SPATIOTEMPORAL VARIATION IN *Plasmodium falciparum* TRANSMISSION IN SELECTED SITES IN WESTERN KENYA

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DECLARATION AND RECOMMENDATION

Declaration:

This thesis is my original work and has not been presented in this University or any other for the award of a degree.

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DEDICATION

I dedicate this work to the memory of my beloved parents, the late Elsa Adek Nyotata and the late Sospeter Oduma Mirowa, and my uncle the late Christopher Odero Otata, who together inspired me to always aim higher and be committed to my studies. Further, this work is dedicated to my siblings Raymond Oduma, Naomy Morphy, Quinter Auma and Benard Suleiman who gave me moral support throughout this journey.

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ABSTRACT

Asymptomatic malaria infections are a threat to elimination of this vector-borne infectious disease. In many endemic regions, malaria transmission is seasonal. However, the impact of seasonality on *Plasmodium falciparum* (*P. falciparum*) gametocyte levels in peripheral blood and their transmission to local mosquito vectors are not well understood. Data describing these parasitological indices across regions of varying transmission intensity is scanty. In addition, malaria transmission can vary significantly over small geographic scales, but the drivers of this heterogeneity are not well understood. This study evaluated the impact of seasonality on *P. falciparum* transmission potential, trends in parasitological indices across areas of differential malaria transmission, and factors that might correlate small scale variation in transmission. Blood samples were collected from individuals living in Homa Bay County (low transmission) and Kisumu County (moderate transmission) in the dry season (n=1116) and rainy season (n=1743). In addition, blood samples were collected from approximately 150 individuals in each of 20 clusters in Busia County (high transmission) in rainy season. Blood samples were screened for *P. falciparum* parasites using quantitative polymerase reaction (qPCR) and microscopy. In Homa Bay and Kisumu the presence and density of blood gametocytes was measured by reverse transcription PCR (RT-qPCR). Differences in parasite and gametocyte densities across seasons were determined by unpaired *t-test*. Differences in the prevalence, proportion of submicroscopic and gametocyte positive infections across study sites were determined by χ^2 *test*. A generalized linear mixed effect model was used to determine predictors of infections. Potential mosquito larval habitats and their number within 250 m of a household were determined by ArcMap. In Homa Bay and Kisumu, mean parasite densities did not differ in dry versus rainy season (P=0.562). Gametocyte densities were 3-fold higher in the rainy than dry season (rainy: 3.46 transcripts/uL blood, dry: 1.05 transcripts/uL, P<0.001). Parasite prevalence and densities, and gametocyte prevalence and densities were highest in the high transmission region. In contrast, the proportion of asymptomatic submicroscopic infections was highest in the low transmission region. Proportion of gametocyte positive infections did not differ across transmission intensities. In Busia County, across the 20 clusters, 3-folds and 4-folds variation in parasites prevalence by qPCR and microscopy respectively was observed. Three to 34 larval habitats per cluster, and 0-15 habitats within a 250m radius around households were observed. Low altitude, kitchen located indoors, open eaves, a lower level of education of the household head, younger age, and being male were significant predictors of higher prevalence. The number of habitats and their proximity to households was not a predictor for prevalence. In conclusion, parasites increase their investment in transmission in the rainy season, reflected by higher gametocyte densities. Seasonal changes of gametocytemia among infections need to be considered when designing malaria control measures. Pronounced variation in prevalence at small scales and the determinants need to be considered for malaria surveillance and control.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACT	Artemisinin combination therapy
ANOVA	Analysis of variance
AP	Artemesinin piperaquine
Ap2-g	Apetala-2 gene
DAHP	Dihydroartemisinin-piperaquine
DDT	Dichloro-diphenyl trichloroethane
DNA	Deoxyribonucleic acid
dPCR	Digital polymerase chain reaction
EDTA	Ethylene diamine tetraacetic acid
EIR	Entomological inoculation rate
ELISA	Enzyme-linked immunosorbent assay
FDR	False discover rate
FSAT	Focal screen and treat
GDV1	Gametocyte development protein 1
GLLM	Generalized linear mixed effect model
GPS	Global positioning system
HBR	Human biting rate
Hda2	Histone deacetylase 2
HP1	Heterochromatin protein 1
HRP2	Histidine rich protein 2
IPTp	Intermittent preventive treatment of malaria in pregnant women
IRS	Indoor residual spraying
ITN	Insecticide treated net
KEMRI	Kenya medical research institute
LDH	Lactase dehydrogenase
LM	Light microscopy
LysoPC	Lysophosphatidylcholine
MDA	Mass drug administration
MDM	Magnetic deposition microscopy
MOR	Median odds ratio

mRNA	messenger ribonucleic acid
MSAT	Mass screen and treat
NASBA	Nucleic acid sequence-based amplification
OR	Odds ratio
P. falciparum	*Plasmodium falciparum*
P. knowlesi	*Plasmodium knowlesi*
P. malariae	*Plasmodium malariae*
P. ovale	*Plasmodium ovale*
P. vivax	*Plasmodium vivax*
PBS	Phosphate buffered saline
PCDT	Passive case detection and treatment
PCR	Polymerase chain reaction
*p*LDH	*Plasmodium* specific lactase dehydrogenase
qPCR	Quantitative polymerase chain reaction
RACD	Reactive case detection
RBC	Red blood cell
RDT	Rapid diagnostic test
RNA	Ribonucleic acid
RT-qPCR	Reverse transcription quantitative PCR
SIR	Sporozoite infection rate
SMC	Seasonal malaria chemoprevention
SP	Sulfadoxine pyrimethamine
UNESCO	United Nations Educational Scientific and Cultural Organization
varATS	*var* gene acidic terminal sequence
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background information

Malaria prevention and control measures have progressed globally over time (Desai *et al*., 2014; Liu *et al*., 2021; Mbacham *et al*., 2019; Nkumama *et al*., 2017). Despite this, malaria remains a major public health concern and is among top contributors to infectious disease morbidity and mortality in tropical and subtropical regions of the world. According to World Health Organization (WHO, 2021) there were about 241 million malaria cases globally with approximately 627,000 recorded malaria mortalities in 2020. Over 90% of malaria cases and deaths occur in Africa. In Kenya, an estimated three-quarters of the population are at risk of malaria infection (WHO, 2021). The distribution of the infections is heterogeneous i.e., some regions experience moderate to high rates of infection while others have low to no infections. Residents of western Kenya experience the highest rate of infection, morbidity and mortality in the country (Alegana *et al*., 2021; Gopal *et al*., 2019; Imbahale *et al*., 2012; Kapesa *et al*., 2018).

Since 2015, progress towards malaria elimination has stalled, and the impact of control efforts reversed in some regions in the recent past (Liu *et al*., 2021; Mbacham *et al*., 2019; Were *et al*., 2019). Malaria elimination efforts are threatened by a combination of factors. *Plasmodium* parasites that are resistant to antimalarial drugs have been reported in multiple countries including neighboring Uganda, Rwanda among others (Adoke *et al*., 2021; Huijben *et al*., 2020; Menard & Dondorp, 2017; Moriarty *et al*., 2021; Rasmussen *et al*., 2017). Also, there are reports of mosquito vectors that are resistant to insecticides (Omoke *et al*., 2021; Orondo *et al*., 2021; Yared *et al*., 2020; Yovogan *et al*., 2021). Mosquitoes that bite and rest oudoor and change their biting time have been observed (Bamou *et al*., 2021; Degefa *et al*., 2017, 2021; Musiba *et al*., 2022; Sherrard-Smith *et al*., 2019). This plasticity in behavior of mosquitoes enables them escape interventions such as bed net use, thus rendering such vector control interventions suboptimal or ineffective. Multiple studies confirm presence of parasites lacking histidine rich protein-2 (*hrp2)* gene (Agaba *et al*., 2020; Kong *et al*., 2021; Mihreteab *et al*., 2021; Vera-Arias *et al*., 2022). These parasites fail to be detected by HRP2-based rapid diagnostic tests (RDTs), resulting in false negatives. Individuals harboring such parasites become important reservoirs of transmission since they are

not treated. Also, there is transmission stemming from asymptomatic malaria reservoirs, and low-density parasite infections that are not detected using field-deployable diagnostics such as microscopy or RDTs, yet are capable of infecting mosquitoes (Bousema *et al.*, 2014; Cheaveau *et al.*, 2019a; Prusty *et al*., 2021).

