiopia in collaboration with University of California and Kenya Medical Research Institute (KEMRI)
- Sept. 2011-July 2014: MSc in Medical Parasitology, Jimma University, Ethiopia
- Sept. 2006-July 2009: BSc in Medical Laboratory Technology, University of Gondar, Ethiopia
- Sept. 2004-Jun. 2006: Preparatory School, Didea Seconday and Preparatory School, Robe District, Arsi, Oromia, Ethiopia
- Sept. 2002-Jun. 2004: High School, Sude Senior Secondary School, Sude District, Arsi, Oromia, Ethiopia
- Sept. 1995-Jun. 2002: Attended primary School at Halila Primary School, Halila, Sude District, Arsi, Oromia, Ethiopia

Employer

- September 2009 to Present: Jimma University, Ethiopia

Academic Rank

- December 2020-Present: Assistant Professor of Medical Parasitology at School of Medical Laboratory Sciences, Faculty of Health Sciences, Jimma University, Ethiopia
- 2014-2020: Lecturer of Medical Parasitology at School of Medical Laboratory Sciences, Jimma University, Ethiopia
- 2011-2013: Assistant Lecturer at School of Medical Laboratory Sciences, Jimma University , Ethiopua
- 2010-2011: Graduate Assistant II at School of Medical Laboratory Sciences, Jimma University, Ethiopia
- 2009-2010: Graduate Assistant I at School of Medical Laboratory Sciences, Jimma University, Ethiopia

Work Experience

- *Teaching (Sept. 2009–Present):* Have tought undergraduate Medical Laboratory and Nursing students, and Postgraduate Medical Parasitology students. I delivered different courses including Medical Parasitology, Techniques in Diagnostic and Experimental Parasitology, Introduction to Medical Laboratory Sciences, Professional Ethics, Professional Practice and Health Laboratory Management.
- *Advising Students on their Research Work:* Have been advising undergraduate Medical Laboratory and Postgraduate Medical Parasitology students on the their thesis
- *Research Project Coordination:* Worked as a coordinator for research projects entitled "*Molecular Epidemiology of Vivax Malaria in Ethiopia*" in 2014 and "*sub-Saharan International Center of Excellence for Malaria Research (ICEMR)*" from 2018 to 2021.
- *Research work:* Have been working research on malaria and vectors since 2014 in collaboration with researchers from different countries including Ethiopia, Kenya, Cameroon and USA. Some of the research areas covered so far include: Malaria vector behaviours and residual malaria transmission, Development and evaluation of vector surveillance and control tools, Asymptomatic malaria, Molecular epidemiology of malaria, Malaria parasite population genomics, insecticide resistance, impact of environmental modifications, population movement and urbanization on malaria epidemiology, and modeling.

- *Laboratory work experiences:* Have experiences on different molecular and immunological techiques including PCR for vector species identification, Kdr PCR for characterizing mechanism of insecticide resistance, sporozoite ELISA and blood meal ELISA.

Current responsibilities

- Assistant Professor of Medical Parasitology at School of Medical Laboratory Sciences, Faculty of Health Sciences, Institute of Health, Jimma University, Jimma, Ethiopia
- Head, School of Medical Laboratory Sciences, Faculty of Health Sciences, Institute of Health, Jimma University, Jimma, Ethiopia

Workshop & Training Certificates

- Participated on a workshop entitled "*Workshop to orient regional experts on vector surveillance in the context of epidemics preparedness and response*" at the Institute Pasteur in Dakar, Senegal, organized by World Health Organization (WHO) from October 22-November 02, 2018
- Training certificate on "*Protecting Human research Participants"* from the US National Institutes of Health (NIH) Office of Extramural Research in 2013, 2016 & 2020
- Training certificate on Training of Trainers (TOT) on "*Malaria Laboratory Dignosis and Quality Assurance*" organized by Ethiopian Public Health Institute (EPHI) in collaboration with ICAP Columbia University in Ethiopia and PMI/USAID Ethiopia, August 2019, Adama, Ethiopia.
- Training certificate on "*Electronic Library Resources training workshop for DAAD scholars Ethiopia*" by German Academic Exchange Service (DAAD), October 16-19, 2018, Addis Ababa, Ethiopia
- Training certificate on "*Heirarchical Linear and Non-Linear Modeling"* from Jimma University in collaboration with Ohio State University, 2013.
- Training certificate on "*Effective Teaching Skill Training*" Organized by Jhpiego Ethiopia in collaboration with Jimma University, Ministry of Health and Minstry of Education, January 18-22, 2010.
- Training certificate on "*Student Performance Assessment Triaining*" Organized by Jhpiego Ethiopia in collaboration with Jimma University, Ministry of Health and Minstry of Education, February 1-3, 2010.

Conference

- Presented my research findings on Scientific Symposium on World Malaria Day organized by Ministry of Health in collaboration with RollBack Malaria and CDC Ethiopia in Gambella, Ethiopia, April 25, 2017.

Publications

1. **Degefa T**, Githeko AK, Lee M-C, Yan G, Yewhalaw D. Patterns of human exposure to early evening and outdoor biting mosquitoes and residual malaria transmission in Ethiopia. *Acta Tropica*. 2021;216:105837. doi: 10.1016/j.actatropica.2021.105837
2. Abera D, Kibet CK, **Degefa T**, Amenga-Etego L, Bargul JL, Golassa L. Genomic analysis reveals independent evolution of *Plasmodium falciparum* populations in Ethiopia. *Malar J*. 2021;20:129. doi.org/10.1186/s12936-021-03660-y
3. Bamou R**,** Rono M, **Degefa T**, Midega J, Mbogo C, Ingosi P, et al. Entomological and Anthropological factors contributing to persistent malaria transmission in Kenya, Ethiopia and Cameroon. *J Infect Dis*. 2021;223(Supplement_2):S155-S70. doi: 10.1093/infdis/jiaa774
4. Subussa BW, Eshetu T, **Degefa T**, Ali MM. Asymptomatic *Plasmodium* infection and associated factors among pregnant women in the Merti district, Oromia, Ethiopia. PloS One. 2021;16(3):e0248074. doi.org/10.1371/journal.pone.0248074
5. Yimer BB, Otava M, **Degefa T**, Yewhalaw D, Shkedy Z. Bayesian Model Averaging in Longitudinal Studies using Bayesian Variable Selection Methods. *Communications in Statistics- simulation and Computation.* 2021. doi.org/10.1080/03610918.2021.1914088
6. Olkeba BK, Goethals PLM, Boets P, Duchateau L, **Degefa T**, Eba K, et al. Mesocosm experiments to quantify predation of mosquito larvae by aquatic predators to determine potential of ecological control of malaria vectors in Ethiopia. *Int J Environ Res Public Health.* 2021;18:6904. doi.org/10.3390/ijerph18136904
7. **Degefa T**, Yewhalaw D, Zhou G, Atieli H, Githeko AK, Yan G. Evaluation of human-baited double net trap and human-odour-baited CDC light trap for outdoor host-seeking malaria vector surveillance in Kenya and Ethiopia. *Malar J*. 2020;19:174. doi.org/10.1186/s12936-020-03244-2

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2. Professor Guiyun Yan, University of California Irvine, USA, Email: guiyuny@hs.uci.edu
3. Dr. Andrew K. Githeko, Kenya Medical Research Institute (KEMRI), Kisumu, Kenya, Email: githeko@yaoo.com

Appendix 2. Questionnaire

Appendix 2.1A. Questionnaire for household heads intended to assess factors related with vector behaviour, human behaviour and malaria transmission (English version)

Institution and country: _____

Project title: Profiling and Quantifying Residual Malaria Transmission in Kenya and Ethiopia

Date_____

Study site _____ Name of the interviewer _____

House number: _____ Individual ID No _____

Questions		**Answers**	**Remark**
01	Age	_____	
02	Gender	1. Male 2. Female	
03	Family size	_____	
04	Type of house	1. Mud plastered 3. Break (holed) walls 2. Stone walls 88. Others_____	
05	Is there any stagnant water around your dwelling?	1. Yes 2. No 99. Don't know	
06	If your answer is yes for Q05 how far from your house?	1. Less than 1km 2. Greater than 1km 99. Don't now	
07	Do you have Insecticide Treated Nets at your home?	1. Yes 2. No	
08	If yes how many ITNs do you have?	1. 01 2. 02 3. 03 4. >3	
09	If yes, what is the status of the ITN	1. New 2. Old	
10	If old is (are) there hole (s) on the net (s)	1. Yes 2. No	If yes mention number of holes per net
11	Who uses the ITNs?	1. Children only 2. Mother only 3. Father only 4. Father and mother only	

		5. The whole family 6. Children & mother only	
12	Have you sprayed chemicals to control mosquitoes?	1. Yes 2. No	
13	If yes how frequent?	1. Once in a year 2. Twice in a year 3. More than twice in a year 88. other	
14	Do you have domestic animal that lives in your house/compound?	1. Yes 2. No	
15	If you answer 'yes' to question number 14, Which of the domestic animals do you have?	1. Cattle 5. Donkey 2. Sheep 6. Dog 3. Goat 7. Chicken 4. Horse 88. Others	Please mention numbers for each
16	What time do you (your family members) usually go to indoor from outdoor in the evening?	_____	
17	What time do you (your family members) go to bed for sleeping?	_____	
18	What time do you usually leave your house in the morning?	_____	
19	What activities keep you outdoor every evening and morning?	1. _____ 2. _____ 3. _____	
20	Do you (any of of your family members) sleep outdoors at night?	1. Yes 2. No	If yes, Why?
21	What is the peak biting time of mosquitoes	1. Evening (time …….?) 2. Midnight 3. Early morning (time ……?)	

Appendix 2.1B. ቃለመጠይቅ (Amharic version)

የምርምሩ ርዕስ: Profiling and Quantifying Residual Malaria Transmission in Kenya and Ethiopia

መግቢያ

የዚህ ምርምር ዋና አላማ የወባ በሽታ እንዲከሰት የሚያደርጉ አጋላጭ ምክንያቶችን ለማጥናት ነዉ። እርስዎም ለዚህ ምርምር የተዘጋጀ ቃለመጠይቅ ላይ እንዲሳተፉ ተጋብዘዋል። በመጠይቁ ወቅት የሚሰጡት መልሶች እና አስተያየቶች በሙሉ በምስጥር የተጠበቁ ይሆናሉ። ስለዚህ የተባለዉን ግንዛቤ ዉስጥ አስገብተዉ መልካም ፍቃደኝነትዎን በመፈረም እንዲገልጹልኝ እጠይቅዎታለሁ።በዚህ ምርምር በመሳተፍዎ በጣም እናመሰግንዎታለን።

በዚህ ምርምር ለመሳተፍ ፍቃደኛ ነዎት? ***1.*** አዎን ***2.*** አይደለዉም

ቀን_____

የተጠያቂዉ ስም _____ የጠያቂዉ ስም _____

ቀበሌ: _____ የቤት ቁጥር _____

ተ.ቁ	**ጥያቄ**	**መልስ**	**አስተያየት**
01	ዕድሜ	**-----**	
02	ፆታ	***1.***ወንድ ***2.***ሴት	
03	የቤተሰብ ቁጥር	----------	
04	የቤት ዓይነት	***1.***የጭቃ ቤት ***3.***ግርግዳዉ የተሰነጣጠቀ (ክፍተት ያለዉ) ***2.***ቭይላ ቤት ***88.***ሌላ------	
05	የታቆረ ዉሃ በአካባቢዎ አለ?	***1.***አዎ ***2.***የለም ***99.*** አላዉቅም	
06	ለጥያቄ ቁጥር ***05*** ምላሽዎ አዎ ከሆነ ከቤተዎ ምን	***1.*** ከ***1*** ኪሜ በታች ***2.*** ከ***1*** ኪሜ በላይ ***99.*** አላዉቅም	

	ያህል ይርቃል?		
07	በፀረ ትንኝ መዲሃኒት የተነከረ የአልጋ አጎበር አለዎት?	**1.** አዎ **2.** የለም	
08	ለጥያቄ ቁጥር **07** ምላሽዎ አዎ ከሆነ ስንት አጎበር አለዎት?	**1. 1** **3. 3** **2. 2** **4. 4**	
09	ለጥያቄ ቁጥር **07** ምላሽዎ አዎ ከሆነ የአጎበሩ አይነት?	1. አዲስ 2. አሮጌ	
10	አሮጌ ከሆነ አጎበሩ ቀዳዳ አለዉ?	1. **1.**አዎ **2.** የለዉም	
11	በፀረ ትንኝ መዲሃኒት የተነከረ የአልጋ አጎበር የምጠቀመዉ ማነዉ?	**1.**ልጆች ብቻ **2.** እናት ብቻ **3.**አባት ብቻ **4.** እናትና አባት **5.**ሁሉም የቤተሰብ አባላት **6.**እናትና ልጆች ብቻ	
12	የወባ ትንኝን ለመቆጣጠር የፀረ ትንኝ ኬሚካል ተጠቅማችሁ ታዉቃላችሁ?	**1.**አዎ **2.** አይደለም	
13	ለጥያቄ ቁጥር **12** ምላሽዎ አዎ ከሆነ በየስንት ግዜዉ ይረጫሉ	**1.**በአመት አንዴ **2.**በአመት ሁለቴ **3.** በአመት ከሁለት ግዜ በላይ	
14	የቤት እንሰሳት አልዎት?	1. አዎ 2. የለንም	
15	ለጥያቄ ቁጥር **14** ምላሽዎ አዎ ከሆነ የትኞቹ?	1. ከብቶች **5.** አህያ 2. በጎች **6.** ዉሻ 3. ፍየሎች **7.** ዶሮ 4. ፈረስ **88.** ሌላ	ብዛታቸዉን ይጥቀሱ

16	ማታ ማታ በስንት ሰዓት ነዉ ወደ ቤት የሚትገቡት?	_____	
17	ማታ ማታ በስንት ሰዓት ነው የሚተኙት?	_____	
18	ጠዋት ጠዋት በስንት ሰዓት ነዉ ከቤት የሚትወጡት?	_____	
19	ጠወት እና መታ ከቤት ዉጪ ምን አይነት ስራ ነዉ የሚትሰሩት?	1. _____ 2. _____ 3. _____ 4. _____ 5. _____	
20	ከበተሰብዎ አባል ለሊት ከቤት ዉጪ የሚተኛ አለ?	1. ***1.***አዎ ***2.*** የለም	ካለ ለምን?
21	የወባ ትንኞች መቼ መቼ ነዉ በጣም የሚያስቸግሩት,	1. ማታ (ሰዓት _____?) ***2.*** ለሊት 3. ጠዋት (ሰዓት _____?)	

Appendix 2.1C. Gaaffilee abbootii warratiif dhiyaate (Afan Oromo version)

Mata duree Qorannichaa: Profiling and Quantifying Residual Malaria Transmission in Kenya and Ethiopia

Seensa

Kaayyoon qorannoo kanaa dhukkubni busaa akka daddarbuuf wantoota sababa ta'an qorachuufi dha. Isinis gaafii fi deebii qorannichaaf jecha dhiyaate kana irratti akka hirmaattan affeeramtanii jirtu. Yoo kan hirmaattan ta'e iccitiin deebii isin nuuf kennitannii kan eeggamu ta'a. Hirmaannaa keessaniif dursinee isin galatooffanna.

Qorannoo kanarratti hrmaachuuf fedhii qabduu? 1. Eeyyeni B. Lakki

Guyyaa_____

Maqaa gaafatamaa _____ Maqaa gaafataa: _____

Ganda _____ Lakk Manaa_____

Gaaffilee		**Deebiwwan**	**Yaada**
01	Umrii	_____	
02	Saala	1. Dhiira 2. Dubara	
03	Baay'ina maatii	_____	
04	Akaakuu manaa	1. Supheen kan marigame 3. Qaawwa qaba 2. Mana shaklaa 88. Kan biraa_____	
05	Naannoo mana jireenya keessanii bishaan ciisan jiraa?	1. Eeyyeni 2. Lakki 99. Hin beeku	
06	Jira yoo ta'e, hammam fagaata?	1. Km tokkoo gadi 2. Km tokkoo oli 99. Hin beeku	
07	Saaphana siree qabduu?	1. Eeyyeni 2. hin qabnu	
08	Gaaffi Lakk "07"f deebin keesssan "eeyyen" yoo ta'e, saaphana siree meeqa	1. 01 2. 02 3. 03 4. >3	

	qabdu?		
09	Gaaffi Lakk “07”f deebin keesssan “eeyyen” yoo ta’e, saaphana siree kan akkamiiti?	1. Haaraa 2. Moofaa	
10	Gaaffi Lakk “09”f deebin keesssan “moofaa” yoo ta’e, qaawwa qabaa?	1. Eeyyeni 2. Lakki	Baay’ina qaawwanii?
11	Saaphana siree eenyutu fayyasdama?	1. Ijoollee qofa 2. Haadha manaa qofa 3. Abbaa manaa qofa 4.Abbaa manaa fi haadha manaa qofa 5. Matii hunda 6.Ijoollee fi haadha manaa qofa	
12	Mana keessan keemikaala farra bookee busaa itti biiftanii?	1. Eeyyeni 2. Lakki	
13	Itti biifame yoo ta’e, yeroo hammam hammamiitin biifama?	1. Waggaatti al tokko 2. Waggaatti al lama 3. Waggaatti al lamaa ol 88. Kan biraa	
14	Beelladoota manaa qabduu?	1. Eeyyeni 2. Lakki	
15	Beelladoota manaa ni qabdu yoo ta’e, kam fa’i?	1. Loon 5. Harree 2. Hoolota 6. Saree 3. Re’oota 7. Lukkulee 4. Farda 88. Kan biraa	Baay’ina isaani?
16	Galgala galgala sa’aa meeqatti alaa olgaltu?	_____	
17	Galgala galgala sa’aatii meeqatti raftu?	_____	
18	Ganama ganama sa’aa	_____	

	meeqatti manaa baatu?		
19	Galgalaa fi ganama hojiiwwan akkamiitiif ala turtu?	1. _____ 2. _____ 3. _____ 4. _____ 5. _____	
19	Maatii keessan keessa halkan namni manaan ala rafu jiraa?	1. Eeyyeni 2. Lakki	Yoo jiraate, maaliif?
20	Bookeen busaa baa'inaan yeroo akkamii nama hidditi?	1. Galgala (sa'aa …….?) 2. Halkan 3. Gara barii (sa'a ……?)	