A mosquito blood meal needs to contain at least one female and one male gametocyte to be infective (Paul *et al*., 2000; Reece *et al*., 2008; Soumare *et al*., 2021; Talman *et al*., 2020). Over the course of the intraerythrocytic cell cycle, while a large proportion of *P. falciparum* ring-stage parasites are committed to the development of asexual parasites (Talman *et al*., 2004; Venugopal *et al*., 2020) , a small proportion *P. falciparum* rings are committed to develop into gametocytes, the sexual form of the parasite (Alano, 2014; Sinden, 1983), a phenomenon refered to as sexual conversion. The gametocytes are taken up by mosquitoes during a blood meal. The ingested gametocytes develop into oocysts and after approximately two weeks, into sporozoites that are transmitted to the vertebrate human host through a bite of an infective female anopheles mosquito (Bruce *et al*., 1990). Gamecytogenesis takes place in extravascular sites in the bone marrow (Talman *et al*., 2004; Venugopal *et al*., 2020). Red blood cells containing mature stage IV to V gametocytes are released from the bone marrow into the peripheral circulation after approximately 10-12 days (Eichner *et al.*, 2001; Farfour *et al*., 2012; Paul *et al*., 2000). Thus, gametocytes are rarely detected in peripheral blood during the first two weeks following sporozoite inoculation.

Gametocyte density in the blood is governed by the conversion rate, i.e., the proportion of early ring stage parasites committed to sexual vs. asexual replication. Changes in the density of mature gametocytes could be achieved through different strategies, e.g., a change of the conversion rate, achieving a higher density of asexual parasites before genetic commitment to gametocyte development, prolonged circulation of mature gametocytes, or a combination of these factors (Greischar *et al*., 2016). In all cases, a higher density of gametocytes is expected to increase malaria transmission if vectors are present. On the other hand, the investment in gametocytes is lost if gametocytes are not taken up by mosquitoes.

Parasite investment in transmission i.e., ‘decision’ to either prioritize gametocyte production or asexual replication in the blood, have been shown to be induced by internal stressors including

antimalarial drug treatment (Barnes *et al*., 2008; Portugaliza *et al*., 2020; Rajapandi, 2019), clinical symptom(s) e.g., fever (Barry *et al.*, 2021; Usui *et al*., 2019) and host anemia (Birget *et al*., 2017), or external stressors including spatial (geographical) changes in transmission intensities (Mobegi *et al.*, 2014; Rono *et al*., 2018), and changes in transmission intensity following successful implementation of interventions (Maude *et al*., 2014; Parobek *et al*., 2016). However, the impact of seasonality on the parasite investment in transmission has not yet been investigated.

"Asymptomatic" malaria reservoirs (i.e., widely defined as individuals with detectable blood stage parasitemia without clinical symptoms, e.g., primarily fever within 48 hours before diagnosis, and have not received treatment with antimalarial drugs in the past week) are prevalent in communities in all transmission settings. Malaria transmission stemming from asymptomatic individuals has gained attention as an increasing number of countries aim for malaria elimination. Asymptomatic cases serve as infectious reservoirs that support ongoing transmission through local mosquito vectors (Debash *et al*., 2023; Ibrahim *et al*., 2023), yet they are neither diagnosed through passive screening that involves checking for clinical malaria cases among those presenting to health facilities nor treated. Hence, such individuls represent a major obstacle to malaria elimination (Galatas *et al*., 2016; Prusty *et al*., 2021; Zhang & Deitsch, 2022). A recent study in western Kenya estimated that 95% of transmission was due to asymptomatic carriers (Sumner *et al.*, 2021). Mosquito infectivity studies in multiple countries corroborated the notion that asymptomatic infected individuals are significant drivers of transmission in the community (Barry *et al.*, 2021; Chaumeau *et al.*, 2019; Gouagna *et al*., 2004; Tadesse *et al.*, 2018).

Also, an interaction between *Plasmodium* parasite, human and animal hosts, and mosquito in their environment may result into varying outcomes including asymptomatic *Plasmodium* infection, symptomatic malaria disease, severe malaria or even death. Severe malaria could manifest as metabolic acidosis, severe anemia or cerebral malaria (Cottrell *et al*., 2015; Maketa *et al*., 2015; Matangila *et al*., 2014). In all the scenerios, the infected individual's health becomes adversely affected. If not clinically managed and cleared, some of the asymptomatic infections progress to symptomatic malaria, or to severe malaria (Lindblade *et al*., 2013; Njama-Meya *et al*., 2004). Equally, there is risk of hospitalization for symptomatic disease or sever malaria. This highlights

the need to develop complementary programs aimed at identifying and treating asymptomatic malaria reservoirs.

Untreated infections can persist for several months unless interrupted by host immune responses (Moormann *et al*., 2013; Nassir *et al*., 2005; Rodriguez-Barraquer *et al*., 2018). During this time, parasite densities fluctuate and are often below the limit of detection by microscopy or malaria rapid diagnostic tests (RDTs) (Björkman, 2018; Bousema *et al*., 2014). Gametocytes may continue to circulate for up to 2-3 weeks after antimalarial treatment (Bousema *et al.*, 2010; Omondi *et al.*, 2019; Roth *et al*., 2018), and their chances of being infectious is increased (Portugaliza *et al*., 2022). Gametocyte densities can be used to predict the infectiousness of humans to mosquitoes (Churcher *et al*., 2013; Gonçalves *et al*., 2017; Ouédraogo *et al*., 2009) and thus to evaluate the effect of interventions that aim at reducing transmission (Da *et al*., 2015; Kosasih *et al*., 2021).

Most malaria interventional tools, such as bed nets and indoor residual spraying (IRS), were developed and tested to reduce the number of clinical cases (Alhassan *et al.*, 2022; Hamel *et al*., 2011; McCann *et al*., 2020; West *et al*., 2014). Their impact on asymptomatic infections is poorly understood. Approaches to specifically identify and/or treat *Plasmodium* parasite infections in the community, such as focal screen-and-treat (Conner *et al.*, 2020), mass screen-and-treat (Kim *et al*., 2021), seasonal chemoprevention (Cairns *et al*., 2015, 2021), reactive case detection (Jaiteh *et al.*, 2021; Stuck *et al*., 2020; Sturrock *et al.*, 2013), mass drug administration (Deng *et al*., 2018; Kyaw *et al*., 2021; Li *et al*., 2021), or combinations thereof (Hsiang *et al.*, 2020) are increasingly being trialed or implemented. These approaches require data on malaria prevalence and risk factors of infection to inform on their best usage to reduce transmission in the community.

In parallel to interventions aimed at reducing the asymptomatic reservoir, vector control is key to reduction of malaria transmission. Knowledge on how mosquito larval habitats occurrence impact malaria transmission would help to design integrated vector control interventions. Mosquito larval habitats are key drivers of adult vector populations (Gimnig *et al.*, 2001, 2002; Paaijmans *et al*., 2008). In western Kenya, the dominant vectors are *An. gambiae* sensu stricto (s.s), *An. arabiensis* and *An. funestus* (Githinji *et al*., 2020; Machani *et al*., 2020; Minakawa *et al*., 2012). Some areas in western Kenya experience perennial malaria transmission with peaks in vector density and

transmission occurring during and shortly after the rainy season. A slight variation in this pattern across sub-regions of western Kenya is often observed. In general, seasonal heavy rains in western Kenya often occur between April – June, and lighter rains in October – November (Kipruto *et al*., 2017; Machani *et al*., 2020; Otambo *et al*., 2022).

In many settings with pronounced seasonality in rainfall, *Anopheles* mosquitoes are sparse in the dry season as opposed to the rainy season when they are plentiful, resulting in transmission primarily occurring during and shortly after the rainy season (Hamad *et al*., 2002; Huestis & Lehmann, 2014; Jawara *et al*., 2008; Ouédraogo *et al.*, 2008). It is not known whether *P. falciparum* adapts its transmission potential to changes in vector abundance across seasons. Adaptations to increase transmission potential when chances for onward transmission are high could maximize the fitness of the parasite population. Understanding such adaptations are crucial when introducing transmission-reducing interventions.

Small-scale heterogeneity in malaria prevalence has long been observed (Amratia *et al*., 2019; Baidjoe *et al*., 2016; Bannister-Tyrrell *et al*., 2018; Githeko *et al*., 2006; Gul *et al*., 2021; Kangoye *et al*., 2016; Zhou *et al.*, 2021). However, the drivers of such heterogeneity are not well understood. Understanding where infection prevalence and transmission potential are higher is needed to develop targeted control interventions. In the East African highlands, significant reductions in the proportion of *P. falciparum* infected mosquitoes or *Anopheles* vector abundance were observed with increasing altitude spanning a large range of 600-1400m difference (Attenborough *et al*., 1997; Bødker *et al*., 2003; Daygena *et al*., 2017; Eyong *et al*., 2016). Data from health facilities show a decline in the number of clinical malaria cases with increasing altitudinal transects (Reyburn *et al*., 2005; Siya *et al*., 2020). The extent of this decline, and whether similar changes in asymptomatic prevalence occur, are not well understood. Likewise, little data is available on whether those changes directly reflect altitude and are caused by temperature gradients, or whether differences in land use and larval habitats are the main drivers.

Over the past two decades, control programs have resulted in 88% reduction in malaria at the national level (Macharia *et al*., 2018). However, the marked reduction in prevalence nationally is not experienced locally in western Kenya (Were *et al*., 2019), where malaria transmission is

substantially higher (Alegana *et al*., 2021; Bashir *et al*., 2019; Zhou *et al*., 2011). This necessitates understanding factors that continue to drive transmission in this region to help design measures to decrease malaria transmission.

In the current study, three malaria endemic sites with considerable varying malaria transmission intensities were selected. Specifically, Teso South in Busia County (high transmission), Chulaimbo in Kisumu County (moderate to high transmission) and Kimira –Oluch in Homa Bay County (low transmission) were selected. Knowledge gain on how the relative abundance of asymptomatic malaria reservoirs, the proportion of infected population not captured during routine malaria surveillance, and the proportion of infected individuals harboring gametocytes, might differ across areas of varying transmission intensity is useful to inform on targeted control measures.

This thesis aimed to understand whether malaria transmission potential varies across seasons. Also, the relative abundance of asymptomatic malaria reservoir, proportion of submicroscopic infections, and proportion of infected individuals harboring gametocytes across areas of differing transmission intensity were compared. In addition, this work investigated whether the number and proximity of larval habitats factors, and individual-, or household- characteristics impact transmission.

1.2 Statement of the problem

Malaria poses a huge economic, social and health burden to the world population. Recent data show that the disease is responsible for about 627,000 deaths and about 241 million cases per year globally. Africa accounts for 94% of the cases and deaths. An estimated three-quarters of the population of Kenya are at risk of malaria infection, and over 3.5 million cases and 10,000 deaths were registered in the recent past. In Kenya, malaria disease burden is highest in western Kenya region. Despite continued implementation of interventions and strategies to prevent malaria transmission, little reduction in malaria cases in this region has been realized. This suggest that the interventions may not be optimally effective. Failure to understand the drivers of malaria transmission during transmission period (i.e., rainy season) leads to suboptimal application of control programs. Also, small scale geographic variation in malaria infection risk might reduce effectiveness of interventions applied at wider geographical scale. Progress towards malaria

elimination has been hampered by asymptomatic malaria reservoirs that sustain transmission cycle in the community.

1.3 Objectives

1.3.1 General Objective

To investigate temporal and spatial trends in malaria transmission and drivers of malaria transmission among asymptomatic malaria reservoirs in endemic regions of western Kenya.

1.3.2 Specific Objectives

i. To investigate changes in *Plasmodium falciparum* parasite transmission potential through quantification of gametocytes in response to seasonality in individuals from malaria endemic regions of differing transmission intensity in western Kenya.
ii. To identify *Plasmodium falciparum* asexual parasite and gametocyte reservoirs across a range of malaria transmission intensities in western Kenya.
iii. To explore small scale geographic variation in malaria transmission risk across altitudinal transects and factors explaining the variations in malaria transmission in western Kenya.

1.4 Hypotheses

i. There is no significant difference in *Plasmodium falciparum* parasite transmission potential in response to seasonality in individuals from malaria endemic regions of differing transmission intensity in western Kenya.
ii. There is no difference in the magnitude of *Plasmodium falciparum* asexual parasite and gametocyte reservoirs across a range of malaria transmission intensities in western Kenya.
iii. There is no significant difference in determinants of malaria transmission risk across altitudinal transects in western Kenya.

1.5 Justification

Despite the gains made in reducing clinical cases of malaria, transmission stemming from asymptomatic infections is increasingly being recognized as a threat to malaria elimination (Chen *et al*., 2016; Hassanpour *et al*., 2017). The temporal persistence of asymptomatic reservoirs offers the parasite and vector combination an opportunity to maximize transmission potential. In addition, there is evidence of an increased risk for symptomatic malaria upon subsequent infection

among previously asymptomatic individuals (Sumner *et al*., 2021). Apart from being drivers of local transmission, if untreated, the infections can lead to adverse health outcomes including anemia, premature birth and malnutrition (Cottrell *et al*., 2015; Maketa *et al*., 2015; Matangila *et al*., 2014). Moreover, infection densities may progress to higher levels (Nguyen *et al*., 2018) and thereby result in adverse health outcomes.

Understanding gametocyte densities in the human host is essential to quantify the contribution of the subclinical infectious reservoirs to transmission (Bradley *et al.*, 2018; Churcher *et al.*, 2013). Increasing gametocyte production over asexual parasite densities during the rainy season will result in higher transmission. Understanding the impact of seasonality on gametocyte densities versus asexual parasite densities trade-off will help design control measures to reduce transmission. Also, knowledge gain on the impact of seasonality on parasite transmission potential across regions of differing transmission intensity will inform on application of the findings across the transmission spectrum.

Data on parasite and gametocyte reservoirs e.g parasite prevalence, the proportion of gametocyte positive infections, and the proportion of submicroscopic infections help to identify population at risk of infection, to guide deployment of targeted programs including mass drug administration, and focal screen-and-treat. The parasitological parameters were compared across areas of varying transmission intensity for detailed understanding of malaria transmission dynamics across range of epidemiological environments.

Small scale variation in geographic features such as proximity of households to larval breeding sites and altitude may impact human malaria infection rates (Atieli *et al*., 2011; Kabaghe *et al*., 2018; Rumisha *et al*., 2014). The variation represents a challenge to effective malaria control and, ultimately, elimination. In areas where malaria transmission intensity differs over small scales, control and elimination strategies applied at general level may not achieve optimal utility or effectiveness (Gari & Lindtjørn, 2018; Hemingway *et al.*, 2016; Kaehler *et al*., 2019; Tizifa *et al*., 2018). Thus, understanding the factors contributing to small scale variation in malaria prevalence are critical for designing programs tailored to the local environment.

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology and burden of malaria

There are many *Plasmodium* species that infect vertebrate animals, but only five species i.e., *Plasmodium falciparum* (*P. falciparum)*, *Plasmodium malariae* (*P. malaraie*), *Plasmodium vivax* (*P. vivax*), *Plasmodium ovale* (*P. ovale*) and *Plasmodium knowlesi* (*P. knowlesi*) are known to cause malaria in humans. Globally, , *P. falciparum* and *P. vivax* are the primary cause of blood stage infection in humans and account for the most morbidity and mortality due to *Plasmodum* infection (Bousema & Drakeley, 2011; Howes *et al*., 2016; Nkumama *et al*., 2017). *P. falciparum* is responsible for majority of severe clinical malaria syndromes (Snow *et al*., 2005) that are, in part, due to erythrocyte cytoadherence to vascular endothelium resulting in organ dysfunction (Plewes *et al*., 2018). In sub-Saharan Africa, *P. falciparum* is the dominant malaria species (Guerra *et al*., 2008; Snow *et al*., 2017). Notably, there have been an increasing number of reports of *P. vivax* (Howes *et al*., 2015; Liu *et al*., 2014; Poirier *et al*., 2016; Prugnolle *et al*., 2013) and *P. malariae* in the region (Hawadak *et al*., 2021; Oboh *et al*., 2020; Twohig *et al*., 2019). In East Africa, *P. falciparum* is the main malaria parasite (Akala *et al*., 2021; Alegana *et al*., 2021).