Appendix 3. Consent forms

3.1. Informed consent form for household heads

Appendix 3.1A. Informed consent form for household heads (English version)

Name of the principal investigator: **Teshome Degefa**

Name of the organization: _____

Introduction: This information sheet is prepared by group of researchers whose aim was to conduct research entitled **"Profiling and Quantifying Residual Malaria Transmission in Kenya and Ethiopia."** The aim of the study is to develop and evaluate new methods for surveillance of malaria vectors. The information obtained from this study will be useful in recommending the use of appropriate malaria vector surveillance and control tools. The investigators include a PhD student from Jimma University and academic supervisors from Jimma University, Kenya Medical Research Institute and University of California, USA. We would like to request you to allow us to use your house for mosquito collection. If you agree to help in the study, your help will be needed for up to 48 nights. We will test different mosquito collection methods around your house using one method each night. The methods will be used to collect mosquitoes as follows:

1. **Human-odour-baited CDC light trap (HBLT):** A pipe, with fan, will be connected from your sleeping room to outdoor mosquito catching station through small hole of approximately two inch. We may make the hole on your house-wall or may use window as appropriate. The pipe will pump human-odour from the room (from the sleeper) to the outdoor station. The odour attracts mosquitoes to the outdoor station. We will set CDC light trap in the evening at the outer end of the pipe to collect attracted mosquitoes. The trap will be removed in the morning.

2. **Human-baited double net trap (HDNT):** We will construct a small shed outdoor beside your house. We will place a bed in the shed. Volunteer individual will rest/sleep on the bed at night and fully protected by a small untreated bed net which will be hung over the bed to the ground. A larger untreated bed net will be hung over the smaller net and raised 30 cm above the ground to allow mosquito entrance. CDC light trap will be set between the two nets to collect mosquitoes attracted to the sleeper.

3. **Human Landing catch (HLC):** Volunteer data collectors will collect mosquitoes from inside your house (sitting in your salon) and outdoor in your compound at night.

Risks: We may make small hole on your house-wall on the side of your sleeping room, but we will repair it to its normal status after we finish our experiment. Volunteers will spend nights in your compound during our experiment. All volunteers (workers) will be selected from your community. If you do not feel comfortable with them at any time of the study, we will replace them immediately.

Benefits: There are no direct benefits to participating in this study. Information obtained from the experiment will assist the government in the implementation of appropriate malaria surveillance and control strategy.

Confidentiality: We will protect your privacy and confidentiality. All data obtained from your house/your compound will be kept strictly confidential and will not be disclosed to anyone other than the principal investigator. Your name will not be in any reports or journals.

Compensation: There are no costs to be in this activity and therefore, you will not be offered payment for being in this study

Right to refuse or withdraw: Participation is voluntary, which means you are free to take part or not to take part. Also, you are free to remove your house from the study and if so, we shall stop immediately and remove all our materials from your compound.

Whom to contact: If you have any questions, you may ask the principal investigator, Teshome Degefa (Jimma University, email: teshedege@gmail.com, Tel: +251910891214) at any time, even after the study has started. Further information can also be obtained from Professor Delenasaw Yewhalaw (Jimma University, email: delenasawye@yahoo.com, Tel: +251917804352), Dr. Andrew Githeko (Kenya Medical Research Institute, email: githeko@yahoo.com, Tel: +254722849382) and Prof Guiyun Yan (University of California, guiyuny@uci.edu). If you wish to ask questions later, you may contact any of the following:

This proposal was reviewed by Institutional Review Board (IRB) of the Health Institute of Jimma University, Ethiopia and Kenya Medical Research Institute (KEMRI). These are committees that make sure that research participants are protected from harm. If you wish to find

out more about the ethical review board, contact Professor Zeleke Mekonnen, Director, Research and Post Graduate Office, Institute of Health, Jimma University, Jimma, Ethiopia. P.O.Box 378, Tel: 0917765427. Secretary, KEMRI Ethics Review Committee, P. O. Box 54840-00200, Nairobi; Telephone numbers: 020-2722541, 0722205901, 0733400003; Email address: erc-secretariat@kemri.org.

Consent

Name of the household head: ---------- Age---------- Sex----------

Country: ---------- Village: ---------- House no: ---------

I have been informed about a study entitled **"Profiling and Quantifying Residual Malaria Transmission in Kenya and Ethiopia"** which aimed at developing and evaluating new tools for surveillance of outdoor malaria vectors. The study contributes by recommending appropriate tools for surveillance and control of malaria vectors. I was requested to allow the investigator to use my house and compound for mosquito collection. I understand that I am free to choose to be in this study and that saying "NO" will have no effects for me or my household. It is therefore, with full understanding of the situation that I gave my informed consent for my house/compound to be used for mosquito collection.

Name (household head) ----------Signature ----------Date ---------

Name (investigator) ----------Signature ----------Date ----------

Name (Witness) ----------Signature ----------Date ----------

Thank you for your participation!

Appendix 3.1B. Fomu ya maelezo ya makubaliano kwa wenye nyumba kushiriki katika utafiti (Kiswahili version)

Kielezo cha utafiti

Kutengeza na kutathmini aina ya mtego wa taa unaotumia harufu ya binadamu kama kivutio na wenye uwezo wa kuwashika mbu wanaonyonya damu ya watu wakiwa nje ya nyumba katika magharibi mwa Kenya.

Orodha ya watafiti

Teshome Degefa (Msc)[1,2], Delenasaw Yewhalaw (PhD)[2], Harrysone Atieli (PhD)[3], Andrew Githeko (PhD)[1], Guiyun Yan (PhD)[4].

Wanakofanyia kazi watafiti

[1]Centre for Global Health Research, Kenya Medical Research Institute (KEMRI), PO Box 1578, Kisumu, Kenya

[2]Department of Medical Laboratory Sciences and Pathology, College of Health Sciences, Jimma University, Ethiopia

[3]School of Public Health, Maseno University

[4]Program in Public Health, College of Health Sciences, University of California at Irvine, Irvine, CA 92697, USA

Maeneo yatakayofanyiwa utafiti: Ahero katika kauntu ya Kisumu na Iguhu katika kauntu ya Kakamega.

Malengo ya utafiti: Lengo kuu la utafiti huu ni kuweza Kutengeza na kutakmini aina ya mtego wa taa unaotumia harufu ya binadamu kama kivutio na wenye uwezo wa kuwashika mbu wanaonyonya damu ya watu wakiwa nje ya nyumba.

Maelezo ya utafiti: Ukikubali kupeana usaidizi katika huu utafiti, usaidizi wako utahitajika kwa siku 20. Tutajaribu njia aina mbili za kushika mbu kila usiku kama ifuatavyo:

1. **Mtego wa taa unaotumia harufu ya binadamu kama kivutio:** Kipande cha bomba chenye banka kitaunganishwa kwa kutoka chumba cha malazi mpaka kituo cha kushika mbu nje kupitia kitundu kidogo chenye takriban upana wa inchi mbili. Tutaweza tengeza kitundu hiki kwenye ukuta wa chumba cha malazi au kwenye dirisha. Hili bomba

litapuliza harufu ya binadamu aliyelala kutoka chumbani hadi kwenye kituo cha kushikia mbu nje. Hii harufu ya binadamu itawavutia mbu kwa hiki kituo cha kwashika mbu nje. Kuanzia jioni tutaweka mtego wa kutumia taa kwa upande wa bomba ulioko nje na mtego huu utaondolewa ifikapo asubuhi.

2. **Mtego wa neti unaotumia harufu ya binadamu kama kivutio:** Tutaweka kijukwaa kidogo nje ya nyumba yako na tuweke kitanda ndani. Mtu wa kujitolea atalala kwenye hiki kitanda usiki na atakingwa kutokana kuumwa na mbu kwa kulala ndani ya neti ndogo iiyokua na dawa. Baadae neti kubwa pia isiyokua na dawa itafungwa juu ya neti ndogo na na kuinuliwa kwa kimo cha 30 cm kutoka aridhini ili kuruhusu mbu kuingia. Mtego wa taa wa kushika mbu utawekwa katikati ya neti hizi mbili ili kushika mbu watakaovutiwa na mtu aliyelala ndani.

Tahadhari: Tutaweza kutoboa kitundu kidogo kwenye ukuta wa chumba cha malazi lakini tutarekebisha katika hali yake ya zamani tutakapomaliza utafiti. Watu watakaojitolea kufanya hii kazi watalala kwenye sehemu ya nje ya uwanja wako. Watu hawa wote watatoka kwenyejamii yenu. Tutakuonyesha vitambulisho vyao na wenye hutakua huru nao tutawabadilisha mara moja.

Mapato tarajiwa: Hakuna mapato ya moja kwa moja kwa kushiki katika huu utafiti. Habari zitakazo patikana kutoka kwa utafiti huu zitaisadia serikali katika utekelezaji wa mpango wa kitaifa wa kupambana na ugonjwa wa malaria.

Siri: Tutalinda hali yako ya siri na jina lako halitakua kwenye ripoti yeyote.

Malipo ya gharama: Hakuna gharama yeyote kwa kushiriki katika huu utafiti na kwa hivyo hakuna malipo yeyote ya gharama.

Kushiriki katika huu utafiti: Kushiriki katika huu utafiti ni kwa kujitolea kumaanisha uko huru kushiriki au la. Pia utakua huru kuondoa nyumba yako kwenye utafiti na endapo utafanya hivyo basi tutaacha kutumia nyumba yako mara moja na tutaondoa vyombo vyetu kutoka uwanja wako mara moja.

Habari ya unavyoweza kuwasiliana ukiwa na maswali na dukuduku : Ukiwa na maswali kuhusu huu utafiti ama kama utakua umedhurika kwa kushiriki katika huu utafiti basi uaweza

kuwasiliana na Dr. Andrew Githeko kutoka ofisi ya KEMRI, kijiji cha Kisian, kando ya barabara ya Kisumu-Bondo, Kisumu. Nambari yake ya simu ni 072-28-49382. Endapo utakuwa na maswali kuhusu kushiriki kwako katika huu utafiti na haki zako, wasiliana na mwandishi, kamitii ya KEMRI ya kuchunguza maadili ya utafiti, P.O.Box 54840-00200, Nairobi; Nambari za simu: 020-2722541, 0722205901, 0733400003; barua pepe: erc-secretariat@kemri.org

Makubaliano

Jina la kiongozi wa nyumba ---------- Umri---------- Jinsia----------

Kaunti----------Kijiji ----------Nambari ya nyumba ----------
Nimeelezwa kuhusu utafiti kwa jina “Kuzindua aina ya mtego wenye uwezo wa kuwashika mbu wanaonyonya damu ya watu wakiwa nje ya nyumba katika mangharibi mwa Kenya” .

Utafiti huu unachangia kwa kupendekeza njia mwafaka za kuwashikia mbu wakusambaza malaria wanaonyonya damu kwa watu walio nje ya nyumba. Nimeulizwa kuwaruhusu watafiti kutumia nyumba na uwanja wangu katika huu utafiti. Naelewa kuwa niko huru kuchagua kuhusika katika huu utafati nay a kwamba kusema “HAPANA” hakutakua na madhara yeyote kwangu au nyumba yangu. Na kwa ufahamu wa jambo hili nimepeana makubaliano ya kuhusika kwa nyumba/uwanja wangu kutumika kwa shuhuli ya kushika mbu.

Jina la kiongozi wa nyumba ----------Sahihi ----------Tarehe ----------
Jina la kiongozi wa mtafiti ----------Sahihi ----------Tarehe ----------
Jina la shahidi---- ----------Sahihi ----------Tarehe ----------
Asante sana kwa kushiriki!

Appendix 3.1C. Informed consent form for household heads participating in the study (Luo version)

Kothor mar projectni

Development and evaluation of a novel human-odour-baited CDC light trap for outdoor host-seeking malaria vector surveillance in western Kenya

Jononroni

Teshome Degefa (Msc)[1,2], Delenasaw Yewhalaw (PhD)[2], Harrysone Atieli (PhD)[1,3], Andrew Githeko (PhD)[1], Guiyun Yan (PhD)[4].

Investigators institutional affiliations

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[2]Department of Medical Laboratory Sciences and Pathology, College of Health Sciences, Jimma University, Ethiopia
[3]School of Public Health, Maseno University
[4]Program in Public Health, College of Health Sciences, University of California at Irvine, Irvine, CA 92697, USA

Kama itimoe nonroni: Ahero e Kisumu kaunti kod Iguhu e Kakamega kaunti.

Gima omiyi itimo nonroni: Gima duong ma omiyi itimo nonroni dongo kendo fwaro yo manyien Mar duoko chien suna malando tuo mar malaria.

Chenro mar timo nonroni: Ka iyie konyo e nonroni, konyni biro dwarore kuom ndalo manyalo piero ariyo. Wabiro timo nonro ariyo mag choko suna e aluora mar odi ka watiyo kod yore ariyo ma opogore e otieno ka otieno. Chenro ariyo mag choko suna gi ibiro tiyo godo e mako suna gi oko e aluora mar odi:

1. **Human-odour-baited CDC light trap (HBLT):** Paip kod rakwe yamo ibiro tudi ka owuok e I odi kor nindo ka dhi oko e kama olosi mar mako suna kokalo e otuchi matin madirom inji ariyo. Wanyalo loso otuchi matin e kor odi kata tiyo kod dirisani. Paip biro dhiro tik mar jachiwre (manindo) kowuok e I ot kadhi oko e kama olosi mar mako suna.

Tigni biro yuayo suna kakelo kar mako suna oko mar ot. Wabiro keto CDC light trap godhiambo e tung mar paip mabiro mako suna ma ochokore. Gima yuayo sunani ibiro gol gokinyi.

2. **Human-baited double net trap (HDNT):** Wabiro gero tipo matin e bath odi kendo wabiro keto otanda e tipono. Jachiwre biro nindo e otandano gotieno ka imiye arita motegno kod net matin ma ok othiedhi mar otanda ma ibiro liero e wi otanda nyaka piny e lowo. Net maduong mar otanda bende ma ok othiedhi ibiro lier e wi matin cha kendo ibiro ngawe gi malo fut manyalo romo sentimita piero adek ewi lowo mondo owe ne suna kar donjo. CDC light trap ibiro keti e dier nede ariyogi mondo ochok suna ma oywa kod janindo.

Hinyruok kod rach manyalo wuok e bedo achiel e nonroni: Wabiro loso otuchi matindo e kor odi e kor nindo Kata kamano wabiro duoke maber machal gi kaka ne wayude ka wasetieko nonroni. Jachiwre biro nindo e aluora mari gotieno ka chenroni dhi nyime. Jachiwre duto(jotich) mabiro nindo oko e aluora mari kata choko suna e aluora mari gin jogwengu. Wabiro timonu ngeruok mongith kodgi kendo ka chunyi odagi ngato e so asaya ka chenroni dhi nyime, wabiro wilogi mapiyo kaka nyalore.

Yuto: Onge yuto moro amora kuom chiwruok e nonroni. Duoko ma omakore kod nonroni ibiro tiyogo e konyo sirikal e chenro mar duoko piny, gengo kod thiedho tuo mar malaria. Pando wach ma e kindwa kodi: Wabiro rito wach man e kindwa kodi ma onge ngat mabiro winjo. Nyingi ok bi ti godo e ripode kata e oboke ma ibiro ndiki gi duoko ma owuok e nonroni.

Duoko : Onge chiwo kata yuto moro amora kuom chiwori e nonroni ema omiyo onge duoko moro amora ma ibiro duokni.

Bedo achiel mar jakanyo: Bedo achiel mar jakanyo en ratiro mari, tiende ni in thuolo bedo jakanyo kata tamori bedo jakanyo. Bende in thuolo golo odi e nonroni to ka iwuok e nonro wabiro chungo tich kendo golo gikwa mag tich duto mapiyo kaka nyalore e aluora mari.

Adres: Ka in kod penjo kuom chenroni losi kod Dr Andrew Githeko e KEMRI office, e gwenge mag Kisian, Kisumu-Bondo, Kisumu. Bende oyudore e nambani 072-28-49382. Ka in kod penjo ma omakore kod ratiro e nonroni tudri kod jagoro, KEMRI Ethics Review Committee, P. O. Box 54840-00200, Nairobi; Telephone numbers: 020-2722541, 0722205901, 0733400003; Email address: erc-secretariat@kemri.org.