Anopheles gambiae (An. gambiae), *Anopheles funestus (An. funestus)*, and *Anopheles arabienses* (*An. arabiensis*) are the major mosquito vectors sustaining malaria transmission in East Africa (Kabbale *et al*., 2016; Kaindoa *et al*., 2019; Keating *et al*., 2005; Kweka *et al*., 2020; Morgan *et al.*, 2010). *An. gambiae* and *An. funestus* largely bite and rest indoor (Doucoure *et al*., 2020; Ototo *et al*., 2015; Sinka *et al*., 2010; Sougoufara *et al*., 2014) while *An. arabiensis* bite and rest outdoor (Govella *et al*., 2023; Mlacha *et al*., 2020; Moiroux *et al*., 2012; Salomé *et al*., 2023). However, the vectors have been shown to alter their biting time and place of resting in response to external cues (Mathania *et al*., 2020; Milali *et al*., 2017; Mponzi *et al*., 2022).

Malaria remains a major public health concern. Approximately 50% of the world's population is at risk of malaria. In 2021, there were about 241 million cases of malaria globally with 627,000 malaria-related deaths. Malaria is endemic in areas of Central and South America, West and Central Africa, Asia, Eastern Europe, and the South Pacific. The majority of cases, over 94%,

occur in tropical and subtropical areas of the world, with Sub-Saharan Africa bearing greatest brunt of the disease (Hay *et al*., 2010; WHO, 2021). Clinical malaria incidence and mortality is highest in children younger than 5 years compared to other age groups (Dao *et al*., 2021; Roberts & Matthews, 2016; Sarfo *et al*., 2023; Workineh *et al*., 2021). This age group accounts for 80% of malaria deaths in sub-Saharan Africa. The higher incidence in children is due to lack of naturally acquired immunity that requires repeated malaria exposures that occur with increasing age (Biswas *et al*., 2008; Travassos *et al*., 2018). Other high-risk populations for severe malaria include pregnant (Ai *et al*., 2022; Eijk *et al*., 2015) and malaria niave travelers from regions of the world where malaria is not endemics (Bunn *et al*., 2004; Hahn & Pottinger, 2016; Marasinghe *et al*., 2020; Massad *et al*., 2009).

The malaria burden is disproportionately high in the sub-Saharan Africa region (Chilot *et al*., 2023; Kamau *et al.*, 2020; Leal *et al.*, 2023) due to a combination of factors that include i) the abundance of highly efficient competent mosquito vectors such as *An. gambiae*, *An. arabiensis* and *An. funestus* (Akpan *et al.*, 2019; Qureshi & Connolly, 2021; Sougoufara *et al*., 2017); ii) the predominant *P. falciparum and P. vivax* that are responsible for majority of uncomplicated and severe malaria cases and deaths (Howes *et al.*, 2016; Nkumama *et al*., 2017); iii) the local weather conditions that favor mosquito breeding (Akpan *et al*., 2019; Thomson *et al*., 2018) with year-round transmission; and iv) limited financial and health resources in many countries that impede efforts for optimal national malaria control programs (Head *et al*., 2017; Snow *et al*., 2010)**.**

Over the recent years, over 3.5 million new clinical cases of malaria and over 10,000 deaths have been reported annually in Kenya. An estimated over 70% of the population in Kenya are at risk of malaria infection (WHO, 2021). The malaria infection burden is heterogeneous i.e., some regions have higher prevalence of infcetions compared to others. According to Kenya Malaria Indicator survey, malaria transmission is classified into four epidemiological zones, namely: highland epidemic prone areas, endemic areas, semi-arid and seasonal malaria transmission areas, and low risk malaria areas. The highest malaria burden and at-risk population is found in the Lake Victoria endemic region in western Kenya (Alegana *et al*., 2021; Bashir *et al.*, 2019; Zhou *et al*., 2011). The western Kenya region is administratively made up of seven counties such as Busia, Kisumu and Homa Bay counties among others.

Despite continued deployment of various control and prevention programs including treatment options, bed net use, and vector density control measures such as indoor residual spraying, progress to eliminate malaria have been slow in many high malaria burden African countries (Liu *et al*., 2021). Over the period 2000-2019, global malaria cases dropped by 29%, but only by 2% in the period 2015-2019. Deaths dropped by 60% over the period 2000-2015, and only by 15% in the period 2015-2019 (Cibulskis *et al*., 2016; WHO, 2021). Complementary or alternative strategies are needed to reinvigorate existing control and prevention programs to eliminate malaria (Balakrishnan, 2022; Gari & Lindtjørn, 2018; Hemingway *et al*., 2016; Kaehler *et al*., 2019; Moonen *et al*., 2010).

2.2 Diagnosis of Malaria

Multiple approaches are available for the diagnosis of malaria. Broadly, *P. falciparum* blood stage infection can result in clinical malaria or subclinical malaria. Clinical malaria diagnosis is done by examining for the presence of clinical symptoms that include fever, headache, muscle ache, loss of appetite, diarrhea, vomiting, chills, tiredness, nausea, or a combination of thereof. Clinical malaria is often diagnosed at the health facility. In most health facilities, once clinical symptoms are identified, a follow-up confirmatory test for presence of malaria parasites in a blood film, e.g., microscopy, is done, as recommended by the World Health Organization. Microscopy is the gold standard and primary choice for malaria diagnosis in health facilities (Kavanaugh *et al*., 2021; Oboh *et al.*, 2021; Tangpukdee *et al*., 2009). An alternative test, e.g., rapid diagnostic test (RDT) (Aidoo & Incardona, 2022; Cunningham *et al*., 2019; Wongsrichanalai *et al*., 2007; Yimam *et al.*, 2022), is done in the situations where there is no electricity to perform microscopy or no experienced microscopists or when a facility run out of stock for microscopy related supplies.

Subclinical malaria, the lack of clinical symptoms in the presence blood stage parasitemia, is often detected during community surveys. RDTs for *Plasmodium* parasites is the most feasible test method for diagnosis in the field (Abba *et al*., 2014; Kojom *et al*., 2021). Laboratory-based methods, i.e., microscopy and molecular techniques such as polymerase chain reaction (PCR) are often used to screen for the parasites. Microscopy involves visualization of Giemsa-stained *Plasmodium* parasites in a blood smear. RDT involves visualization of *Plasmodium* parasite species specific protein antigens e.g histidine rich protein 2 (HRP2) or lactase dehydrogenase

(pLDH) or aldolase that are represented by a band on a test strip. HRP2 protein is specific for *P. falciparum* (Feleke *et al.*, 2021; Marquart *et al*., 2022; Noedl *et al*., 2002), while pLDH protein is expressed by all malaria causing *Plasmodium* parasite species (Barney *et al*., 2022; Brown *et al*., 2004; Iqbal *et al*., 2004; Simpalipan *et al*., 2018). With the advent of new technologies, *Plasmodium* species specific multiple bands RDTs that distinguishes between or among the species are now available for use in diagnosis (Ashton *et al*., 2010; Yerlikaya *et al*., 2018).

The use of RDTs to screen for the *P. falciparum* parasite in countries with ongoing transmission is threatened by increasing numbers of parasites with *hrp2* gene deletions being reported in multiple countries (Beshir *et al.*, 2017; Gupta *et al*., 2017; Kumar *et al*., 2013; Li *et al*., 2015; McCaffery *et al*., 2021; Oreh *et al*., 2022; Pati *et al.*, 2018; Thomson *et al*., 2019; Vera-Arias *et al*., 2022). The *hrp2* gene deletion affects the performance of malaria HRP2 based RDTs, resulting in missed diagnosis i.e., false negative results (Gatton *et al.*, 2020; Kumar *et al*., 2013; Oreh *et al*., 2022). Also, false negative RDT may occur due to excessive levels of either antigen or antibodies in circulation that block binding sites, a phenomenon refers to as the prozone effect (Gillet et al., 2009; Luchavez *et al*., 2011). The individuals harboring the otherwise undetected parasites are not treated and contribute to onward malaria transmission in the community. These individuals are considered among key obstacles to malaria elimination.