Nyiso yieruok

Nying mar wuon ot ---------- Higa---------- Sex---------- kaunti ----------Gweng----------Namba ot----------

Olerna kuom nonro mar **"Development and evaluation of a novel human-odour-baited CDC light trap for outdoor host-seeking malaria vector surveillance in western Kenya"** ma jiwo dongruok kod fwaro yor mako suna ma lando tuo mar malaria. Nonroni changia migawo maduong mar duoko chien kecho oko mar suna ma lando malaria. Ne okwaya ayie jononro mondo oti kod oda kaachiel gi aluorana. Awinjo ni an thuolo bedo e nonro kata tamora bedo e nonro bi bedo gi chochruok kuoma kata jooda. Ema omiyo, kaluwore gi winjona achiwo oda/aluora mara mondo otigo e yor mako suna.

Nying(wuon ot) ----------Sei----------Tarik ---------
Nying (janonro) ----------Sei----------Tarik ----------
Nying (Janeno) ----------Sei----------Tarik----------
Wagoyoni erokamano kuom bedo jakanyo e nonroni

Appendix 3.1D. Bwibali khu vene Inzu vasanganga Muvuhenzi yuvu (Luhya Version)

Murwi kwi Lisomo Yili

Khutsililitsa nukhuhenzeshitsa mureko kwu vulavu ku tsisuna tsimenyanga ilwanyi mu livanda lya mumbo mwa Kenya tsileranga malaria.

Mira ga vahenzi

Teshome Degefa (Msc) [1,2], Delenasaw Yewhalaw (PhD) [2,] Harrysone Atieli (PhD) [1,3], Andrew Githeko (PhD) [1], Guiyun Yan (PhD) [4.]

Mikanda chya vahenzi

[1]Lisaka lyu khuhenza vulama mushivala; mukanda kwuvulamu mu Kenya Lisanduku lyi posta 1578 Kisumu

[2]Lisaka lyukhuhenza malwale nivipimu,lyuvulama; lisomero lya sayansi yuvulamu mulisomero lya kushii (Ethiopia)

[3]Lisomelo lyu bulamu bwa vandu Maseno

[4]Lisomelo lya Carlifornia mu bulamu vwa vandu ha Irvrine, Irvrine, CA 92697, USA

Masaka Kubuhenzi: Ahero Mulukongo kwa Kisumu Nende Iguhu-Lukongo lwi isheyu.

Shivune shu Buhenzi: Lichomo likali ni khukava injila indeyi yukhulondelela tsisuna tsileranga malelia khurula ilwanyi.

Liva lyi lisoma yili: Nikava waliyema khuhambana nakhutsi,walakhukhonya khu matukhu shilini. Khularumishila tsinjira tsiviri hanzu yoyo,injira shaindala khuvutukhu vulala. Tsinjira tsieneyitsi tsilarumikha khukumila tsisuna ilwanyi winzu ndinangwa:

1. **Mureko kwuvulabu numwayu kwumundu:**
   Khulahutula bwiina vutititi manya tsi inji tsivili mwitichi hashikoro khuremu luseshe nishihunzi khutukha ilwanyi hamureko kwitsisuna kali anoho mwitilisha. Umwika kwa oyo ukoni kwalaviriramu mpaka ilwanyi. Khuvalavikha mureko halukolova ilwanyi,makhwihuli mabwebwe.
2. **Mureko kwi tsineti Hali khaviri tsia vandu:**
   Kwalumbakha shikoro ilwanyi winzu yoyo. Makhuvishimu na vukono. Mundu wukhwirulitsa,alakonamu vutukhu nakhulavikhamu ineti yakhashilikhwa numusala

kwitsisuna. Khulavoha ineti ingali vuchila musala ikulu wi ineti indi khu 30cm khurula hasi khunjilitsa tsitsuna. Murego kwakhanga kulavikhwa hakari hatsyo khukumila tsisuna tsivira khununa masahi khu woyo ukonanga.

Vuchehelu: Khulayeva rwina rutititi ilwanyi hashikoro shosho, nikhali khwalilitsa kwakhamala vuhenzi vweru. Yavo virulitsi valahonga hango hoho vutukhu vwosi mulisoma yili. Vahinziri yava nivulusoma lweneyulu. Khulakumanyia vipande vyavo,niva shuvayanza tawe khwalatera vandi vwangu.

Vuleyi vwa vwangu: Shikuli nende buleyi vwa hanene vulunji tawe. Nikali marivuli kiliichomo, kalakhonya iserikali khurwekhitsa vulondeleli nu vushinji vwa mushyalo.

Isili yoyo: Khulalinda Isili yoyo, malaha koko shikalolekhakhutawe mumaripoti keru.

Mirungu: Burumishi vwitsisendi shivuliho tawe, kulwayako lupapulo shuluhandikwa tawe.

Buhambani: Khuhambana mulisoma yili nu bwirulitsi vwovwo. Ulinumunwa khukuva halala anoho wambakane,khulahaminya vindu vieru vwangu hango hoho niva ulakaya.

Bulondeleli: Niva ulinamarevo khulondekhana numuyumu yuku: yanza ukhuvili Daktari Andrew Githeko muofisi, lusoma lwa kisiani-muhanda kwa Bondo-ishisumu-khu inamba yiyi: 0722849382. Khandi nuva namarevo na kashiganga tsihaki tsyotsi khuvira muhandichi wishikato KEMRI Ethics Review Lisanduku lyi posta 54840-00200,Nairobi. Lung”unyo:020-2722541,0722205901,0733400003, Email erc-secretarial@kemri.org.

Buhuchilili:

Lilaha lya mwene Hango: …… Mihiga …….Mwikula/mukhali

Lukongo …… Lusoma……Inamba yinzu…….

Khulondekhana nu vwivali vuleyi khu muyinzi kwi mireko chu vulavu nende mwayu kwu mundu khukumira tsitsuna tsya mareria mu Lusaka lwa mumbo mwa Kenya. Lisomo yili limanya injira indahi imbakha yukhu henzeshiza nukuchimira tsisuna tsia malelia. Nasaywa khuhuchilila vahenzeshizi khurumishila inzu yanje na hango hanje,ninzu khurumikha khukumila tsisuna.

Lilaha (mwene hango)……. Isaini …… Mweli …….

Lilaha (mhenzeshitsi) …… Isaini …… Mweli …….

Lilaha (Mushahidi) ……. Isaini …… Mweli …….

Urio muno khukholera halala nakhutsi!

Appendix 3.1E. ጥናቱ ላይ ለሚሳተፉ አባወራዎች የተዘጋጀ የስምምነት ቅጽ (Amharic version)

የተመራማሪዉ ስም: **----------**

የተቋሙ ስም: **----------**

መግቢያ: ይህ የመረጃ ቅጽ ርዕሱ “Profiling and Quantifying Residual Malaria Transmission in Kenya and Ethiopia” ለተሰኘ ጥናት በተመራማሪዎች የተዘጋጀ ሲሆን፤ የጥናቱ ዋና ዓላማ አዲስ የዎባ ትንኞች ማጥመጃ (መሰብሰቢያ) መሳርያዎችን ለመስራት እና እነዚህ መሳርያዎች በትክክል መስራታቸዉን ለማረጋገጥ እንዲሁም የወባ ትንኞቹ ምን ያክል ዎባን እያስተላለፉ እንደሆነ ለማጥናት ነዉ። ከዚህ ጥናት የሚገኘዉ መረጃ ለወደፊት ትክክለኛ የዎባ ትንኞች ማጥመጃና መቆጣጠርያ መሳርያዎችን ለማመላከት ይጠቅማል። ምርምሩ በጅማ ዩኒቨርሲቲ የPhD ተማሪ እንዲሁም ከጅማ ዩኒቨርሲቲ፣ ከኬኒያ ሜዲካል ሪሰርች እንስትትዩትና ከካሊፎርንያ ዩኒቨርሲቲ ከተወጣጡ ከፍተኛ ተመራማሪዎች የሚሰራ ይሆናል። አርስዎም በዚህ ምርምር እንዲሳተፉ የተጋበዙ ስሆን፤ ፍቃደኛ ከሆኑ የዎበ ትንኞችን ለመሰብሰብ የርስዎን ቤትና ግቢ እንድንጠቀም እንድፈቅዱልን በትህትና እንጠይቅዎታለን። ፍቃደኛ ከሆኑ ይህ ስራ ለ***48*** ለሊት የሚሰራ ይሆናል። በስራዉ ግዜ የተለያዩ የዎባ ትንኞች ማጥመጃ ዘዴዎች የሚንጠቀም ሲሆን በአንድ ለሊት በእርስዎ ግቢ/ቤት እንዱን የማጥመጃ ዘዴ ብቻ የሚንጠቀም ይሆናል። የማጥመጃ ዘዴዎቹ የሚከተሉት ናቸዉ።

1. **Human-odour-baited CDC light trap (HBLT):** የአየር ቱቦ ከርስዎ የመኝታ ክፍል ወደ ዉጭ (በረንዳ) የሚንዘረጋ ይሆናል። ቱቦዉን በቤትዎ ገርግዳ ላይ ትንሽ ቀዳዳ (***2*** ኢንች) በመፍጠር ወይም የቤትዎ መኝታ ከፍል መስኮት ካለዉ መስኮቱን በመጠቀም የምንዘረጋ ይሆናል። ቱቦዉ በ***ፈን*** (Fan) አመከኝነት የሰዉን ተንፋሽ ከመኝታ ክፍሉ ወደ በረንዳ (ከቤት ዉጭ) ያወጠዋል። ወደ ዉጭ የሚዎጣዉ የሰዉ ትንፋሽ ትንኞችን ወደበረንዳዉ ይስባቸዋል። ትንኞቹ በረንዳዉ ላይ (የቱቡ መጨረሻ ላይ) CDC light trap የሚባል ወጥመድ ከመሬት ***1.5*** ሜትር ከፍ አርገን በመስቀል የሚሰበሰቡ ይሆናል። ይህ የማጥመጃ ዘዴ ማታ ተሰቅሎ ጠዋት የሚነሳ ይሆናል።

2. **Human-baited double net trap (HDNT):** የቤትዎ ግቢ ዉስጥ ትንሽ ድንኳን በመስራት በዉስጡ ትንሽ አልጋ ከነፍራሹ እናስቀምጣለን። ፍቃደኛ የሆነ አንድ የአከባቢ ሰዉ (ወንድ፣ እድሜዉ ከ*18* ዐመት በላይ፣ የእርስዎ የቤተሰብ አባል ወይም ጎሮቤት የሆነ ሰዉ) አልጋዉ ላይ ለሊት የሚተኛ ሲሆን ከትንኞች ለመከላከል እልጋዉ ኬሚካል ባልተነከረ ትንሽ አጎበር ሙሉ በሙሉ የሚሸፈን ይሆናል። ሌላ ትልቅ ኬሚካል ያልተነከረ አጎበር ከትንሹ አጎበር ከፍ ተደረጎ እና ከአጎበሩና መሬት መካከል የ*30*ሜንትሜትር ክፍተት እንዲኖር ተደርጎ ይሰቀላል። በተኛዉ ሰዉ ትንፋሽ ተስበዉ ወደ ትንሹ አጎበር የሚመጡ ትንኞች CDC light trap በሁለቱ አጎበሮች መካከል በመስቀል የሚሰበሰቡ የሆናል። ለደህንነት ድንኳኑ ዙሪያዉን የሚታጠር ይሆናል።

3. **Human Landing catch (HLC):** ፍቃደኛ የሆኑት መረጃ ሰብሳቢዎች ለሊት ለሊት (ከምሽት*12፡00-ጠዋት12፡00 ሰዓት*) የቤትዎ ሳሎን ዉስጥ እና ከቤትዎ ዉጭ (በረንዳ ላይ) በመቀመጥ የወባ ትንኞች እግራቸዉ ላይ ልክ እንዳረፉ ከመናደፋቸዉ በፊት ወድያዉኑ አስፓይሬተር (Aspirator) በሚባል መሳሪያ የሚሰበስቡ የሆናል።

ተጋላጭነት: በምርምሩ ወቅት በቤትዎ ግርግዳ ላይ ትንሽ ቀዳዳ ልንሰራ እንችላለን። ምርምሩ ካለቀ በኋላ ግን ወደነበረበት እንጠግነዋለን። የወባ ትንኞች ለመሰብሰብ ሲባል ፍቃደኛ የሆኑት መረጃ ሰብሳቢዎች በርስዎ ገቢ ወይም በቤትዎ ሳሎን ዉስጥ ለሊት ሊያሳልፉ ይችላሉ። መረጃ ሰብሳቢዎቹ ከርስዎ ቤተሰብ ዉስጥ ወይም ከጎሮቤት ሕብረተሰብ ዉስጥ የሚመረጡ ስሆን ከመረጃ ሰብሳቢዉች መካከል እርሰዎን ቅር የሚያሰኝ ከተገኘ ወዲያዉኑ በሌላ ሰዉ የሚተካ ይሆናል።

ጥቅማጥቅም: በዚህ ምረምር በመሳተፍዎ የሚያገኙ ቀጥታ ጥቅም አይኖረም። ነገር ግን ከምርምሩ የሚገኘዉ መረጃ ለወደፊት ዎባ በሽታን ለመከላከል ይጠቀማል።

ምስጥራዊነት: የርስዎ ሚስጥራዊነት የተጠበቀ ነዉ። ማንኛዉም ከርስዎ ቤት/ግቢ የሚገኘዉ መረጃ ሚስጥራዊነቱ የሚጠበቅ ስሆን መረጃዉ ከዋናዉ ተመራማሪ በስተቀር ለሌላ ሰዉ አይጋለጥም። ከርስዎ ቤት የሚሰበሰብ መረጃ ላይ የርስዎ/የበተሰብዎ ስም አይኖርም።

መካካሻ፡ በዚህ ምርምር ላይ በመሳተፍዎ ምንም አይነት ወጪ የማያስወጣዎት ሰለሆነ ለርስዎ የሚከፈል ክፍያ አይኖረም።

ያለመሳተፍ ወይም የማቋረጥ መብት

በዚህ ጥናት የመሳተፍ ወይም ያለመሳተፍ መብትዎ የተጠበቀ ነዉ። በዚህ ጥነት ላይ ባይሳተፉም ከዚህ በፊት ያገኙ የነበረ መንኛዉንም አገልግሎት ያገኛሉ። የርስዎ በዚህ ጥነት ላይ መሳተፍ ሙሉ በሙሉ በርስዎ ፍቃደኝነት ላይ ብቻ የተመሰረተ ነዉ። በጥናቱ ላይ መሳተፍ ከጀመሩ በኋላም ቢሆን ሀሳብዎን መቀየር ከፈለጉ በርስዎ ሀሳብ መሰረት ከቤትዎ የዎባ ትንኞች መሰብሰባችንን እናቋርጣለን።

ጥያቄ ከለዎት

በማንኛዉም ግዜ ጥናቱን በተመለከተ ጥያቄ ከለዎት የጅማ ዩኒቨርሲቲ መምህርና የPhD ተማሪ የሆኑት አቶ ተሾመ ደገፋን በ+251910891214 በመደወል ማናገር ይችላሉ። ተጨማሪ መረጃም ከፈለጉ ሌሎች የዚህ ምርምር አባል የሆኑትን ፕ/ር ደልነሳዉ የኋላዉ (ጅማ ዩኒቨርሲቲ፣ ስልክ፡+251917804352)፣ ዶ/ር አንድሪዉ ግቴኮ (Dr. Andrew Githeko, Kenya Medical Research Institute, email: githeko@yahoo.com,ስልክ ቁ፡ +254722849382) እና ፕ/ር ጉዩን ያን (Prof Guiyun Yan, University of California, eamail: guiyuny@uci.edu) ማናገር ይቻላል። ይህ ምርምር በጅማ ዩኒቨርሲቲ እና በኬንያ ሜድካል ሪሰርች እንስትትዩት Institutional Review Board (IRB) ተገምግሞ ፈቃድ ያገኘ ሲሆን ተጨማሪ ጥያቄ ከለዎት በጅማ ዩኒቨርሲቲ ጤና እንስትትዩት የድህረ ምረቃና ምርምር ዳይሬክተር የሆኑትን ፕ/ር ዘለቀ መኮንን በ+251***917765427*** በመደወል መጠየቅ ይችላሉ። አሁንም ቢሆን ጥየቄ ካለዎት እኔን መጠየቅ ይችላሉ። ጥያቄ አለዎት?

ስምምነት

የተሳታፊዉ (አባወራ) ስም፡ ---------- እድሜ--------- ጾታ---------

ሀገር፡ ---------- ቀበሌ፡ --------- የበት ቁጥር፡ -------

እኔ ---------- በአከባቢያችን ለመካሄድ ስለታቀደዉ ጥናት “Profiling and Quantifying Residual Malaria Transmission in Kenya and Ethiopia” በቂ መረጃ ተሰቶኛል። የጥናቱ ዋና አላማ የወባ ትንኞችን ለመሰብሰብ እና ለመቆጣጠር የሚያስችሉ መሳሪያዎች ለመስራት እና እነዚህ

መሳሪያዎች በትክክል መስራታቸዉን ለማጥናት መሆኑ ተነግሮኛል። ጥናቱ ለወደፊት የወባ ትንኞችን በደንብ ለመከላከልና ለመቆጣጠር እንደሚጠቅምም ተነግሮኛል። ለዚህም ጥናት ይረዳ ዘንድ የወባ ትንኞችን ከበቴና ከግቢዬ ዉስጥ ከማታ እስከ ጠዋት ለመሰብሰብ ፍቃደኝነቴን ጠይቀዉኛል።

በዚህ ጥናት ምንም አይነት የገንዘብ ጥቅም የማላገኝ መሆኔን እና ከመፈረሜ በፊት እንዳስብበት በቂ ግዜ ተሰጥቶኝ የተስማማዉ መሆኔን በፍረማዬ ለማረጋገጥ አወደለሁ።

የተሳታፊዉ ስም ---------- ፍርማ ---------- ቀን ----------

የአጥኚዉ ስም ---------- ፍርማ ---------- ቀን ----------

የምስክር ስም ---------- ፍርማ ---------- ቀን ----------

በጥነት እና ምርምሩ በመሳተፍዎ እናመሰግናለን።

Appendix 3.1F. Guca walii galtee abbootii warraatiif dhiyaate (Afan Oromo version)

Maqaa qorataa: **----------**

Dhaabbata (Yuunivarsiitii) ----------

Seensa: Ibsi waliigaltee kun qorannoo mata dureen isaa "Profiling and Quantifying Residual Malaria Transmission in Kenya and Ethiopia" jedhuuf qorattootaan kan dhiyaate yommuu ta'u, kaayyoon qorannichaas meeshalee bookee busaa ittiin funaanuf tajaajilu qalaquufi tajaajilummaa meshaalee kanaa qorachuu akkasumas haala tatamsa'ina dhukkuba busaa naannnoo keenyaa qorachuufi. Ragaan qorannoo kanarraa argamu gara fuula duraatti tatamsa'ina dhukkuba busaa ittisuuf ni tajaajila. Qorannoon kun barataa doktoreetii yuunivarsiitii Jimmaa fi hayyuulee qorannoo Yuunivarsiitii Jimmaa, Dhaabbata qorannoo fayyaa Keeniyaa fi Yuunivarsitii Kaalifoorniyaa irraa walitti babahaniin kan raaw'atamu ta'a. Isinis qorannoo kanarratti hirmaachuuf kan affeeramtan yoo ta'u, hirmaachuuf fedhii kan qabdan yoo ta'e, bookee busaa mana keessan keessaafi dallaa keessan keessaa akka funnaanuuf akka nuuf eeyyamtan kan isin gaafannu ta'a. Yoo kan nuuf eeyyamtan ta'e hojiin kun halkan 48tiif kan hojjetamu ta'a. Yeroo ibsame kana keessatti maloota bookee busaa ittiin funaanan garagaraa kan fayyadamnu yoo ta'u, halkan tokkotti mala tokko qofa fayyadamna. Malootni bookee busaa funaanuuf itti fayyadamnus kanneen armaan gadiiti:

1. **Human-odor-baited CDC light trap (HBLT):** Ujummoon qilleensaa daree hirriibaa keessanirraa gara alaatti (barandaatti) kan diriirfamu ta'a. Ujummoon kun girgiddaa kutaa hirribaa keessan irratti ulaa xinnoo (iinchii 2) uumudhaan kan diriirfamu ta'a. Ujummichi gargaarsa meeshaa fan jedhamuun hafuura namaa kutaa hirriibaa irraa gara alaatti (barandaatti) basuuf tajaajila. Afuurichimmoo bookee busaa gara barandaatti harkisa. Bookeewwan dhufan meeshaa CDC light trap jedhamu fuuldura ujummichaatti (gara alaatin) lafarraa meetira 1.5 olkaasuun fannisuudhaan kan funaanaman ta'a.

2. **Human-baited double net trap (HDNT):** Dallaa keessan keessatti dunkaana xiqqaa ijaruun dunkaanicha keessa siree firaasha waliin kan keenyu ta'a. Namni tokko fedhii isaatiin (dhiira, umriin isaa waggaa 18 olii fi miseensa maatii keessanii ykn hawaasa keessan keessaa kan filatamu) dunkaanicha keessa halkan kan rafu yommuu ta'u, bookeen busaa halkan akka isa hin hiddineef sireen inni irra rafu guutumaan guututti agoobara xiqqaa keemikaala hin cuubaneen kan haguugamu ta'a. Dabalataanis agoobara guddaa keemikaala

hin cuubamne agoobaricha isa xinnaadhaa olitti kan haguugamu yommuu ta'u, lafaafi agoobara isa guddich jidduu ulaan seentimaatira 30 kan jiraatu ta'a. Hafuuraa nama dunkaanicha keessa rafeen harkifamuun bookeewwan gara jidduu agabara isa guddichaa fi xinnicha gidduu seenan meeshaa CDC light trap jedhamu jedduu agoobara lamaanitti fannisuudhaan kan funaanaman ta'a. Nageenya nama dunkaanicha keessa rafuuf jecha naannawa dunkaanichaatti dallaan cimaan kan ijaaramu ta'a.

3. **Human Landing catch (HLC):** Namoonni fedhii isaanitiin bookee busaa funaanan halkan halkan (galgala sa'aatii 12:00 hanga ganama sa'aatii 12:00) mana keessan keessa (saaloonii) fi dallaa keessan keessa taa'uun bookeen busaa yommuu miila isaanirraa qubattu dafanii (osoo bookeen sun isaan hin hiddine) meeshaa aspirator jedhamuun kan funaanan ta'a.

Saaxilamummaa: Qorannichaaf jecha qaawwa xinnoo girgiddaa mana keessanii irratti kan uumnu ta'a. Akkuma qorannoon kuni xumurameen garuu akka duraan turetti kan isiniif suphamu ta'a. Bookee busaa funaanuuf jecha namoonni mana keessan keessa (saalonii keessa) fi dallawa keessan keessa kan taa'an ta'a. Haat'au malee namoonni kun akkuma fedhii keessaniitti miseensa maatii keessanii keessaa ykn hawaasa keessan keessaa kan filataman ta'a. Erga qorannoon jalqabamee boodas yoo akka tasaa komii namoota kanarraa qabaattan dafnee namoota birootiin kan bakka isinii buusnu ta'a.

Faayidaa: Qorannoo kanarratti hirmaachudhaan faayidaan kallattiidhaan isin argattan hin jiraatu. Haata'u malee, bu'aan qorannicha kanarraa aragmu gara fuulduraa tatamsa'ina dhukkuba busaa ittisuuf faay'idaa ni qabaata.

Iccitii: Iccitiin keessan kamiiyyuu sirrtti kan isiniif eegamu ta'a. Ragaan mana keessan fi dallaa keessan keessaa funaanamu kamiiyyuu iccitiidhaan kan olkaa'amu ta'a. Iccitii keessaniif jecha ragaan isin biraa argamu yommuu galmeeffaamu maqaa keessaniin osoo hin ta'in koodiidhaan kan galmeeffamu ta'a.

Beenyaa: Qorannoo kanarratti hirmaachuun baasii kan isin hin baasisne waan ta'eef kaffaltiin isiniif kaffalamu hin jiraatu

Mirga hirmaachuu dhiisuu ykn hirmaannaa addaan kutuu

Qorannoo kanarratti hirmaachuuf ykn hirmaachuu dhiisuuf mirga qabdan. Sababa qorannoo kanarratti hirmaachuu dhiisuu keessaniitiin tajaajilli kana dura argachaa turtan kamiiyyuu isinirraa kan hafu hin jiraatu. Hirmaannaan keessan fedhii keessaniin qofa waan ta'eef, hirmaachuuf erga murteeessitaniin boodas yoo ta'e yaada keessan jijjirruuf mirga guutuu qabdan.

Gaaffii yoo qabaattan: Gaaffii yoo qabaattan qorataa dursaa hojii kanaa kan ta'e obboo Tashoomaa Daggafaa (Yuunivarsiitii Jimmaa, Lakk bilbilaa: +251910891214) haasofsiisuu dandeessan. Hubannoo dabalataas yoo barbaaddan miseensota qorannoo kanaa kan ta'an Prof Dilnassaaw Yewhaalaaw (Jimma University, Tel: 0917804352), Dr. Andrew Githeko (Kenya Medical Research Institute, email: githeko@yahoo.com, Lakk bilbilaa: +2544722849382) fi Prof Guiyun Yan (University of California, guiyuny@uci.edu) gaafachuu dandeessu. Yoo barbaaddan ammas ykn boodas gaafii yoo qabaattan nagaafachuu dandeessu.

Piroopoozaalli kun hayyoota Yuunivarsiitii Jimmaa fi Dhaabbata qorannoo fayyaa Keeniyaatin kan gulaalamee fi eeyyame argatee dha. Gaafii waa'ee eeyyamaa fi kan biraas yoo qabaattan Pirof. Zallaqaa Makonnin, Daareektar qorannoo dhaabbata fayyaa Yuunivarsiitii Jimmaa, P.O.Box 378, Tel: 0917765427 bilbilaan gaagfachuu dandeessu.

Waliigaltee

Maqaa hirmaataa (Abbaa warraa) ---------- Umrii --------- Saala----------

Biyya: ---------- Ganda: ---------- Lakk manaa: ---------

Ani (maqaa) ---------- waa'ee qorannoo matadureen isaa **"Profiling and Quantifying Residual Malaria Transmission in Kenya and Ethiopia"** jedhu irratti hubannoon gahaan naaf kennamee jira. Kaayyoon qorannichaa meeshalee bookee busaa dubarsan ittiin sassaaban kalaquu fi tajaajilummaa isaanii madaaluu akka ta'e natti himamee jira. Qorannichi mala bookee busaa ittiin walitti qabanii akkasumas ittisan gumaachuurratti gahee olaanaa akka qabu natti himamee jira. Qorannon kanaaf jecha manaa fi qa'ee kiyya keessaa bookee busaa funaanuuf eyyama kan nagaafatan yoo ta'u, eeyyamuus eeyyamuu dhiisuufis mirga guutuu akkan qabu natti himamee jira. Kanafuu hubannoo armaan olii kanarratti hunda'uun waliigaltee eeyyamaa kennuu kiyyaa mallattoo kiyyaan akka armaan gadiitti agarsiisee jira.

Maqaa Abbaa warraa ----------Mallattoo ----------Guyyaa ---------

Maqaa qorataa ----------Mallattoo ----------Guyyaa ----------

Ragaa ----------Mallattoo ----------Guyyaa ----------

Hirmmaannaa Keessaniif galatoomaa!

3.2. Informed consent form for volunteer mosquito collectors

Annex 3.2A. Informed consent form for volunteer mosquito collectors (English version)

This informed consent form has two parts:

- Information sheet (to share information about the research with you)
- Certificate of consent (for signature if you agree to participate)

You will be a given a copy of the full informed consent form.

Part I: Information sheet

Introduction: My name is Teshome Degefa, PhD student from Jimma University, Ethiopia. I and my supervisors (researchers) from Jimma University, Kenya Medical Research Institute and University of California are planning to conduct a research entitled **"Profiling and Quantifying Residual Malaria Transmission in Kenya and Ethiopia."** We are going to invite you to participate as a volunteer to be involved in Anopheline mosquito collection in this study and we are going to give you information about the purpose and procedure of the study. You do not need to decide now whether you will participate in this research. Before you decide, you should understand and talk to anyone you like about the research.

Purpose of the study: The aim of the study is to develop and evaluate new methods for surveillance of malaria vectors (*Anopheles* mosquitoes) and to assess vector behaviour and malaria transmission in southwestern Ethiopia and western Kenya. The information obtained from this study will be useful in recommending the use of appropriate malaria vector surveillance and control tools.

Participant Selection: You are chosen to participate in this study as you are inhabitants of the study site and are eligible. We want to recruit 4-5 volunteers from the study site for the study. All males who are inhabitants of the study site, above the age of 18 years and who can give consent will be included in the study. However, individuals having sinus and epilepsy will be excluded from the study.

Description of the procedures and protocol

As a participant you will be asked to collect mosquitoes using either Human landing catch or Human-baited double net trap method.

Human landing catch (HLC): You will be asked to collect host-seeking mosquitoes both indoor and outdoor from desk to mid-night or from mid-night to dawn sitting inside or outside the selected houses. First, you will be trained on mosquito collection by aspiration. You will sit on stool having your aspirator and torch, and expose your lower legs and feet by rolling up your trousers. You will then catch mosquitoes before the mosquitoes land on your skin to avoid nuisance biting and subsequent infection. You will keep the mosquitoes you collect each hour in labelled paper cups.

Human-baited double net trap (HDNT): We will construct a small shed outdoor beside selected houses. We will place a bed with mattress in the shed. We will ask you to sleep on the bed at night (from 6:00pm to 6:00am). You will be fully protected by a small untreated bed net which will be hung over the bed to the ground. A larger untreated bed net will be hung over the smaller net and raised 30 cm above the ground to allow mosquito entrance. CDC light trap will be set between the two nets to collect mosquitoes attracted to your odor. You will be asked to do this for four nights each month for 12 months.

Participant protection against malaria or other vector-borne diseases

In the first place you will be provided an appropriate and effective chemoprophylaxis (mefloquine) to avoid risk of infection or avoid contracting malaria when you participate in mosquito collection by HLC and HDNT as per the national malaria treatment guidelines of Ethiopia. You will also be trained to collect mosquitoes using these methods.

For the purpose of the safety and privacy, as much as possible houses with 3 rooms (living room, preparation room and bed room) will be carefully selected for HLCs and you will sit quit in the living room with your aspirators for the incoming mosquitoes or host seeking mosquitoes. Mosquito collection by HLC and HDNT does not expose you to other vector-borne diseases like dengue which has never been reported from the selected study area.

Information on Chemoprophylaxis: If you wish to participate in this study especially in HLC and HDNT, you will be provided to take full dose of chemoprophylaxis (mefloquine) per week starting at two weeks before you engage in mosquito collection. This drug is known to protect you from contracting malaria. It is recommended by the World Health Organization, Ethiopian

Ministry of Health and Kenya Ministry of Health to be used as chemoprophylaxis. It has no major side-effects. However, this drug has side-effects in individuals with sinus and epilepsy.

Duration: This study will extend until December 2018 and if you agree to continue participating in this study you are allowed to stay with the research team for the study perod.

Risk and benefits: There will no risk of malaria infection as long as you take the recommended dose of the prophylaxis and with good drug adherence but in case you feel ill or febrile, you will be advised to visit nearby health facilities and will be treated for free of charge. The research team will follow up your health status. You will not get any other benefit from participating in the study. The findings of this study will be useful in recommending the use of appropriate malaria vector surveillance and control tools in your country.

Incentives: You will get a fair allowance on daily basis as a result of participation in the research.

Confidentiality: We will protect your privacy. Your name will not be used in any reports.

Right to refuse or withdraw**:** Your participation is voluntarily**.** You do not have to take part in this research if you do not wish to do so. You may also stop participating in the research at any time you choose. It is your choice and all of your rights still will be respected. There will not be any cohercive action which makes you to participate in the research.

Whom to contact: If you have any questions you may ask the principal investigator Teshome Degefa (Jimma University, Tel: +251910891214) at any time, even after the study has started. Further information can also be obtained from Prof. Delenasaw Yewhalaw (Jimma University, Tel: +251917804352), Dr. Andrew Githeko (Kenya Medical Research Institute, email: githeko@yahoo.com, Tel: +254722849382) and Prof Guiyun Yan (University of California, guiyuny@uci.edu).

This proposal was reviewed by Institutional Review Board (IRB) of Institute of Health of Jimma University, Ethiopia and Kenya Medical Research Institute (KEMRI). These are committees that make sure that research participants are protected from harm. If you wish to find out more about the ethical review board, contact Prof Zeleke Mekonnen, Director, Research and Post Graduate Office, Institute of Health, Jimma University, Jimma, Ethiopia. P.O.Box 378, Tel:

+251917765427. Secretary, KEMRI Ethics Review Committee, P. O. Box 54840-00200, Nairobi; Telephone numbers: 020-2722541, 0722205901, 0733400003; Email address: erc-secretariat@kemri.org.

Part II: Certificate of consent

I read the forgoing information, or it has been read to me. I have had the opportunity to ask about it, and any questions that I asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research and understand that I have the right to withdraw from the research at any time without in anyway affecting my medical care.

Participant Name _____ Signature_____ Date: _____

If Illitrate: I have witnessed the accurate reading of the information sheet to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print Name of witness:_____ Thump print of participant:_____

Witness Signature:_____ Date: _____

Statement by the researcher or other person taking consent:

I have accurately read out the information sheet to the potential participant and to the best of my ability made sure that the participant understands that he will participate in the research. I confirm that the participant was given an opportunity to ask questions about the study and all the questions asked by the participant were answered correctly to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Print name of researcher_____ Signature _____ Date_____

A copy of this informed consent form has been given to the participant.

Appendix 3.2B. Ushiriki kwa kusanya mbu. form ya Ridhaa (Kiswhali Version)

Jina la mradi wa utafiti: Kuendeleza na kutathmini aina ya mtego wa taa unaotumia harufu ya binadamu kama kivutio na wenye uwezo wa kuwashika mbu wanaonyonya damu ya watu wakiwa nje ya nyumba katika magharibi mwa Kenya.

Orodha ya wakaguzi

Teshome Degefa (Msc)[1,2], Delenasaw Yewhalaw (PhD)[2], Harrysone Atieli (PhD)[1,3], Andrew K Githeko (PhD)[1], Guiyun Yan (PhD)[4].