Similarly, false positive results have been reported from malaria RDTs use. Parasite antigens may remain in blood circulation for extended periods of time even after parasite clearance by antimalarial treatment. In such instances, RDTs give false positive results (Dalrymple *et al.*, 2018; Tiono *et al.*, 2014). Also, false positive results from malaria RDTs have been observed in persons with African trypanosomiasis or rheumatoid factor (Gatton *et al*., 2018; Unterborn *et al*., 2022).

Molecular techniques involve detection of parasite DNA using specific primers in reaction mixture (Aschar *et al.*, 2022; Lazrek *et al*., 2023; Taylor *et al.*, 2011). Detection is confirmed by the presence of a band in a gel following conventional PCR amplification, or by the presence amplification signals in real-time PCR. Other approaches for identification of malaria parasite in peripheral circulation include serology, by which circulating antibodies against parasite antigens are measured through techniques including enzyme linked immunosorbent assay (ELISA)

(Chaorattanakawee *et al*., 2013; Kwenti *et al*., 2017) or magnetic bead-based immunoassay (Rogier *et al*., 2017; Varela *et al*., 2018).

The methods used for parasite screening vary in their ability to detect the parasite in peripheral blood. PCR is the most sensitive method with lower limit of detection of approximately 0.5-5 parasite per microliter of blood (Berzosa *et al.*, 2018; Hofmann *et al*., 2015; Schoone *et al*., 2000; Wu *et al*., 2015) while microscopy and conventional RDTs have relatively similar lower limit of detection of approximately 50-100 parasites per microliter of blood (Azikiwe *et al*., 2012; Moody, 2002; Tangpukdee *et al*., 2009). Ultrasensitive RDTs with higher sensitivity in comparison with the conventional RDTs have been developed (Niyukuri *et al*., 2022; Slater *et al.*, 2019). While it is possible to identify various species of the parasite by PCR, RDT and microscopy, only PCR and microscopy can be used to quantify density of the parasite in blood.

Factors influencing detection limit of a test diagnostic include the volume of blood screened, type of sample used in detection, and properly targeted genetic material. The higher the volume of blood screened, the higher the sensitivity (Hofmann *et al*., 2015; Holzschuh & Koepfli, 2022). Sensitivity is increased by screening whole bloods compared to saliva, plasma, serum and other body fluids (Oguonu *et al*., 2014; Samal *et al*., 2017). Higher sensitivity for *P. falciparum* detection has been reported by targeting multicopy gene e.g *var* gene acidic terminal sequence (*var*ATS) compared to single copy gene e.g 18S rRNA parasite genetic material (Hofmann *et al.*, 2015).

2.3 Approaches for malaria control and prevention

Comprehensive and targeted measures related to parasite, mosquito vectors, and human behavior are being employed to prevent and control malaria.

2.3.1 Parasite control and prevention interventions

Measures related to the parasite include case management that involves malaria diagnosis and treatment. The treatment options are guided by *Plasmodium* species, history of treatment, parasite resistance or susceptibility to a drug, type of illness or clinical status of patient (e.g., severe malaria or uncomplicated malaria).

Approaches to identify and treat malaria parasite infections in the community are increasingly trialed or implemented. These include mass screen and treat (MSAT) (Conner *et al.*, 2020; Cook *et al*., 2015; Kim *et al*., 2021), focal screen and treat (FSAT) (Hsiang *et al.*, 2020; Searle *et al*., 2021), seasonal malaria chemoprevention (SMC) (Cairns *et al.*, 2021; Chotsiri *et al*., 2022; Nikiema *et al*., 2022), mass drug administration (MDA) (Deng *et al*., 2018; Eisele, 2019; Newby *et al*., 2015), reactive case detection (RACD) (Perera *et al.*, 2020; Stuck *et al*., 2020), or a combination thereof (Hsiang *et al*., 2020). Other approaches include intermittent preventive treatment of malaria in pregnant women (IPTp) (Azizi *et al*., 2018; Kakuru *et al.*, 2020; Peter, 2013; Quakyi *et al*., 2019), passive case detection and treatment (PCDT) (Dharmawardena *et al*., 2019; Rios-Zertuche *et al*., 2021; Singh *et al*., 2016; Zemene *et al*., 2018).

In MSAT strategy, individuals in the community are conveniently screened for malaria parasite. The FSAT strategy is a form of MSAT strategy where individuals within defined locality, usually targeted small geographical area, are screened for malaria parasite. Positively identified parasite carriers by either strategy are then given standard treatment for uncomplicated malaria according to National Guidelines for Malaria Treatment. In PCDT, persons are prompted by onset of clinical symptoms for malaria then present themselves to health facility to seek treatment. Treatment is administered after confirmed diagnosis. In some scenerios, the persons exhibiting malaria symptoms visit health care providers in the community e.g trained community health volunteers who give them antimalarial drugs.

SMC involves monthly administering of curative regimen during or shortly after the rainy season when transmission peaks, to children aged between 3 months to 5 years irrespective of clinical symptoms or presence/absence of infection, in areas that experience moderate-to-high but seasonal malaria transmission. Sulfadoxine-pyrimethamine (SP) in combination with amodiaquine are the recommended regimens (Ambe *et al.*, 2020; Cairns *et al*., 2021; Yaméogo *et al*., 2021).

In MDA, antimalarial drug is administered to individuals in a selective population or defined geographical locality irrespective of clinical symptom or presence/absence of infection. Usually, the treatment is repeated at intervals, and each treatment phase is done over a short time span. Dihydroartemisinin-piperaquine (DHAP) or artemisinin-piperaquine are recommended regimens

(Brady *et al*., 2017; Kagaya *et al.*, 2019; Li *et al.*, 2021; McLean *et al*., 2021). MDA is often done in near elimination, or low malaria transmission areas. In IPTp, curative dose of antimalarial drug is administered to pregnant women at predefined intervals during the pregnancy irrespective of malaria infection status. SP is the approved drug for IPTp. Also, DHAP has proven to be effective for IPTp (Andronescu *et al*., 2021; Banda *et al.*, 2022; Chotsiri *et al*., 2021; Hill *et al*., 2020).

In RACD, an index case, i.e., a passively identified patient presenting with clinical symptoms of malaria, is used as reference contact to guide on prompt tracing of other people who are likely at risk of infection. The traced persons, typically members of index case or neighboring households, are then screened, and positive cases treated according to Malaria National Treatment Guidelines of a given country. RACD is typically done within a specified radius around the index case and is mostly employed in areas where malaria transmission has substantially reduced e.g., very low-to-near elimination regions (Abdelmenan *et al*., 2022; Das *et al*., 2022; Fontoura *et al*., 2016; Hustedt *et al*., 2016; Jaiteh *et al*., 2021; Perera *et al*., 2020; Sturrock *et al*., 2013).

Clearance of sexual stage parasites, e.g., gametocytes, is achieved primarily by standard malaria treatment. Gametocyte clearance is somewhat faster with artemisinin combination therapies (ACTs) than non-ACTs (Okell et al., 2008; Zou *et al.*, 2022). Use of low-dose primaquine, tafenoquine or methylene blue in combination with primary treatment for malaria resulted in significant reduction and shorter clearance time of gametocytes compared to when a standard treatment is administered alone (Dicko *et al.*, 2018; Graves *et al*., 2018).

Also, various malaria vaccines are being developed or being trialed to measure their efficacy in reducing malaria disease and burden. Among them include “RTS, S” malaria vaccine that is administered in four doses and have demonstrated a significant efficacy among children under 5 years. A complete dose of the vaccine reduced clinical malaria cases by 39% and severe malaria by 30% (WHO, 2021). This vaccine was approved in 2021 by WHO for administration to children under 5 years in malaria endemic countries of moderate to high *P. falciparum* transmission. The vaccine targets circumsporozoite protein on the surface of sporozoite and targets the parasite before it infects liver cells (Laurens, 2020). Another vaccine, “R21”, has demonstrated substantially higher efficacy among younger children (Datoo *et al*., 2022). The vaccine also targets

sporozoite stage of the *P. falciparum* parasite. The "R21" vaccine is yet to get full approval from WHO for administration to the public.