Taasisi za Wakaguzi

[1]Centre for Global Health Research, Kenya Medical Research Institute (KEMRI), PO Box 1578, Kisumu, Kenya

[2]Department of Medical Laboratory Sciences and Pathology, College of Health Sciences, Jimma University, Ethiopia

[3]School of Public Health, Maseno University

[4]Program in Public Health, College of Health Sciences, University of California at Irvine, Irvine, CA 92697, USA

Maeneo ya utafiti: Ahero in Kisumu county and Iguhu in Kakamega county.

Madhumuni ya Utafiti: Lengo kuu la utafiti huu ni kuendeleza na kutathmini mbinu mpya kwa ajili ya ufuatiliaji wa mbu wa malaria wanaouma ukiwa nje ya nyumba.

Nini tutafanya: Tutajenga kibanda ndogo nje kando ya nyumba zilizochaguliwa.Tutaweka kitanda kwa kibanda.Tutakuomba ulala kwenye kitanda wakati wa usiku kwa masaa 12. Utajikinga kwa chandarua ndogo ambayo haijatibiwa na iliyo ninginia juu ya kitanda adi sakafuni.Chandarua kubwa ambayo haijatibiwa itaninginizwa juu ya chandarua ndogo na kuinuliwa sentimita 30 kutoka sakafuni kwa ajili ya kuruhusu mbu kuingia. Mtego wa mwanga wa CDC utategwa kati ya chandarua mbili kukusanya mbu waliovutwa na harufu yako**.**Utaombwa ulale kwa kibanda kwa siku nne kila juma kwa majuma matano Tutajenga uzio kuzunguka kitanda kutumia waya wenye matundu kukulinda kutokana na wanyama pori. Ingawa majaribio haya hayaruhusu mbu kuuma,utapewa dawa ambayo inapunguza uwezekano wa kuambukizwa na vimelea vya malaria. Kabla ya utafiti kuanza Tutachukua sampuli za damu kwa mchomo wa kidole ili kuchunguza kama unao vimelea vya malaria. Utaombwa uje kwenye kituo

cha afya kila baada ya wiki 2 kuchunguzwa kama unao vimelea vya malaria kutoka mwanzo wa utafiti hadi wiki 4 baada ya mwisho wa utafiti. Utafiti utajumuisha watu 12 waliojitolea.

Faida: Utapata utambuzi wa malaria na matibabu bure wakati wa utafiti.

Hatari: Utaombwa ukae nje kwa kitanda wakati wa usiku na unaweza kujisikia na kuwa na wasiwasi kwa sababu ya hali ya baridi. utajikinga kwa chandarua ambayo haijatibiwa unapolala,lakini unaweza umwa na mbu. Utapewa dawa ili kupunguza hatari hii. Dawa utakaopewa iitwayo mefloquine ili kuzuia malaria, inaweza kuwa na baadhi ya madhara. Athari za kawaida ni maumivu ya tumbo, kichefuchefu, kuharisha, kutapika na maumivu ya kichwa. Kunaweza kuwa na hatari ya uhalifu kutokana na kukaa nje wakati wa usiku. Wakati wa kuchukua sampuli za damu, kunaweza kuwa na maumivu kidogo kwenye kidole ambapo damu imechukuliwa. Tutafuatilia kiwango cha utaratibu uliowekwa wakati wa kuchukua sampuli za damu kutoka kwa kidole chako.

Faragha na usiri: Jina lako halitatumika katika ripoti yeyote au majarida.Maelezo kukuhusu yanaweza kuwa pamoja na wachunguzi na kamati ya maadili kutoka Kenya Medical Taasisi ya Utafiti.

Haki zako kushiriki, kutoshiriki, au kujiondoa: Ushiriki ni kwa hiari, kumaanisha una huru kushiriki au kutoshiriki. Pia, una huru wa kujiondoa wakati wowote.

Gharama na fidia kwa kushiriki katika utafiti: Ikiwa wewe ni mgonjwa, utapata utambuzi wa Malaria na matibabu ya bure katika kituo cha afya. Ikiwa umepatikana na ugunjwa wa malaria baada ya utambuzi, tutagharamia usafiri unapotembelea kituo cha afya Hatutagharamia usafiri au ada ya matibabu ya magonjwa mengine ambayo chanzo chao si kushiriki katika utafiti.

Kuwasiliana: Kama unao maswali kuhusu utafiti huu tafadhali wasiliana na Dr Andrew Githeko katika KEMRI ofisi, kijiji cha Kisian, mbali Kisumu-Bondo barabara, Kisumu. Unaweza kumfikia kwa nambari ya simu 072-28-49382. Kama una maswali kuhusu athari za ushiriki na haki zako katika utafiti huu, tafadhali wasiliana na Katibu Mkuu, Kamati ya Maadili na kutathmini KEMRI, S.L.P. 54840-00200, Nairobi; namba za simu: 020-2722541, 0722205901, 0733400003; Barua pepe: erc-secretariat@kemri.org.

Ridhaa: kama umekubali kushiriki katika utafiti huu, tafadhali tia sahihi na Jina lako chini ikiwa:

1. Umeambiwa sababu za utafiti,
2. Umeambiwa hatua za kufuatiliwa katika utafiti,
3. umejibiwa maswali yako kuhusu utafiti huu,
4. umeambiwa hatari na faida utakozopata kwa kushiriki katika utafiti,
5. Umeamua kushiriki katika utafiti huu kwa ihali yako.

Jina la Mshiriki: _____Sahihi: _____ tarehe: _____

Jina la Shahidi : _____ Sahihi: _____ Tarehe: _____

Nimeelezea lengo la utafiti huu kwa mshiriki. Kadri ya ufahamu wangu, yeye ameelewa lengo, taratibu, hatari na faida ya utafiti huu.

Tarehe: _____ Jina la Msomaji _____ Sahihi: _____

Appendix 3.2C. Volunteer mosquito collector informed consent form (Luo version)

Kithor mar nonro e projektni:

Development and evaluation of a novel human-odour-baited CDC light trap for outdoor host-seeking malaria vector surveillance in western Kenya

Jononro

Teshome Degefa (Msc)[1,2], Delenasaw Yewhalaw (PhD)[2], Harrysone Atieli (PhD)[1,3], Andrew K Githeko (PhD)[1], Guiyun Yan (PhD)[4].

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Kama itimoe nonroni: Ahero e kaunti ma kusimo and Iguhu e Kakamega kaunti

Gima omiyo itimo nonroni: Gima duoung ma omiyo watimo noroni e dongruok kod fwaro yore manyien mag duoko kecho mar suna mar malaria oko mar ot.

Gima wabiro timo: Wabiro gero tipo matin oko etiend ute ma ochiw. Wabiro keto kitanda e tipo. Wabiro kwayi mondo inindi ekitandano gotieno kuom seche apar gi ariyo. Ibiro gengi motegno gi net matin ma ok othiedhi ma ibiro ngaw e wi otanda nyaka e lowo. Net maduong ma ok othiedhi ibiro ngaw ewi net matin kendo ibiro tinge malo e wi lowo mondo suna oyud kaka donjo. CDC light trap ibiro keti e dier nedego mondo ochok suna ma oywa kod tik. Wabiro kwayi mondo itim kama kuom ndalo angwen e wik kuom wige abich. Wabiro keto rit motegoe aluora mar otanda kod waya ma gengi kuom le makech mag bungu. Kata kamano nyanonro ni ok keti mondo suna okayi, wabiro miyi yath mabiro gengi kuom thuolo mag yudo tuo mar malaria. Kapok wachako nyanonro ni wabiro kawo remo matin ka ichuowo lweti ka ipimo ka dibed gi kute mag malaria. Bende wabiro kwayi mondo idhi e kar thieth tok wige ariyo modo

opimi malaria ka ichako nonroni nyaka wige angwen tok ka nonro oserumo. Nonroni oriwojochiwre ma di rom ji apar gi ariyo.

Yuto: Ibiro yudo pim mar malaria ma nono.

Hinyruok : Wabiro kwayi mondo inindi oko e otanda gotieno kedo ok ibet thuolo nikech koyo mangich. Ibiro gengi kod net matin ma ok othiedhi ka inindo to kata kamano suna pod nyalo kayi. Yath Wabiro miyi, yath magengo malaria ma iluongo ni Mefloquine, nyalo bedo gi hinyruok. Hinyruok ma ngenyie mongere gni kaka piny ich maremo, chuny malepo, ngok to kod wich bar. Hinyruok moko nyalo yudore kaluwore kod timbe mag njore momakore gi nindo oko saa gotieno. Kawakawao remo mar pim, inyalo winjo rem matin e lith lweti kama ibiro kawo e remo. Wabiro luwo chenro mar golo remo.

Kano wach man e kindwa kodi: Wabiro gengi kendo nyingi ok bi tigo e ripode kod oboke ma ibiro ndiki. Duoko mar nonroi inyalo tiyogo gi jononro ma opogore opogore.

Ratiro mar chiwruok kata tamruok kata wuok: Chiwruok enonro en yiero mari tiende ni in kod ratiro mar chiwruok, dagi kata weyo bedo jakanyo e nonro sa asaya.

Yuto ne chiwruok e nonroni: Ka ituo ibiro timi pim kod thieth mar malaria ma oge chudo moro amora kar thieth. Ka opimi kod masin mar pimo malaria ma oyud ni in kod malaria ibiro duokni pesa mar matoka ma ne idhi godo e kar thieth. Ok bi duokni pesa kata yudo thieth ma onge chudo ka oyudi kod touché mamoko ma opgore gi malaria ma iyudo koyiengore kod chiwruokni e nonroni.

Adres ma inyalo losogo kata penjogo kuom nonroi: Ka in kod penjo kuom nonroni okwayi ni inyalo penjo Dr Andrew Githeko ka en e apisi mar KEMRI, gwenge mag Kisian, e apaya mar Kisumu-Bondo. Onyalo yudore e 072-28-49382. Ka In kod penjo momakore kod hinyruok kuom chiwruok e nonroi komakore kod ratiro mari los kod jagoro, KEMRI Ethics Review Committee, P. O. Box 54840-00200, Nairobi; Telephone numbers: 020-2722541, 0722205901, 0733400003; Email address: erc-secretariat@kemri.org

Consent: **Ka iyie kendo ichiwri e nonroi, wakwayi I iket sei kod nyingi piny kae ka:**

1. Oseyangini gima omiyo itimo nonroni,
2. Oseyangni chenro kata okenge mag nonroni ,

3. Oseduok penjo magi duto ma omakore kod nonroni,
4. Oseyangni hinyruok kod ber ma ibiro yudo ka ikonyoe nonroni
5. KOD yiero mondo ikony e nonroni ka yiero e mari.

Nying jachiwre: _____Sei: _____ Tarik: _____

Nying janeno: _____ Sei: _____ Tarik: _____

Aselero gima omiyo watimo nonroni e jachiwre kaka anyalo, kendo osewinjo maber gima omiyo, kaka itime, hinyruok kod yuto mari e nonroni888

Tarik: _____ Nying jasomo _____ Sei jasomo: _____

Appendix 3.2D. Liyama lyu khwirulitsa khukumila tsisuna (Luhya Version)

Murwi kwi lisoma yili: Khutsililitsa nukhuhenzeshitsa mureko kwu vulavu nu mwayu kwu mundu khu isuna ya malelia mu Kenya yimbo.

Mira ga Vahenzi:

Teshome Degefa (Msc)[1,2], Delenasaw Yewhalaw(PhD)[2] Harrysone Atieli (PhD)[1,3], Andrew K Githeko (PhD)[1], Guiyun Yan (PhD)[4].

Mikanda chya Vahenzi

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Masaka Kubuhenzi: Ahero mulukongo lwa Kisumu nende Iguhu Lukongo Lwa Isheyu

Shibune Shukhuhenzeshitsa: Lichoma likali ni khukhava injila indeyi yukholondelela tsisuna tsiluma vandu nukhulera malaria khurula ilwanyi.

Khulakola ndinangwa: Kwa lumbakha litili ilwanyi witsinzu tsya varevuli makhuremu shitali. Khulakusaya ukone mu khu masa kumi na mbili, na khula khurelamu ineti indititi ya khashilikwa yitsisuna. Khulavikamu ineti ingali ifuti indala khurula khu khaneti khatititi khu tsitsuna tsinjilimu. Mureko kwu bulavu kwalavikhwa hakari hatsineti khukumira tsitsuna tsihulila mwayu kwokwo. Khulakusaya ukonemu khu vutukhu vunene vuli lisitsa,khu masitsa karano.Khwalumbakha lukaka kulwaya lwishichunjihe kushitali khushinga tsisolo tsye mbulimu. Khalindyo,muyinzi yuku shukukhulomba ulwale malelia tawe, khulakuha musala kwukhuchehiza malwale ka malelia. Nukhushile khuranga, khulavukula musahi mushitere khupima malelia. Khulakhusaya utsyi musivitali shavuli masitsa kaviri upinwi malelia mpaka lwa khumala lisoma. Lisoma yili lya lavunjelitsa viirulitsi kumi na vaviri.

Buyeli bwa Hamleli: walapimwa malelia na ulashilikhwa vuswa lisoma litsilila

Vuchelelu: Khulakusaya ukone ilwanyi khuvushindu,ulashingwa ni ineti nukonanga nikhali isuna inyala khuluma. Ulahelwa musaala kwukhushinga malelia kulangwa Mefloquine-kuri nende shinyasyo manya indakhuluma. Khwenya khusala,khunyala khusala nende murwi khuluma khunyala khuvoha nende munyangano shichila khuhonga ilwanyi vutukhu. Khulalonda vuchusi vwu khurulitsa musayi kwu kupima khu mulala avulikhuhulila vululu.

Isili yoyo: Khulalinda isili yoyo. Malaha koko shikalolekhanakhu tawe mumaripoti keru. Marivuli koko kanyala khuvambulwa na vahenze shitsi nende shikhalilu chya mukanda kwukhuhenzeshitsa vulamu Mukenya.

Tsihaki tsyotsyo khulimasia kwiyama inoho,khwambakhana: Khulimasia nukhwirulitsa,mbu unyala khuhuchilila anoho wirutitsimu isa yosi.

Mirungu khuva halala mumuyumu yuku: Niva ulalwala,ulapimwa shukhya malelia ma ushulishwi musivitali. Nikava ulanyolekha na malelia khuvirila mutuluvini,khulakhuha mirungu chya khukalukha musivitali.Nikhali malwale kandi kakharulana nende lisoma yili, shukhuhana tsilupia tawe.

Vulondeleli vwa marevo nende kakhuminanga: Wava nilirevo lyosi khulondekhana khu lisoma yili, khuvira Daktari Adrew Githeko wa KEMRI mu ofisi,Lusoma lwa Kisian-Muhanda Kwa Bondo,Kisumu khunamba yiyi: 0722849382. Khandi wava namarevo kashinganga tsihaki tsyotsyo, khuvila muhandichi wishikhalilu shia miima-Lisanduku lyi posta 54840-00200 Ilori.Isimu ni:020-2722541,0722205901,0733400003,Likuyakuyi-erc-secretariat@kemri.org.

Khuhuchilila: Niva wiyami khulimasia mumuyumu yuku,yanza uvikhi shitere niva:

1. Uvolelwi shivune/vibune vyi lisoma yili.
2. Uvolelwi vikhaa vyukhulonda.
3. Marevo koko vachipi vuleyitsa.
4. Uvolelwi maleyi namatinyu ka yivi khukhonyana mu lisoma yili
5. Khurevula khukhonyana khulienya lyolyo.

Lilaha lya mwirulitsi ……. Isaini …… Mweli ……

Lilaha lya mushahidi …… Isahini …….. Mweli ……

Khulondekhana khu machesi kanje,niivali lichomo lyu muyinzi kunu khu uyu uvahalala mukwo na avele nilimanya, vikha,vutinyu nende vuleyi vwi lisomo yili.

Mweli…….. Musomi ……. Isaini ……..

Appendix 3.2E. የዎባ ትንኞችን ለሚሰበስቡ ተሳታፊዎች የተዘጋጀ የስምምነት ቅጽ (Amharic version)

ይህ የስምምነት ቅፅ ሁለት ክፍል አለዉ፤

- የመረጃ ሺት (ስለጥናቱ መረጃ ለተሳታፊዎች ለማካፈል)
- የስምምነት ሰርትፍኬት (የስምምነት ፊርማ)

የዚህ የስምምነት ቅፅ ግልባጭ የሰጦታል

ክፍል ሀ፡ የመረጃ ሺት

መግቢያ፡ ስሜ ተሸመ ደገፋ ይባላል። በጅማ ዩኒቨርሲቲ የPhD ተማሪ ነኝ። እኔና ሌሎች አማካሪዎቼ (ተመራማሪዎች) ከጅማ ዩኒቨርሲቲ፣ ኬንያ ሜዲካል ሪሰርች እንስትትዩት እና ካሊፎርኒያ ዩኒቨርሲቲ "Profiling and Quantifying Residual Malaria Transmission in Kenya and Ethiopia" ለተሰኘ ርዕስ ምርምር ለመስራት አቅደናል። በዚህ ምርምር የወባ ትንኞችን ለመሰብሰብ በፍቀገኝነት እንዲሳተፉ የተጋበዙ ሲሆን ለመሳተፍ ፍቃደኛ ከሆኑ ስለ ምርምሩ አለማና የስራ ሀደት መረጃ እንሰጦታለን። በጥናቱ ላይ ስለመሳተፍዎ የግድ ዛሬ መወሰን አይጠበቅቦትም። ከመወሰንዎ በፊት ከማንም ጋር ስለ ምርምሩ ጉዳይ መወያየት ይችላሉ።

የጥናቱ አላማ፡ የጥናቱ ዋና ዓላማ አዲስ የዎባ ትንኞች ማጥመጃ (መሰብቢያ) መሳርያዎችን ለመስራት እና እነዚህ መሳርያዎች በትክክል መስራታቸዉን ለማረጋገጥ እንዲሁም ስለ ትንኞቹ ባሓሪ እና የዎባ ስርጭት መጠን በእትዮጵያና ኬንያ ለማጥናት ነዉ። ከጥነቱ የሚገኘዉ ዉጤት የዎባ ባሽታ ለሜጥፋት የሚደረገዉበ ጥረት ከግቡ እንዲደርስ ያግዛል።

የተሳታፊዎች ምርጫ፡ እርስዎ የዚህ አከባቢ ነዋሪ ስለሆኑ በዚህ ምርምር እንዲሳተፉ ተጋብዘዋል። የምርምር አባሎቻችን ለቀበሌያችሁ ካስጣወቁ በኋላ እርስዎ በጥናቱ ላይ እንዲሳተፉ ይመረጣሉ። ከዚህ ምርምሩ ከሚካሄድበት አከባቢ ***4-5*** ፍቃደኛ የሆኑ ተሳታፊዎችን እንመርጣለን። ሁሉም እድሜያቸዉ ከ ***18*** ዓመት በላይ የሆኑት የዚህ ቀበሌ ነዋሪ ፈቃደኛ ወንዶች የመሳተፍ እድል ኣላቸዉ። ነገር ግን ሳይነስና የሚጥል በሽታ ያለባቸዉ በዚህ ጥናት ላይ አይሳተፉም።

የምርምሩ ሂደትና ቅድመ ተከተል ገለፃ

እንደ ተሳተፊ የወባ ትንኞችን Human landing catch ወይም Human-baited double net trap በሚባል መንገድ እንድሰበስቡ እንጠይቆታለን።

Human landing catch (HLC): ቤት ዉስጥና ከቤት ዉጪ በመቀመጥ ከማታ እስከ እኩለ-ለሊት ወይም ከእኩለ-ለሊት እስከ ጠዋት እንዲለቀሙ እንጠይቆታለን። በመጀመርያ ትንኞች በ**ናሮቂቅሽቂሮቋቂቂን** እንዴት እንደሚሰበሰቡ በቂ ስልጠና እንሰጦታለን። እግርዎ እንዲጋለጥ ሱሪዎትን ትንሽ ወደ ላይ በመሰብሰብ ወንበር ላይ የቀመጣሉ። ትንኞቹ እግርዎ ላይ እንዳረፉና ከመናደፋቸዉ በፊት በ“aspirator” ቶሎ ይሰበስባሉ። በየሰዓቱ የተሰበሰቡትነ ትንኞች በተዘጋጀለት እቃ ዉሰጥ በማስቀመጥ ይመዘግባሉ።

Human-baited double net trap (HDNT): ትንሽ ድንኳን የተመረጡት ግቢ ዉስጥ በመስራት በዉስጡ ትንሽ አልጋ ከነፍራሹ የሚናስቀምጥ ስሆን እርስዎ ድንኳኑ ዉስጥ በተቀመጠዉ አልጋ ላይ ለሊት እንዲተኙ እንጠይቆታለን። እርስዎን ከትንኞች ለመከላከል እልጋዉ ኬሚካል ባልተነከረ ትንሽ አጎበር ሙሉ በሙሉ የሚሸፈን ይሆናል። ሌላ ትልቅ ኬሚካል ያልተነከረ አጎበር ከትንሹ አጎበር ከፍ ተደረጎ እና ከአጎበሩና መሬት መካከል የ**30**ሜንትሜትር ክፍተት እንዲኖር ተደርጎ ይሰቀላል። በርስዎ ትንፋሽ ተስበዉ ወደ ሁለቱ አጎበረሮች መሓል የሚገቡ ትንኞች CDC light trap በሁለቱ አጎበሮች መካከል በመስቀል የሚሰበሰቡ የሆናል። ለደህንነትዎ ስባል ድንኳኑ ዙሪያዉን የሚታጠር ይሆናል። ይህን ስራ በየወሩ ለ**4** ለሊት ለአንድ ዓመት እንዲሰሩ እንጠይቆታለን።

ተሳታፊዎችን ከወባና ሌሎች ተላላፊ በሽታዎች መከላከል

በመጀመርያ ለወባ ተጋላቨጭነትን ለመቀነስ የፀረ-ወባ መድሀኒት (mefloquine) የወስዳሉ። በተጨማረም ስለስራዉ (HLC እና HDNT) በቂ ስልጠና የሰጦታል። በ HLC መንገድ የወባ ትንኞችን ላመሰብሰብ ትንኞቹ እግርዎ ላይ እንዳረፉና ከመናደፋቸዉ በፊት “aspirator” በሚባል መሳርያ ቶሎ ይሰበስባሉ። ለርስዎ ሰላምና ምስጥራዊነት ስባል ሶሰት ክፍል (ሳሎን፣ ማብሰየ ክፍልና መኝታ ክፍል) ያሉት ቤቶች የመረጣሉ። እርስዎ ሰሎን ዉስጥ ተቀምጦ ወደ ቤቱ የሚመጡትን ትንኞች HLC በሚባለዉ መንገድ ይሰበስባሉ። ይህ ስራ እንደ ደንጌ ላሉት ሌሎች ተላላፊ በሽታዎች አይጋለጥም ምክንያቱም ደንጌ ምርምሩ በሚሰራበት አከባቢ የለም።

ስለወባ ቅድመ መከላከያ መድሀኒት (mefloquine) መረጃ

ይህን ስራ ከመጀመርዎ ከ ሁለት ሳምንት በፊት ***ጀምሮ በየሳምነቱ*** የወባ መከላከያ መድሀኒት ይሰጦታል። ይህ መድሀኒት እርስዎን ከወባ የሚከላከልና በአለም አቀፍ የጤና ድርጅትና በእትዮጵያ ጤና ምኒስቴር የተፈቀደ ነዉ። መድሀኒቱ የጎላ ተዉኖ የለዉም። ነገር ግን ሳይነስ እና የሚጥል በሽታ ያለባቸው ሰዎች ላይ ጉዳት ሊያስከትል ይችላል።

የምርምሩ ግዜ/ቆይታ፡ ይህ ምርምር እስከ ታህሳስ ***2011*** ድረስ የሚቆይ ሲሆን እርስዎም ለመሳተፍ ፍቃደኛ ከሆኑ እሰከዛ የኛ የምርምር አባል የሆናሉ።

ተጋላጭነትና ጥቅሞች፡ የቅድመ መከላከያ መድሀኒት በአግባቡ እስከተወሰደ ድረስ ይህ ምርምር ለወባ አያጋልጥም። ነገር ግን ድንገት የወባ ምልከት ከታየቦት ቅርብ ወዳለበት የጤና ተቋማት ሄደዉ ተገቢዉን ህክምናና መድሀኒት እንዲወስዱ ይደረጋል። የምርምሩ አባላት የርስዎን የጤና ሁኔታ ይከታተላሉ። ከዚ ባለፈ ግን ሌላ ጥቅማጥቅም አያገኙም። ከጥነቱ የሚገኘዉ ዉጤት እትዮጵያ ዉስጥ የሚከሰተዉን የወባ ባሽታን የመጥፋት ጥረት ከግቡ እንዲደርስ ያግዛል።

መካካሻ፡ በጥናቱ ለይ በመሳተፍዎ ምክንያት የቀን የዉሎ አበል ያገኛሉ።

ምስጢራዊነት፡ የርስዎ ምስጥራዊነት የምጠበቅ ይሆናል። ስምዎ የምርምሩ ዉጤት ዘገባ ዉስጥ አይካተትም።

ያለመሳተፍ ወይም የማቋረጥ መብት፡ በዚህ ጥናት የመሳተፍ ወይም ያለመሳተፍ መብትዎ የተጠበቀ ነዉ። በዚህ ጥነት ላይ ባይሳተፉም ከዚህ በፊት ያገኙ የነበረ የጤና አገልግሎት ያገኛሉ። የርስዎ በዚህ ጥነት ላይ መሳተፍ ሙሉ በሙሉ በርስዎ ፍቃደኝነት ላይ ብቻ የተመሰረተ ነዉ። በጥናቱ ላይ መሳተፍ ከጀመሩ በኋላም ቢሆን ሀሳብዎን መቀየር ይችላሉ ወይም ማቋረጥ ይችላሉ።

ጥያቄ ከለዎት

በማንኛዉም ግዜ ጥናቱን በተመለከተ ጥያቄ ከለዎት የጅማ ዩኒቨርሲቲ መምህርና የPhD ተማሪ የሆኑት አቶ ተሾመ ደገፋን በ+251910891214 በመደወል ማናገር ይችላሉ። ተጨማሪ መረጃም ከፈለጉ ሌሎች የዚህ ምርምር አባል የሆኑትን ፕ/ር ደልነሳዉ የኋላዉ (ጅማ ዩኒቨርሲቲ፣

ስልክ፡+251917804352)፣ ዶ/ር አንድሪዉ ግቴኮ (Dr. Andrew Githeko, Kenya Medical Research Institute, email: githeko@yahoo.com, Tel: 254722849382) እና ፕ/ር ጉዩን ያን (Prof Guiyun Yan, University of California, eamail: guiyuny@uci.edu) ማናገር ይቻላል። ይህ ምርምር በጅማ ዩኒቨርሲቲ እና በኬንያ ሜድካል ሪሰርች እንስትትዩት Institutional Review Board (IRB) ተገምግሞ ፈቃድ ያገኘ ሲሆን ተጨማሪ ጥያቄ ከለዎት በጅማ ዩኒቨርሲቲ ጤና እንስትትዩት የድህረ ምረቃና ምርምር ዳይሬክተር የሆኑትን ዶ/ር ዘለቀ መኮንን በ+***251917765427*** በመደወል መጠየቅ ይችላሉ። አሁንም ቢሆን ጥየቄ ካለዎት እኔን መጠየቅ ይችላሉ። ጥያቄ አለዎት?

ክፍል ለ፡ የስምምነት ሰርትፍኬት

ከላይ የቀረበዉን መረጃ አንብቤ ተረድቻለሁኝ ወይንም ተነብቦልኛል። ግልፅ ስላልሆነልኝ ጉዳዮችም ሲኖሩ ጥያቄ እንድጠይቅ እድል የተሰጠኝ ሲሆን ለጥያቄዎቼም መልስ ተሰቶኛል። ስለዚህ በጥናቱ ላይ የመሳተፍ ፈቃደኝነቴን ስገልፅ በማንኛዉም ሰዓት የማቋረጠጥ መብቴ የተጠበቀ መሆኑን በመረዳትም ጭምር ነዉ።

የተሳታፊዉ ስም_____ ፊርማ _____ቀን _____

ላልተማሩ ተሳታፊዎች፡ ለተሳታፊዉ ስለጥናቱ ስነበብ አይአቻለሁ። ተሳታፈም ስላልገባዉ ጥያቄ ጠይቆ መልስ አግኝቷል። ተሳታፊዉ በፍቃዱ ለመሳተፍ መወሰኑን ምስክር ነኝ።

የምስክር ስም:_____ የተሳታፊዉ የጣት አሻራ:_____

የምስክር ፊርማ:_____ቀን: _____

የተመራማሪዉ መግለጫ

ለተሳታፊዉ ስለ ጥናቱ አላማ በሚገባዉ መልኩ አንብቤለታለሁ። ተሳታፊዉም በደንብ ተረድቶታል። ተሳታፉዉ ስላልገባዉ ጉዳይ ጥያቄ እንዲጠይቅ እድል የተሰጠዉ ሰሆን ጥያቄዎቹም በስረዓቱ ተመልሶለታል። ተሳታፊዉ በምርምሩ ላይ አንዲሳተፍ ያልተገደደ እና በፍቃደኝነቱ ለመሳተፍ መወሰኑን አረጋግጫለሁ።

የተመራማሪዉ ስም_____ የተመራማሪዉ ፊርማ_____ቀን _____

የዚህ የስምምነት ቅፅ ግልባጭ በተመራማሪዉ አማካይነት ለጥናቱ ተሳታፊ ተሰቷል

Appendix 3.2F. Guca walii galtee hirmaattota bookee busaa funaanamiif dhiyaate (Afan Oromo version)

Gucni waliigaltee kun kutaa lama qaba:

- Kutaa ibsa waay'ee qorannichaa (ibsa waay'ee qorannichaa ilaalchisee hirmaattotaaf)
- Sartifikeetii waliigaltee (mallattoo waliigaltee)

Garagalchi guca waliigaltee kanaa isiniif kan kennamu ta'a

Kutaa I: Ibsa waay'ee qorannichaa

Seensa: Maqaan koo Tashoomaa Daggafaa jedhama. Barataa digrii lammaffaa (PhD) Yuunivarsiitii Jimmaati. Anii fi gorsaawwan koo Yuunivarsiitii Jimmaa, Dhaabbata Qorannoo Fayyyaa Keeniyaa fi Yuunivarsiitii Kaalifoorniyaa irraa walitti babahan qorannoo matadureen isaa **"Profiling and Quantifying Residual Malaria Transmission in Kenya and Ethiopia"** jedhu hojjechuuf karoorfannee jirra. Qorannoo kanarratti bookee busaa funaanuudhaaf fedhii keessaniin akka hirmaattan affeeramtanii jirtu. Waay'ee kaayyoo fi haala qorannichi itti adeemsifamus isinitti himuuf dhufne. Hirmaachuufi hirmaachuu dhiisuu keessan amma murteessun dirqama miti. Hirmaachuudhaaf murteessuu keessaniin dura waay'ee qorannoo kanaa hubachuun akkasumas gaafii yoo qabaattan gaafachuun gaarii dha.

Kaayyoo qorannichaa: Kaayyoon qorannoo kanaa meeshalee bookee busaa ittiin funaanuf tajaajilan qalaquufi tajaajilummaa meshaalee kanaa qorachuu akkasumas amaloota bookee busaa fi haala tatamsa'ina dhukkuba busaa naannnoo keenyaa qorachuufi. Ragaan qorannoo kanarraa argamu gara fuula duraatti tatamsa'ina dhukkuba busaa ittisuuf ni tajaajila.

Filannoo hirmaattotaa: Jiraataa naannoo kanaa waan taataniif qorannoo kanarratti akka hirmaattaniif filatamtanii jirtu. Qorannoo kanaaf hirmaattota 4-5 tu barbaachisa. Jiraattonni naannoo kanaa saalaan dhiira ta'an, umriidhaan wagga 18 oli fi hojicharratti hirmaachuuf fedhii qaban hunduu carraa hirmaachuu ni qabaatu. Haata'u malee, namoonni rakkina saaynasii fi martoo qaban qorannicharratti hirmaachuuf ulagaa hin guutan.

Ibsa wantoota hojjetamuuf karoorfaman

Akka hirmaataa qorannichaatti bookee busaa mala 'Human landing catch ykn Human-baited double net trap' jedhamuun akka funaantan ni gaafatamtu.

Human landing catch (HLC): Mala HLC jedhamuun bookee busaa funaanuuf surree keessan hanga jilbaatitti ol sassaabuun mana keessa ykn ala (barandaa) teessorra teessu. Bookeen busaa yoo miila keesssan irra qubattu osoo isin hin hiddin meeshaa 'aspirator' jedhamuun qabuu barbaachisa. Hojiin kun halkan halkan sa'aatii ja'aaf (galgala sa'aatii 12:00 hanga sa'aatii 6:00 ykn halkan sa'aatii 6:00 hanga ganama sa'aatii 12:00tti) kan hojjetamu ta'a.

Human-baited double net trap (HDNT): Mala HDNT jedhamuun bookee busaa funaanuuf, dunkaana tinnoo isaa dallaawwan filataman keessatti ijaaruudhaan dunkaanicha keessa siree firaasha waliin kan keenyu yommuu ta'u, isinis siricharra halkan kan ciistan ta'a (dunkaana tokko keessa nama tokkotu rafa). Bookeen busaa akka isin hin hiddine ittisuuf sireen irra raftan gutumaan guututti agoobara xiqqaa keemikaala hin cuubamneen kan haguugamu ta'a. Dabalataanis agoobara guddaa keemikaala hin cuubamne agoobaricha isa tinnaarraa ol siqee kan haguugamu yommuu ta'u, lafaafi agoobara isa guddicha jidduu ulaan seentimaatira 30 kan jiraatu ta'a. Hafuura keessaniin (hafuura nama dunkaanicha keessa ciisuun) harkisamuun bookeewwan gara agoobara isa guddichaa fi xinnicha gidduu seenan meeshaa CDC light trap jedhamu jedduu agoobara lamaanitti fannisuudhaan kan funaanaman ta'a. Nageenya nama dunkaanicha keessa rafuuf jecha naannawa dunkaanichaatti dallaan cimaan kan ijaaramu ta'a. Hojiin kun ji'atti halkan afuriif, ji'oota 12f kan hoojjetamu ta'a.