2.3.2 Vector control interventions

The primary mosquito vector control intervention tools include insecticide treated nets (ITN) and indoor residual spraying (IRS) (Bath *et al.*, 2021; Kenea *et al*., 2019; Pinder *et al*., 2015; Protopopoff *et al*., 2018; Wagman *et al*., 2021). Both ITN and IRS target adult mosquitoes. The ITN and IRS may be implemented with interventions targeting immature stages of mosquito (larvae and/or pupae) such as larviciding (Antonio-Nkondjio *et al*., 2021; Mutero *et al*., 2020; Runge *et al*., 2021), use of biological control e.g. larvivorous fish (Das *et al.*, 2018; Howard *et al*., 2007; Lou & Zhao, 2011), habitat manipulation or habitat modification (Jacups *et al*., 2011; Martello *et al*., 2022; Muema *et al*., 2017; Tusting *et al*., 2013).

ITNs and IRS help in reducing mosquito longevity and provide protection from mosquito bites. The IRS coats the walls and surfaces thereby killing mosquito when it encounters the contaminated surfaces or walls. The aim of ITN is to reduce contact of human with infected mosquitoes and is most effective for mosquitoes that bites and rest indoor. In the early years, IRS employed use of dichloro-diphenyl-trichloroethane (DDT) as part of Global Malaria Eradication Campaign (1955-1969). However, due to concerns on environmental safety, DDT has since been replaced with other less toxic substances to the environment including pyrethroid and non-pyrethroids insecticides. In addition, mosquito repellents or attractants products are increasing trialed or, used to offer protection against indoor and outdoor mosquito bites (Hao *et al*., 2012; Verschut *et al*., 2019; Xu *et al*., 2019; Zhou *et al.*, 2020).

Larviciding involve application of chemical or biological insecticides to water bodies in areas experiencing ongoing malaria transmission in which optimal ITNs and IRS coverage has been achieved (Antonio-Nkondjio *et al*., 2018, 2021; Choi & Wilson, 2017; Dambach *et al*., 2021; Derua *et al*., 2019). The goal of larviciding is to reduce mosquito vector density, and is recommended when or where larval habitats are few, fixed, findable i.e., during the dry season as opposed to rainy season when larval habitats are likely swept away by running or accumulating waters (Maheu-Giroux & Castro, 2013; WHO, 2019). Thus, the potential larval habitats in the

current study were mapped during the dry season when the larval habitats might provide ideal situation of ongoing transmission in the area, even months after rainy season ends. Compared to high transmission areas, larviciding was found to be more effective in low transmission areas (Runge *et al*., 2021). Implying that in regions of high malaria transmission, larviciding approach needs to be implemented together with other vector and parasite control measures for optimal reduction in transmission.

2.4 Challenges facing malaria control and prevention strategies

Parasites resistant to antimalarial drugs including chloroquine and widely used first line treatment drugs for uncomplicated malaria have been observed in Africa, Asia and South America (Balikagala *et al*., 2021; Dhorda *et al*., 2021; Njiro *et al*., 2022). Also, multiple studies report existence of parasite lacking *hrp2* gene (Oreh *et al.*, 2022; Thomson *et al*., 2019; Vera-Arias *et al*., 2022). Individuals carrying these parasites are falsely identified using RDTs, and thus miss out on treatment offering them a chance to contribute to onward transmission. Also, due to the misdiagnosis, adverse consequences including death may occur. The misdiagnosed individuals result in underestimation of parasite carriers in the population leading to suboptimal effectiveness of resources geared towards combating the disease.

Low-density infections that are missed by field deployable tools including microscopy and RDTs pose a threat to malaria control (Awandu *et al.*, 2019; Drakeley *et al.*, 2018; Hartley *et al.*, 2020; Kumar *et al.*, 2022). These undetected infections are mostly from asymptomatic malaria carriers who are not treated since they do not show up sick at the health facility. Also some infections of clinical patients are below the limit of detection of microscopy or RDT. These infections contribute to onward transmission of malaria (Gonçalves et al., 2017; Sumner et al., 2021; Tadesse et al., 2018), thereby subverting control efforts.

Vectors may exhibit plasticity in their behavior that allow them to bite more frequently such as change in their time of feeding to before people go to bed, or late in the night (Mathania *et al.,* 2016; Milali *et al*., 2017; Mponzi *et al*., 2022; Thomsen *et al*., 2017). Also, increasing malaria transmission from outdoor biting vectors (i.e., outdoor transmission) is being reported (Degefa *et al*., 2017, 2021; Keïta *et al*., 2021; Moshi *et al.*, 2018), and is responsible for significant proportion of infections in Africa including residual malaria transmission (Killeen, 2014; Mwesigwa *et al*.,

2017; Sherrard-Smith *et al*., 2019; Sougoufara *et al*., 2020). The outdoor biting and resting mosquitoes escape bed net intervention. Lastly, there is mounting evidence of mosquitoes that are resistant to pyrethroid insecticides (Glunt *et al*., 2015; Musiba *et al*., 2022; Quiñones *et al*., 2015; Toé *et al*., 2014), rendering the pyrethroids ITN less effective in protecting against bites.

2.5 Life cycle of *Plasmodium falciparum* malaria parasite

Transmission of malaria involves the human host, mosquito vector and the *Plasmodium* parasite (Figure 1). For transmission to occur, mosquitoes must take up at least one microgametocyte (male gametocyte) and one macrogametocyte (female gametocyte) from an infected human during a blood meal. This initiates the sporogenic cycle in the mosquito. Once ingested by a mosquito, female and male gametocytes undergo gametogenesis in the midgut to form gametes. While in the mosquito's midgut, the male gamete fertilizes the female gamete to form zygotes which then develop to ookinetes. Motile ookinetes invade and cross the midgut epithelium of the mosquito where they further develop into an oocyst beneath the basal lamina. The oocysts grow, rupture and release thousands of sporozoites that move to the haemolymph and invade mosquito's salivary glands. The sporogenic cycle takes approximately 10-12 days (Kengne-Ouafo *et al*., 2019; Venugopal *et al.*, 2020).

The infective sporozoites are injected into human host blood stream whenever a female mosquito takes a subsequent blood meal. This initiates the pre-erythrocytic cycle. Once in the blood stream, they migrate and enter blood capillaries, and further move to liver sinusoids and invade hepatocytes. Inside hepatocytes, they multiply to form liver schizonts. Each asexual replication cycle yields a liver schizont containing thousands of merozoites. The pre-erythrocytic cycle takes 5-7 days. The merozoites enter the blood stream in merosomes i.e., membrane-bound structures. They are then released and infect erythrocytes to initiate the intra-erythrocytic cycle. The merozoites develop to ring stages to trophozoite and then to schizonts. Schizonts mature then burst to release merozoites that initiate another asexual replication cycle. Clinical symptoms begin to manifest at this phase. This phase takes 48 hours and produces 16-32 merozoites (Kengne-Ouafo *et al.*, 2019; Venugopal *et al*., 2020).

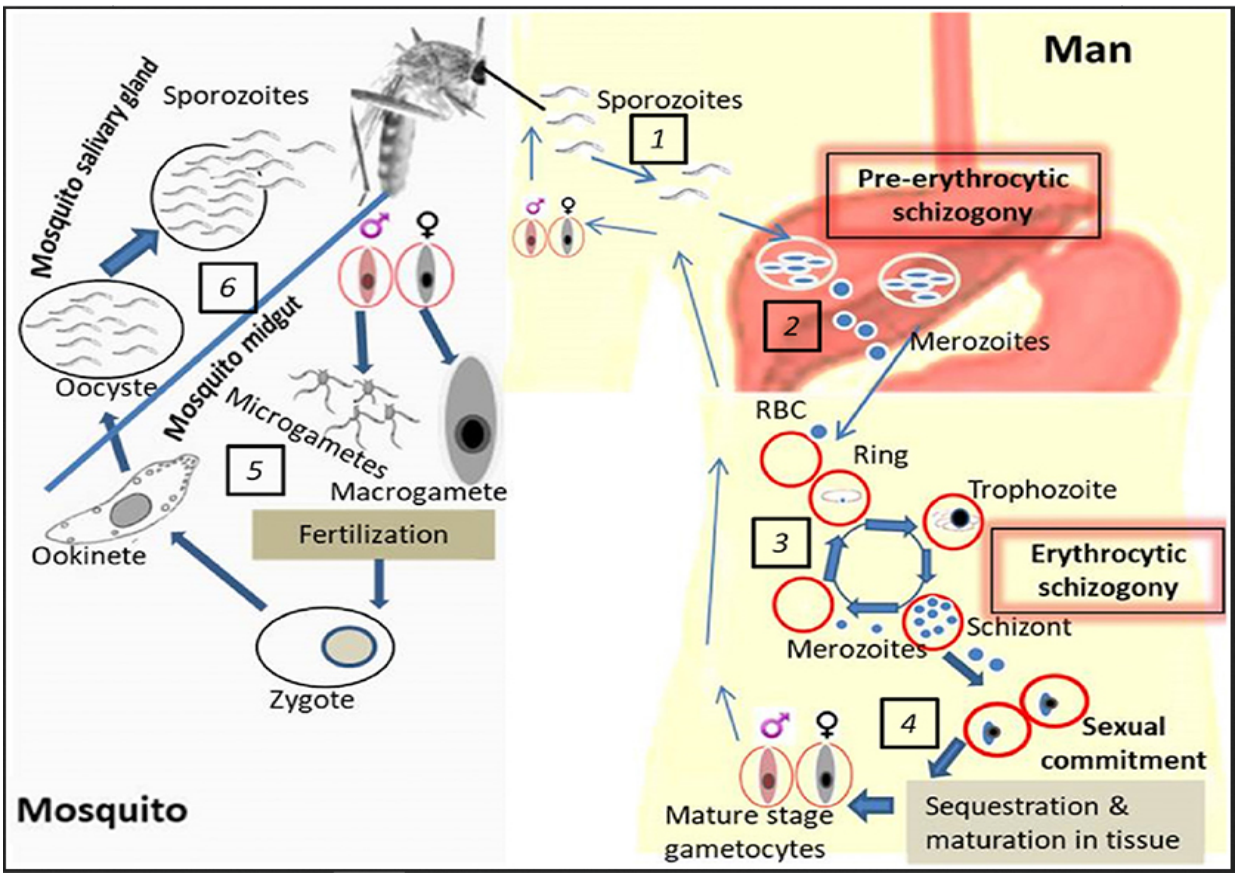

Figure 1. Life cycle of *P. falciparum* in human and mosquito. Adapted from Kengne *et al*. (2019). Step 1. Infected mosquito bite into the skin and inoculate sporozoites into human during blood meal. The sporozoite then migrate into the liver. Step 2. Sporozoites infect liver cells (hepatocytes). Parasite multiply in the liver and infect more hepatocytes. Sporozoites develop to liver schizonts that burst to release merozoites. Step 3. The merozoites develop to ring stages to trophozoite and then to schizonts. Schizonts mature then burst to release merozoites that initiate another asexual replication cycle. Step 4. Subpopulation of schizonts, less than 10%, commit to form gametocytes. Early-stage immature gametocytes sequester in inner tissues and organs until maturity then later egress in peripheral circulation as mature stage V gametocytes where they are available for uptake by mosquito upon feeding on an infected individual. Step 5. Sexual parasite develops in the mosquito midgut. Male gametocyte undergoes exflagellation then fertilize to release zygote that develop into a motile ookinete. Step 6. Oocyts are formed from mature ookinetes. Oocyst rupture to form sporozoites in the salivary gland for inoculation into host blood stream during mosquito bite.

2.6 *Plasmodium falciparum* gametocyte biology

Formation and maturation of *P. falciparum* gametocytes within the human host involves five morphologically distinguishable and transcriptionally distinct stages I-V (Soulard *et al*., 2015). Early ring stage gametocytes freely circulate in peripheral blood (Farid *et al*., 2017). Late stages I-IV undergo structural deformity that enable them to sequester for 7-12 days into deep organs including bone marrow and the spleen until they develop to maturity i.e., stage V gametocytes. Once mature, the stage V egress into the peripheral blood circulation where they become available for uptake by mosquito during a blood meal (Eichner *et al*., 2001; Farfour *et al*., 2012). Once ingested by mosquitoes, each individual gametocyte forms 1 female gamete or up to 8 male gametes. Compared to other *Plasmodium* species, gametocyte development and maturation in *P. falciparum* occurs over relatively lengthy period (8-12 days) (Dash *et al*., 2022; Delves *et al*., 2013; Lawniczak & Eckhoff, 2016). Once in blood circulation, a mature gametocyte requires about 3 additional days to be infective to mosquitoes. Gametocytes may circulate in the peripheral blood of infected individual for a longer period of time. Even after antimalarial treatment, gametocytes may continue to circulate for up to 2-3 weeks (Dunyo *et al*., 2006; Haanshuus & Mørch, 2020; Shekalaghe *et al*., 2007; White, 2017).

2.7 Molecular mechanisms of sexual conversion in *Plasmodium falciparum*

For transmission to occur, the parasite while in its intraerythrocytic cycle requires a switch from asexual replication to sexual differentiation into gametocytes (Brancucci *et al*., 2017; Schneider *et al*., 2018), a phenomenon referred to as sexual conversion (Figure 2). During this cycle, a proportion of schizonts (fewer than 10%) undergo sexual commitment to form gametocytes in each 48-hour cycle. Each committed schizont forms gametocytes of same sex and not a mixture of sexes (Silvestrini *et al.*, 2000; Smith *et al*., 2000). *P. falciparum* apetala-2 (*pfap2*) transcription factor is a key determinant of sexual conversion. The gene is essential for the differentiation of committed schizonts into early gametocytes (Kafsack *et al*., 2014). Expression of *ap2-g* is activated when a repressor protein, e.g., heterochromatin protein 1, is removed from *ap2-g* by nuclear protein gametocyte development protein 1 (GDV1) (Eksi *et al*., 2012; Filarsky *et al*., 2018). In asexual parasites, the *ap2-g* is epigenetically silenced (Coleman *et al*., 2014; Flueck *et al.*, 2009), hence no sexual conversion occurs (Figure 2).