Hirmaattota busaa fi dhukkuboota daddarboo birootirraa ittisuu

Qorannicha osoo hin jalqabin dura qorichi busaa ittisuuf tajaajilu (Mefloquine) kan isiniif laatamu yommuu ta'u, leenjiin ga'aan waa'ee bookee busaa mala HLC fi HDNT jedhamuun funaanan ilaalchisee kan isiniif kennamu ta'a.

Nageenyaa fi icciitii eeguuf jecha hojii kanaaf manneen kutaa sadi (saaloonii, kutaa ciisichaa fi kutaa sooranni itti bilchaatu) qaban kan filataman yoo ta'u, isin kutaa saaloonii taa'uudhaan bookee busaa kan funaantan ta'a. Hojiin kun dhukkuboota daddarboo buroo bookedhaan darbaniif (fkn dengue) nama hin saaxilu. Sababni isaas dhukkuboonni kun naannawa keessan waan hin jirreefi.

Ragaa waay'ee dawaa (mefloquine) isiniif laatamuu: Hojii kana jalqabuu turban lama dursee dawaan mefloquine jedhamu turban torbaniin kan isiniif kennamu ta'a. Dawaan kun dhukkuba busaa irraa nama ittisuuf kan tajaajilu yommuu ta'u, dhaabbata fayyaa addunyaatii fi ministeera

fayyaa Itoophiyaatinis kan eeyyamamu ta'a Dawaan kun miidhaa cimaa namarraan hin gahu. Haa ta'u malee namoota rakkina saaynasii fi martoo qaban irratti miidhaa geessisuu mala.

Yeroo turtii qorannichaa: Qorannichi kun hanga Caamsaa 2011 kan turu yommuu ta'u, hirmaachuudhaaf yoo fedhii qabaattan hanga yeroo sanii nu waliin turuu dandeessan.

Saaxilamummaa fi faayidaawwan qorannichaa: Dawaan fudhatamnaan qorannichi dhukkuba busaatiif nama hin saaxilu. Haa ta'uutii yoo akka tasaa mallattoo dhukkuba busaa isinirratti mul'ate, gara mana yaalaa deemtanii qorannoo fi dawaa bilisaa akka argattan taasifama. Waa'ee fayyaa keessanii miseensonni qorannoo kanaa kan hordofan ta'a. Kanarraa kan hafe garuu faayidaan addaa sababa hirmaannaa keessaniitiin argattan hin jiraatu. Ragaan qorannoo kanarraa argamu gara fuula duraatti tatamsa'ina dhukkuba busaa ittisuuf ni tajaajila.

Durgoo: Hirmaannaa keessaniif durgoo gita hojii kanaa ni argattu.

Iccitii: Iccitiin keessan kan eegamu ta'a. Maqaa keessan gabaasa waa'ee qorannoo kanaa keessatti hin barraahu.

Mirga hirmaachuu dhiisuu ykn addaan kutuu: Hirmaannaan keessan fedhii keessan qofa irratti kan hundaaye dha. Yoo fedhii hin qabaanne hirmaachuu dhiisuu ni dandeessu ykn hirmaannaa erga jalqabdanii boodas yoo ta'e addaan kutuuf mirga qabdu. Hirmaachuu dhiisuu keessaniif midhaan isinirra gahu hin jiru, tajaajilli duraan argachaa turtan kan isinirraa hafus hin jiraatu.

Gaaffii yoo qabaattan: Gaaffii yoo qabaattan qorataa dursaa hojii kanaa kan ta'e obboo Tashoomaa Daggafaa (Yuunivarsiitii Jimmaa, Lakk bilbilaa: +251910891214) haasofsiisuu dandeessan. Hubannoo dabalataas yoo barbaaddan miseensota qorannoo kanaa kan ta'an Prof Dilnassaaw Yewhaalaaw (Jimma University, Tel: +251917804352), Dr. Andrew Githeko (Kenya Medical Research Institute, email: githeko@yahoo.com, Tel: 254722849382) fi Prof Guiyun Yan (University of California, guiyuny@uci.edu) gaafachuu dandeessu. Yoo barbaaddan ammas ykn boodas gaafii yoo qabaattan nagaafachuu dandeessu.

Piroopoozaalli kun hayyoota Yuunivarsiitii Jimmaa fi Dhaabbata qorannoo fayyaa Keeniyaatin kan gulaalamee fi eeyyame argatee dha. Gaafii waa'ee eeyyamaa fi kan biraas yoo qabaattan Dr Zallaqaa Makonnin, Daareektar qorannoo dhaabbata fayyaa Yuunivarsiitii Jimmaa, P.O.Box 378, Tel: +251917765427 bilbilaan gaagfachuu dandeessu.

Kutaa II: Sartifikeettii walii galtee

Ibsa armaan olitti dhiyaate dubbisee jira ykn akka naaf galutti naaf dubbifamee jira. Gaafii akkan gaafadhuufis carraan kan naaf kenname yommuu ta'u, gaafilee kiyyaafis deebii quubsaa argadhee jira. Qorannoo kanarratti hirmachuuf fedhii akkan qabu mallattoo kiyyaan armaan markaneessee jira.

Maqaa hirmaataa _____ Mallattoo_____ Guyyaa: _____

Hirmaattota hin baranneef: Hirmaatichaaf ibsi waay'ee qoranno kanaa sirritti dubbifamuu isaa ragaan baha. Hirmaatichis gaafii akka gaafatuuf carraan kan kennameef yommuu ta'u gaafilee isaa hundeef deebii argatee jira. Hirmaatichi fedhii isaatiin hirmaachuuf akka walii gales ragaan baha.

Maqaa ragaa:_____ ashaaraa qubaa (kan hirmaataa):_____

Mallattoo ragaa:_____ Guyyaa : _____

Ibsa qorataa ykn nama waliigalticha kennee

Ibsa waay'ee qorannoo kanaa hirmaatichaaf sirritti dubbisee jira. Hirmaatichiis gaafii yoo qabaatu akka gaafatuuf carraan kan kennameef yommuu ta'u gaafilee isaa hundaaf deebiin kennameefi jira. Hirmaatichi guutumaan guututti fedhii isaatiin hirmaachuuf akka waliigale markaneessee jira.

Maqaa qorataa_____ Mallattoo _____ Guyyaa_____

Garagalchi waliigaltee kanaa hirmaatichaaf kennamee jira.

Appendix 4. Laboratory Protocols

4.1. PCR protocol for identification of vector species compelexes

i. DNA Extraction

A. Alcohol precipitation method

Reagent preparation

Homogenization buffer:

Final concentration	Measured amount
0.10M NaCl	0.59g NaCl
0.20M Sucrose	6.84g Sucrose
0.01M EDTA	0.37g EDTA
0.03M Trizma base	0.36g Trizma base
PH 8.0	100ml sterile water

Lysis Buffer:

Final concentration	Measured amount
0.25M EDTA	9.28g EDTA
2.5% (W/V) SDS	1.88g SDS
0.5M Trizma base	6.03g Trizma base
0.03M Trizma base	0.36g Trizma base
PH 9.2	100ml sterile water

Grind Buffer: Mix four parts of Homogenization buffer with 1 part Lysis buffer to make Grind buffer. Store all buffers at +4^{o}C for immediate use. Store for longer at -20^{o}C

Procedures

1. Collect single mosquitoes in to separate sterile centrifuge tubes. If using mosquitoes collected and frozen, they should be ground immediately after removing them from the freezer. Whole mosquito or only Legs and wings can be used.
2. One mosquito at a time add 100ul grind buffer to the tube and grind with a sterile pestle until no identifiable mosquito part remain. Immediately place the tube in +65^{o}C water bath and continue with next mosquito until all mosquitoes are in the water bath.

3. Keep tubes at 65^{o}Cfor 15-30 minutes. This step kills nucleases released after grinding the mosquito so they will not degrade the DNA
4. While the tubes are still warm, add 13ul of 8M KAc (OR 18 ul 5M KAc) to achieve a final concentration of 1M. Mix by tapping the tube. Incubate tubes on ice at least 30 minutes (more). The salt solution will precipitate out the mosquito parts and other insuolubles, as well as proteins denatured by SDS
5. Centrifuge tubes at maximum speed at room temperature for 15 minutes. Label new sterile centrifuge tubes to transfer supernatant in to. Immediately after the spin, transfer the supernatant in to the new tubes, being careful not to transfer any of the precipitate.
6. Add 200ul of ice-cold 100% ethanol (EtOH) to the supernatants and mix well by inverting the tubes. Incubate at room temperature for 5 minutes (definitely no more than 10 minutes) to precipitate out the DNA. The tubes can be stored at -20^{o}C or -80^{o}C for long term storage at this point.
7. Centrifuge tubes at maximum speed in a refrigerated centrifuge at +4^{o}C if available (if not, room temperature will be sufficient) for 15-20 minutes to pellet the DNA. Orient all the tubes the same way (hinge up) so that the pellet is in the same place for every tube even if you can't see it. Immediately, pipette or pour off the EtOH being careful not to disturb the pellet. The pellet may look purple if the head of the mosquito has been used. If the pellet becomes dislodged, spin again for 5 minutes before pouring off all of EtOH.
8. Add 200ul of cold 70% or 80% EtOH and spin for 5 minutes at top speed to wash the pellet. Carefully pipette or pour off the EtOH.
9. Add 200ul of cold 100% EtOH and spin for 5 minutes at top speed to wash the pellet. Carefully pipette or pour off the EtOH.
10. All the pellets to air dry on the bench for at least 1 hour (2 is better). Make sure that no traces of EtOH remain. It is okay to leave the pellets to dry on the bench overnight.
11. Create a solution of TE with 1ul/ml of RNase/DNase-free (this is useful to remove any RNA that co-precipitated with the DNA). Re-suspend your DNA in 50-100ul of this solution (use 50ul if you only extracted legs and/or wings). The pellet should dissolve by gentle tapping of the tube. Allow the DNA plenty of time to fully re-suspend before using in further.

B. Qiagen DNA Extraction Method

1. Cut wings and legs of mosquitoes and put them in 1.5 ml Eppendorf tubes individually

2. Add 180 µl of ATL buffer. Add 20 µl of Proteinase K, mix by vortexing and incubate at 56 ^{o}C for 1 hr (until completely lysed). Mix by vortexing
3. Crash the mosquitoes using sterile pestle and mix by vortexing
4. Add 200 µl of AL buffer. Mix thoroughly by vortexing. Incubate the samples at 56 ^{o}C for 10 minutes.
5. Add 200 µl Ethanol (96-100%). Mix thoroughly by vortexing
6. Pipette the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at ≥ 6000 x g (8,000 rpm) for 1 minute. Discard the flow-through and collection tube.
7. Place the spin column in a new 2 ml collection tube, add 500 µl of AW1 buffer. Centrifuge for 1 min at ≥ 6000 x g. Discard the flow-through and collection tube.
8. Place the spin column in a new 2 ml collection tube, add 500 µl of AW2 buffer and centrifuge for 3 min at 20,000 x g (14,000 rpm). Discard the flow-through and collection tube.
9. Transfer the spin column to a new 1.5 ml or 2 microcentrifuge tube.
10. Elute the DNA by adding 100 µl of AE buffer to the center of the spin column membrane. Incubate for 1 min at room temperature (15-25 ^{o}C). Centrifuge for 1 min at ≥ 6000 x g.
11. Optional: Repeat step 8 for increased DNA yield.

ii. PCR protocol for identification of *An. gambiae* species complex

TEMPase

An. gambiae	390bp
An. arabiensis	315bp
An. quadrianulatus/An. amharicus	153bp
An. merus/melas	466/464bp

Multiply:_____

Reagents	**Vol. pr sample**	**Vol. in mix**	**Final conc. per sample**
H_2O	4.0 µl	H2O	
Primers: (1.25uM) UN: 5'-GTGTGCCCCTTCCTCGATGT-3' GA: 5'-CTGGTTTGGTCGGCACGTTT-3' AR: 5'-AAGTGTCCTTCTCCATCCTA-3' QD: 5'-CAGACCAAGATGGTTAGTAT-3' ME: 5'-TGACCAACCCACTCCCTTGA-3'	4.0 µl	Primers	0.25 µM pr. Primer
Tempase Master mix	10 µl	Tempase	

Add. 18 µl Master Mix
2 µl Sample DNA

PCR Cyce

95°C 5 min
94°C 30 sec
50°C 30 sec } x 30
72°C 30 sec
72°C 10 min
4°C Forever

iii. PCR protocol for identification of *Anopheles funestus* group

GreenTaq Master Mix

An. funestus	505bp
An. rivulorum	411bp
An. vaneedeni	587bp
An. parensis	252bp
An. leesoni	146bp :

Multiply:_____

Reagents	**Vol. per sample**	**Vol. in mix**	**Final conc. per sample**
PCR H_2O	8.5 µl	H2O	NA
Primer mix: 10uM UN: 5'TGTGAACTGCAGGACACAT-3' FUN: 5'-GCATCGATGGGTTAATCATG-3' VAN: TGTCGACTTGGTAGCCGAAC-3' RIV: 5'-CAAGCCGTTCGACCCTGATT-3' RIVLIKE: CCGCCTCCCGTGGAGTGGGGG-3' PAR: 5'-TGCGGTCCCAAGCTAGGTTC-3' LEES: 5'-TACACGGGCGCCATGTAGTT-3'	1.0 µl	Primers	0.4 µM per. Primer
Dream Taq Master mix	12.5 µl	Dream Taq master mix	1X

Add. 22 µl Master Mix
3 µl Sample DNA

95°C 5 min
94°C 30 sec
50°C 30 sec } x 40
72°C 40 sec
72°C 10 min
4°C – forever

iv. Protocol Gel Electrophoresis

1. Measure 1.5gm of Agarose 3:1 HRB
2. Measure 75 ml of 1xTAE buffer into pyrex flask (NB. 1XTAE buffer is prepared from 50X TAE buffer by mixing 10ml of 50x TAE and 490 mol of distilled water)
3. Mix the measured agarose with the TAE buffer (boil under microwave for two minutes)
4. Cool the agarose solution under cool running water. Shaking the flask ensures uniform cooling and prevent solidification
5. Add 1µl of Ethidium Bromide and mix it by shaking
6. Prepare gel (20 minutes)
7. Add approximately 500 ml of 1XTAE buffer to Gel Electrophoresis chamber
8. Transfer the gell to the gell tanker
9. Add 12 µl of PCR product (amplicon) for larger comb-holes or 8 µl of the amplicon for the smaller comb-holes
10. Run with 70 volt for 45-50 minutes
11. Read the result (302 nm)

4.2. Blood meal ELISA Protocol

1. Introduction

Blood meal sources of fed female *Anopheles* mosquitoes were determined using direct ELISA (Beier et al., 1988, Beier, 2002). The direct ELISA begins by incubating the blood-meal sample directly in microtiter plate wells. It uses a host-specific antibody-enzyme conjugate to detect homologous IgG in the blood-meal sample and specific substrate to produce a color reaction. Antibodies specific to human, bovine, goat, chicken and dog blood meals were used in this study.

Preparation of regaents

1. Phosphate buffered Saline (PBS)

Phosphate buffered solution was prepared by dissolving PBS tablet in distilled as per the manufacturer instruction and pH was adjusted to 7.4.

2. Blocking buffer (BB): shelf life is one week at 4^{o}C

Component	Volume: 500ml	Volume: 1000ml
Casein	2.5g	5.0g
NaOH, 0.1N	50ml	100g
1xPBS, 10mM pH 7.4	450ml	900g
Phenol red solution, 10µg/ml	0.1ml	0.2ml

1. Bring 0.1N NaOH to a boil in a flask with a stir bar mixing on low
2. Slowly add the casein (Sigma Aldrich C7078) and mix until dissolved in 0.1N NaOH
3. Allow solution to cool at room temperature
4. Slowly add the PBS
5. Adjust the pH to 7.4 with 1N HCl
6. Add the phenol red

3. PBS-Tween 20 washing solution

1. Add 500µl of Tween 20 to 1 liter PBS
2. Mix well and store at 4^{o}C. Shelft life two weeks

4. Antibody conjugate

Conjugate antibodies were received from KPL (http://www.kpl.com) /SeraCare in lyophilized form. Peroxidase labeled antihuman, antigoat, antichicken and antidog antibodies were

reconstituted using KPL's hostradish peroxidase (HRP) stabilizer as per the manufacturer instruction. Phosphatase labeled antibovine antibody was reconstituted using KPL's alkaline phosphatase (AP) stabilizer.

5. ***ABTS:*** substrate for peroxidase labeled antibodies
   - Obtained from KPL(http://www.kpl.com) in solution form (ABTS soulution A and B)
   - Stable for a minimum of 1 year when stored at 2-8^{o}C
6. ***pNPP:*** substrate for phosphatase labeled antibody
   - Consists 100 (5mg) tablets of *p*-nitrophenylphosphate (*p*-NPP) and 100 mL Diethanolamine (DEA) Buffer (5X)
   - Stable for a minimum of 1 year from date of receipt when stored at 2-8°C.

Preparation of mosquito sample

1. Prepare freshly fed female mosquitoes by cutting them transversely at the thorax between the first and third pairs of legs.
2. Place the posterior part (abdomen) of the mosquito in a labeled tube (a single mosquito per tube)
3. Add 50µl of PBS and grind well using a pestle
4. Dilute the sample 1:50 with PBS and store at -20^{o}C until testing
5. Before grinding the next mosquito, rinse pestle in PBS-Tween twice; dry with tissue to prevent contamination between mosquitoes.

Preparation of Controls

- Positive control were prepared by collecting venous blood from human by Medical Laboratory professional and from other vertebrate hosts (cattle, goat, chicken and dog) by Veterinery professionals following standard operating procidures
- The positive controls were diluted (1:500) with PBS
- Unfed mosquitoes were used as negative control and prepared following the same procedures as for mosquito sample preparation

General Procedures

1. Fill out blood-meal ELISA worksheet (template) with sample information (code). Mark the ELISA plate in order to maintain correct plate orientiation.

2. Add 50µl of the diluted sample in wells of ELISA plate. Add 50µl of positive controls and 50µl of negative controls on wells labeled for positive and negative controls respectively. Use separate plate for each host (human, bovine, goat, chicken and dog)
3. Cover with almunium foil and incubate for 2hrs at room temperature
4. At the end of the incubation period, prepare a working solution of conjugate antibodies by adding BB plus 0.025% Tween 20 to the reconstituted conjugate antibodies (1:250 dilution) based on the volumes by host species listed below. Vortex gently

Tube label (host)	**Volume of BB**	**Volume of stock antibody conjugate**
Human	4880µl	20µl
Bovine	4880µl	20µl
Goat	4880µl	20µl
Dog	4880µl	20µl
Chicken	4880µl	20µl

5. Wash the plate two times with PBS-Tween 20
   - ✓ Throw out sample
   - ✓ Add 200µl wash buffer
   - ✓ Wait 1min
   - ✓ Throw out wash buffer
   - ✓ Redo second time

   } **OR** use ELISA plate washer
6. Add 50µl of host-specific conjugate prepared in step 4
7. Cover the plates and incubate for one hr at room temperature
8. Wash three times with PBS-Tween 20
9. Add 100µl of ABTS peroxidase substrate to each well: for human, goat, chicken & dog
10. Add 100µl of pNPP phosphatase substrate to each well, for bovine
11. Read absorbance at 414nm using ELISA reader 30 min after addition of the ABTS (for human, goat, chicken and dog). The dark green positive reaction may also be determined visually
12. Read absorbance at 414nm using ELISA reader 1hr after addition of pNPP (for bovine). The yellow positive reactions may also be determined visually

13. Samples are considered positive if the absorbance values exceeded the mean plus three times standard deviation of four negative controls. Use unfed mosquitoes as a negative control

Procedures for determination of human and bovine blood meal on a single plate

1. Add 50µl of the diluted sample in wells of ELISA plate. On the same plate (different consecutive wells), add 50µl of positive controls (one for human and the other for bovine) and four negative controls.
2. Cover with almunium foil and incubate for 2hrs at room temperature
3. Wash two times with PBS-tween 20
   - ✓ Throw out sample
   - ✓ Add 200µl wash buffer
   - ✓ Wait 1min
   - ✓ Throw out wash buffer
   - ✓ Redo second time

   OR use ELISA plate washer
4. Add 50µl of peroxidase labeled anti-human IgG and 50µl of phosphatase labeled anti-bovine IgG to each well of the plate
5. Cover the plate and incubate for one hr at room temperature
6. Wash three times with PBS-tween 20
7. Add 100µl of ABTS peroxidase substrate to each well
8. Read absorbance at 414nm using ELISA reader 30 min after addition of the ABTS. The dark green positive reaction may also be determined visually and indicates positive reaction for human
9. After reading the result for human, wash the wells three times with PBS-tween 20 and add 100µl of pNPP phosphatase substrate to each well
10. Read absorbance at 414nm using ELISA reader 1hr after the addition of pNPP to determine positive reactions for bovine. The yellow positive reactions may also be determined visually
11. Samples are considered positive if the absorbance values exceeded the mean plus three times standard deviation of four negative controls. Use unfed mosquitoes as a negative control

4.3. Sporozoite ELISA Protocol

Plasmodium falciparum, *P. vivax*-210 and *P. vivax*-247 CSPs were detected in *Anopheles* mosquitoes using a sandwich ELISA.

Principle

The sandwich ELISA begins with adsorption of capture monoclonal antibody (mAb) to wells of microtiter plate. After the capture mAb has bound to the plate, the well contents are aspirated and the remaining sites are blocked with BB. Mosquitoes to be tested are ground in BB containing IGEPAL CA-630 and an aliquot is tested. If CSP is present, it will form an antigen-antibody complex with the capture mAb. After incubation for 2 hrs at room temperature, the mosquito triturate is aspirated and the wells are washed. Peroxidase-labeled mAb is then added, completing the formation of the sandwich. After 1 hr the well contents are aspirated, the wells are washed again and peroxidase substrate solution is added. As the peroxidase enzyme reacts with the substrate, a dark green product is formed. The intensity of the color is directly proportional to the amount of CSP antigen present in the test sample. The results are read visually or at 405-411nm using an ELISA plate reader 30 and/or 60 minutes after the substrate has been added (Beier et al., 1987, Wirtz et al., 1987).

Reagent preparation

1. ***Phosphate buffered Saline (PBS)***

Prepared as described for blood meal ELISA

2. ***Blocking Buffer (BB)***

Prepared as decribed for blood meal ELISA

3. ***Grinding Buffer***

    1. Combine 25ml of BB and 125µl of IGEPAL CA-630. This is sufficient for one plate
    2. Mix well using a vortex to dissolve the IGEPAL CA-630 in the BB
    3. Store at 4^{o}C (shelf life is one week)

4. ***PBS-Tween20 Wash solution***

Prepared as described for blood meal ELISA

5. ***Capture and Conjugate monoclonal antibodies (mAb)***

Capture and conjugate mAb were received from BEI Resources in lyophilized form. The label on vials of the mAb indicates the amount of glycerol:distilled water (1:1) to be added to reconstitute the mAb. Glycerol water allows for storage at -20^{o}C without freeze-thawing.

6. Positive controls

Positive controls were received with mAb in lyophilized form. The label on vails of the positive controls shows the amont of BB to be added to reconstitute the positive controls.

Mosquito Sample Preparation

6. Place the mosquito, head and thorax only, in a labeled 1.5ml microcentrifuge tube
7. Add 50µl grinding buffer and grind well using a pestle
8. Rinse the pestle twice, each time with 100µl of grinding buffer catching the rinses in the tube containing the mosquito triturate. Final volume will be approximately 250µl
9. Before grinding the next mosquito, rinse pestle in PBS-Tween twice; dry with tissue to prevent contamination between mosquitoes
10. Samples may be used immediately or frozen for later analysis

Procedures

1. Fill out sporozoite ELISA worksheet (template) with sample information (code). Mark the ELISA plate in order to maintain correct plate orientiation.
2. Prepare a working solution of mAb capture by adding PBS to the reconstituted capture mAb based on the volumes by species listed below. Vortex gently

Species	**mAb**	**µg/50µl/well**	**µg/5ml**	**µl stock/5ml**
P. falciparum	Capture	0.20 µg/50µl	20.0 µg	40µl stock + 5ml PBS
P. vivax-210	Capture	0.10 µg/50µl	2.5 µg	20µl stock + 5ml PBS
P. vivax-247	Capture	0.10 µg/50µl	2.5 µg	20µl stock + 5ml PBS

3. Place 50 µl of capture mAb solution made in step 2 in each well of the ELISA plate. Use a separate plate for each sporozoite species.
4. Cover plate and incubate for at least 30 min or as long as overnight at room temperature.
5. Aspirate well contents and bang plate upside down on paper towel 5 times holding sides only
6. Fill wells with 200µl BB.
7. Cover plate, leaving space between well and top of lid. Incubate for 1 hour
8. Aspirate well contents and bang plate upside down on paper towel 5 times holding sides only
9. Load samples and control in to wells
    i. Add 50µl mosquito homogenate to wells labeled for samples.

ii. Add 50µl positive and negative control solutions to wells labeled for positive and negative controls repsectively.

iii. Cover plate and incubate for 2 hours at room temperature.

Steps 10-12 can be performed just before the end of the 2 hour incubation

10. Prepare the ABTS - Substrate solution - This solution should be prepared fresh. Mix Solution A and Solution B (hydrogen peroxide) 1:1. Prepare enough to add 100 µl / well.
11. Prepare a working solution of mAb conjugate by adding BB to the reconstituted conjugate mAb based on the volumes by species listed below. Vortex gently.

Species	**mAb**	**µg/50µl/well**	**µg/5ml**	**µl stock/5ml**
P. falciparum	Peroxidase	0.050 µg/50µl	5.0 µg	10µl stock + 5ml BB
P. vivax-210	Peroxidase	0.050 µg/50µl	5.0 µg	10µl stock + 5ml BB
P. vivax-247	Peroxidase	0.050 µg/50µl	5.0 µg	10µl stock + 5ml BB

12. Check enyme activity by mixing 5µl of the mAb conjugate made in step 11 with 100µl of the substrate made in step 10 in a separate tube. Vortex gently. There should be a rapid color change indicating that the peroxidase enzyme and the substrate are functional.
13. Aspirate well contents and bang plate upside down on paper towel 5 times holding sides only
14. Wash wells two times with 200µl PBS-Tween, aspirating and banging plate 5 times with each wash
15. Add 50µl of peroxidase conjugate solution made in step 11 to each well
16. Cover pate and incubate for 1 hour
17. Aspirate well contents and bang plate upside down on paper towel 5 times holding sides only
18. Wash wells three times with 200µl PBS-Tween, aspirating and banging plate 5 times with each wash
19. Add 100µl of substrate solution per well
20. Cover plate and incubate for 30 and/or minutes. Handle plate carefully to avoid splashing
21. Read visually or at 405-411nm. Visually green color indicates positive for circum sporozoite proteins. Quantitatively, samples which have OD values above the cut-off (cut-off = 2 x mean OD of negative controls.

Appendix 5. Mosquito collection and Laboratory forms

i. Mosquito Collection and Species Identification Record Form (for HLC)

Country: _____ Region: _____ District: _____Study site: _____ House No. _____ Lat._____ Long. _____

Elevation _____m House sprayed: Yes ☐ No☐, If sprayed last spray Date: _____ (DD/MM/YY) Insecticide sprayed: _____ ITN use: Yes ☐ No☐ If yes number of ITN used _____ Family size: _____ No human baits used: In _____ Out _____ Date of collection: _____ (DD/MM/YY)

Collection time	Rainfall (mm), Relative humidity (%) and Temperature (°C)					*An. gambiae s.l*		*An. pharoensis*		*An. coustani*		An.		An		An		Hourly anopheline total		Hourly Culicine total	
	Rain fall	Indoor		Outdoor		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
		RH (%)	Temp	RH (%)	Temp																
6-7pm																					
7-8																					
8-9																					
9-10																					
10-11																					
11-12																					
12-1am																					
1-2																					
2-3																					
3-4																					
4-5																					
5-6																					
Total																					

ii. Mosquito Collection and Species Identification Record Form

(CDC light trap, HDNT, HBLT, PSC and Pit shelter)

Country: _____ Region: _____ District: _____Study site: _____ Area sprayed: Yes☐ No☐ , If sprayed last spray date: _____ (DD/MM/YY) Insecticide sprayed: _____ Date of collection: _____ (DD/MM/YY)

House No. (Name of household head)	House type*	Wall type**	Spray status***	# of LLIN	Family size	# of Domestic animals					Method of collection	Place (in/out)	Mosquito species****	Total collected	Females (Feeding status)				Males
						Cows	Sheep	Goat	Donkey	Chicken					Unfed	Fed	Half gravid	Gravid	

**Rectangular with corrugated iron roof (a), Rectangular thatched roof (b), Tukul with thatched roof (c), Tukul with iron roof (d), other (specify)*

***Mud (1), cement (2), thatched (3), other (specify). ***House sprayed (1), House not sprayed (0)*

*****For each house, list Anopheline species (morphologically identified), Culicines or none collected*

Key: *HDNT: Human-baited double net trap (Bed net trap), HBLT: Human-odour-baited CDC light trap (pipe trap)*

iii. PCR Template

Date: _____

PCR Run No._____ Purpose: _____

Samples:

1	**9**	**17**	**25**	**33**	**41**	**49**	**57**	**65**	**73**	**81**	**89**
2	**10**	**18**	**26**	**34**	**42**	**50**	**58**	**66**	**74**	**82**	**90**
3	**11**	**19**	**27**	**35**	**43**	**51**	**59**	**67**	**75**	**83**	**91**
4	**12**	**20**	**28**	**36**	**44**	**52**	**60**	**68**	**76**	**84**	**92**
5	**13**	**21**	**29**	**37**	**45**	**53**	**61**	**69**	**77**	**85**	**93**
6	**14**	**22**	**30**	**38**	**46**	**54**	**62**	**70**	**78**	**86**	**94**
7	**15**	**23**	**31**	**39**	**47**	**55**	**63**	**71**	**79**	**87**	**95**
8	**16**	**24**	**32**	**40**	**48**	**56**	**64**	**72**	**80**	**88**	**96**

iv. Gel setup form

Gel Setup form

Date: _____

Test: _____

Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Sample ID														
Result														

Performed by: _____ Sign. _____

Gel Setup form

Date: _____

Test: _____

Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Sample ID														
Result														

Performed by: _____ Sign. _____

v. ELISA Template

Name of the Research Center (Lab): _____

Mosquito species:_____

Assay Type: _____(Blood meal/Sporozoite) **Plate #:_____**

Name of Technician/s: _____ **Date: _____**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Note_____

DECLARATION

I, the undersigned, declared that this dissertation is my bona fide original work, has never been presented in this or any other University, and that all the resources and materials used for this thesis have been fully acknowledged.

Name of the PhD Candidtate: Teshome Degefa Demie

Signature: 

Date: 02/08/2021

Place: Jimma University, Jimma, Ethiopia

Date of Submission: 02/08/2021

This dissertation has been submitted for examination with my approval as:

Candidate's first Promotor (supervisor)

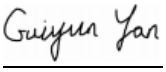
Name: Professor Delenasaw Yewhalaw

Signature: 

Date: 02/08/2021

Candidate's second Promotor (supervisor)

Name: Professor Guiyun Yan

Signature: 

Date: 02/08/2021

Candidate's third Promotor (supervisor)

Name: Dr. Andrew K. Githeko

Signature: 

Date: 02/08/2021

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KEMRI/RES/7/3/1 **July 10, 2015**

TO: **DR. ANDREW GITHEKO,**
PRINCIPAL INVESTIGATOR

THROUGH: **THE STEPHEN MUNGA,**
THE DIRECTOR, CGHR,
KISUMU

14/7/2015

Dear Sir,

RE: SSC PROTOCOL NO. 3005 (*RESUBMISSION 2 OF INITIAL SUBMISSION*): ECOLOGY AND POPULATION GENETICS OF AFRICAN HIGHLAND MALARIA (*VERSION 1.3 DATED MAY 20TH, 2015*)

Reference is made to your letter dated 26th June 2015 and received at the KEMRI Scientific and Ethics Review Unit on 2nd July 2015.

This is to inform you that the Committee notes that the issues raised at the 238th meeting of the KEMRI ERC held on 21st April, 2015 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this **10th July 2015** for a period of one year. Please note that authorization to conduct this study will automatically expire on **July 9, 2016.**

If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **May 28, 2016.**
You are required to submit any proposed changes to this study to the SERU for review and the changes should not be initiated until written approval from the SERU is received.

Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of the SERU and you should advise the SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

PROF. ELIZABETH BUKUSI,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT (SERU)

KENYA MEDICAL RESEARCH INSTITUTE

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E-mail: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 **March 08, 2017**

TO: **TESHOME DEGEFA,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. STEPHEN MUNGA,**
THE DIRECTOR, CGHR,
KISUMU

Dear Sir,

RE: PROTOCOL NO. KEMRI/SERU/CGHR/0057/3363 (*RESUBMISSION2 OF INITIAL SUBMISSION*): DEVELOPMENT AND EVALUATION OF A NOVEL HUMAN-ODOR-BAITED CDC LIGHT TRAP FOR OUTDOOR HOST-SEEKING MALARIA VECTOR SURVEILLANCE IN WESTERN KENYA_ (*VERSION 1.2 DATED FEBRUARY 26, 2017*)

Reference is made to your letter dated 27th February, 2017. The KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on the 1st March, 2017.

This is to inform you that the Committee notes that the issues raised during the 256th Committee B meeting of the KEMRI/SERU held on **19th October, 2016** have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **8th March, 2017** for a period of one year. Please note that authorization to conduct this study will automatically expire on **7th March, 2018**. If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **25th January, 2018**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

DR. EVANS AMUKOYE,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

JIMMA UNIVERSITY
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ቁ/Ref.No JUIRB/2075/18
ቀን/Date 07/01/2018

Institutional Review Board (IRB)
Institute of Health
Jimma University
Tel: +251471120945
E-mail: zeleke.mekonnen@ju.edu.et

To: Mr. Teshome Degefa

Subject: Ethical approval of research protocol

The IRB of institute of health has reviewed your research project entitled:

"Monitoring and Surveillance of Residual Malaria Transmission in Western Kenya and Southwestern Ethiopia"

This is to notify that this research protocol as presented to the IRB meets the ethical and scientific standards outlined in national and international guidelines. Hence, we are pleased to inform you that your protocol is ethically cleared.

We strongly recommended that any significant deviation from the methodological details indicated in the approved protocol must be communicated to the IRB before they are implemented.

With regards!

Zeleke Mekonnen (PhD)
Associate Professor, Health
Research and Postgraduate
Director

Institute of Health
Jimma University