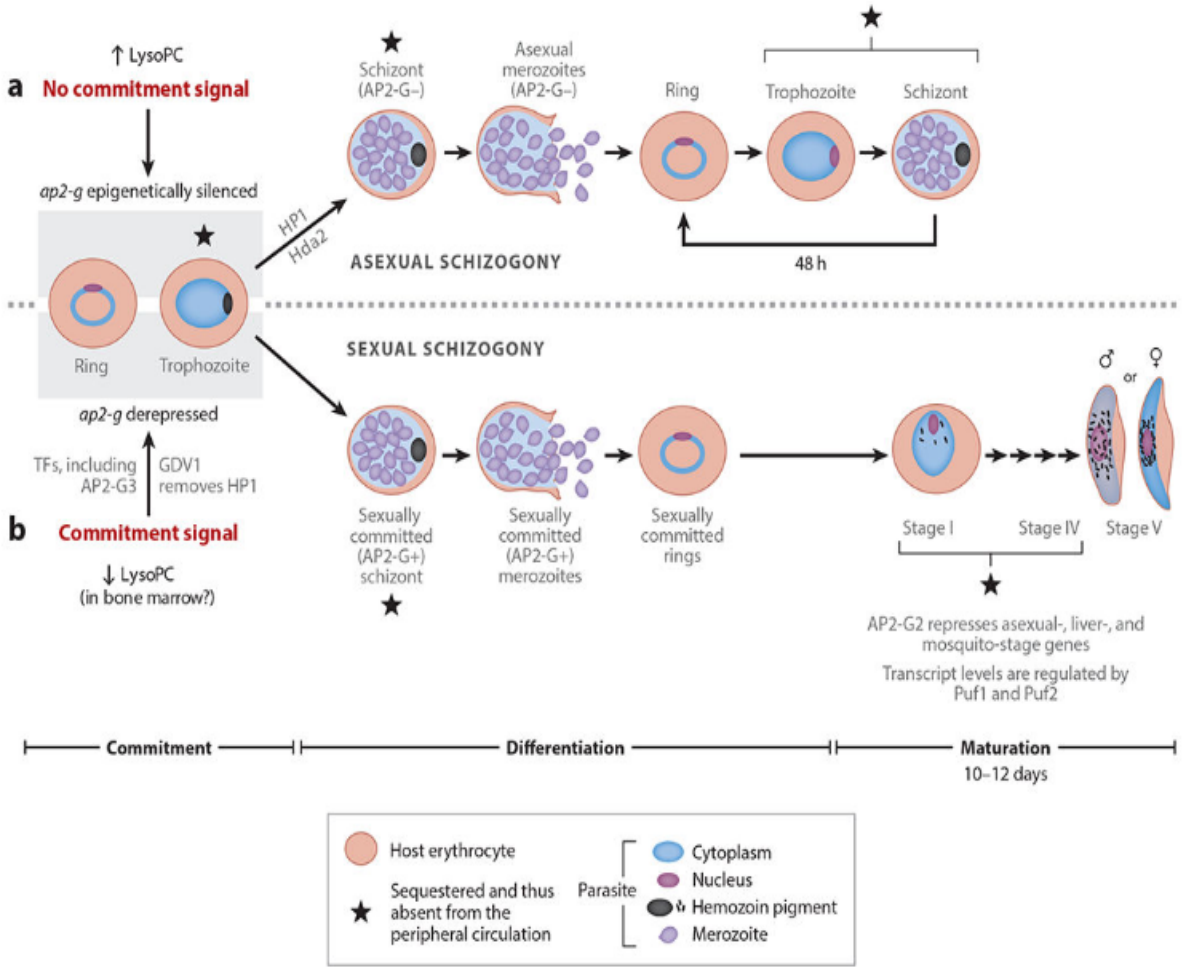

Figure 2. Molecular pathways for *Plasmodium falciparum* sexual conversion versus asexual replication. Adapted from Ikadai *et al*. (2013). The basis of Figure 2 is to illustrate the molecular divergent pathways of the parasite after formation of schizonts. (a). Pathway (a) involves asexual schizogony i.e., schizonts develop to asexual ring stage parasites that develop to trophozoites. In this pathway, apetala-2 gene (*ap2-g*) that is responsible for gametocyte production is silenced. This occurs in the presence of high or elevated concentration of lysophosphatidylcholine (LysoPC) in blood circulation. An interraction of heterochromatin protein 1 (HP1) and histone deacetylase-2 (Hda2) silence the *ap2-g*. This makes the schizonts undergo asexual schizogony where they replicate to form asexual parasites. (b). Pathway (b) involves sexual schizogony i.e., schizont commit to produce gametocyte. In sites where LysoPC concentration is low such as bone marrow, the *ap2-g* expression is activated (i.e., derepressed) through removal of HP1 from *ap2-g* by a combination of gametocyte development protein 1 (GDV1) and transcription factor AP2-G3. The

activated *ap2-g* facilitate formation of sexually committed rings that form gametocytes. Early-stage gametocytes are sequestered in inner organs such as spleen and bone marrow where they undergo cascade of developmental stages to form mature stage V gametocytes that egress into peripheral circulation.

2.7.1 Significance of sexual conversion

The sexual conversion rate, i.e., the proportion of parasites that develop into gametocyte, is critical as it determines the number of schizonts that eventually form gametocytes. After blood stage schizont formation in the erythrocytic cycle, the parasite undergoes a two-arm developmental stage (Figure 2). One arm is the continuation of asexual replication to infect more erythrocytes. In doing so, the parasite enhances its survival chances in the human host by causing more damage to the host. The other arm is that the parasite can increase gametocyte production by adjusting its conversion rate. In doing so, it maximizes its transmission potential (Carter *et al.*, 2013; Reece *et al*., 2009).

2.7.2 Methods for measuring conversion rate

In field isolates, parasites commitment to transmission i.e., conversion rate can be measured directly or indirectly. The direct method involves short-term culture whereby early-stage gametocytes are quantified by microscopy after 2-8 days in culture and comparing their density to parasite density at baseline (day 0) (Usui *et al*., 2019). Indirect methods include studying changes in the parasite genome and transcriptome, quantifying mature late-stage gametocytes in human blood samples by microscopy or molecular methods, conducting mosquito feeding assays, or measuring expression of *ap2* gene (Llorà-Batlle *et al.*, 2020; Tadesse *et al*., 2019).

In natural infections, parasite densities fluctuate over time. Thus, when determining the conversion rate, quantifying parasite density in parallel with gametocyte density is informative as differences in gametocyte densities may merely represent variation in parasite densities. Given that *P. falciparum* gametocytes sequester in inner organs for about 10-12 days then re-emerge in blood circulation (Eichner *et al*., 2001; Farfour *et al*., 2012), their densities at the day of blood collection might represent parasite densities up to at least 10 days earlier.

2.7.3 Factors influencing conversion rate

Conversion rate is likely influenced by several factors such as plasma lysophosphatidylcholine (LysoPC) concentrations, fever, the presence of reticulocytes, antimalarial drug uptake, host parasitemia, and malaria transmission intensity in a region. Sites with low LysoPC concentrations such as bone marrow permit increased conversion rate (Brancucci *et al*., 2017; Usui *et al*., 2019). The impact of antimalarial drug pressure on conversion rate is very complex and remains unclear as drugs differ in mode of action, dosage, and stage of parasite that they target. Several studies that examined the effect of antimalarial drugs on gametocyte production yielded contrasting results (Peatey *et al*., 2009; Portugaliza *et al.*, 2020; Rajapandi, 2019; Schneider *et al*., 2018).

The presence of fever is associated with a decreased conversion rate (Barry *et al*., 2021; Usui *et al*., 2019). A higher conversion rate was observed in hosts with higher parasitemia (Schneider *et al*., 2018; Usui *et al*., 2019). Higher conversion rates were observed in regions where malaria transmission has decreased substantially (Parobek *et al*., 2016; Rono *et al*., 2018; Vantaux *et al*., 2018). By investing more resources in gametocyte production in areas where transmission is low, the parasite partially compensates for fitness lost. In addition, malaria parasites would gain by producing more gametocytes in rainy season when mosquitoes are plentiful to optimally drive transmission. However, there is no evidence that increased production of gametocytes occurs in the rainy season.

2.7.4 Methods for detection and quantification of gametocytes

There are existing methods for detection and quantification of gametocytes such as light microscopy (LM), Magnetic Deposition Microscopy (MDM), nucleic acid sequence based amplification (NASBA), and Reverse Transcription – quantitative Polymerase Chain Reaction (RT-qPCR) (Guiguemde *et al*., 2020; Schneider *et al*., 2015; Sumari *et al*., 2016). Notably, these methods have various drawbacks.

The LM and MDM are widely applicable for diagnosis of gametocytes at moderate-to-high density. Thus, these two approaches fail to identify gametocyte at very low density which are mostly expected in field studies especially in asymptomatic malaria reservoirs. The MDM technique targets hemozoin, a by-product of *Plasmodium* parasite's metabolism after feeding on hemoglobin. The hemozoin may be produced both by asexual parasite and gametocyte thus this

method is not exclusively for gametocyte detection. OptiMAL RDT-based method is also used to detect gametocytes (Mueller *et al.*, 2007), but is equally not suitable in detecting low density gametocytes. The technique targets *Plasmodium* lactate dehydrogenase (pLDH) which is found in both asexual parasite and gametocyte in peripheral circulation thus compromising its accuracy to exclusively detect gametocytes.

Molecular methods such as NASBA and RT-qPCR are based on the detection and quantification of gametocyte-specific *P. falciparum* gametocyte messenger ribonucleic acid (mRNA) transcripts (i.e., molecular markers). These markers include but are not limited to male gametocyte markers *Pfmget*, *Pfs230p* and *Pf13*, early stage ring gametocyte marker *Pfgexp-5*, *Ap2-g*, *Surfin 13.1* and *Surfin 1.2*, both sex gametocyte marker *Pfg17*, and female gametocyte markers *Pfs25* and *Ccp4* and *Pf11.1* (Koepfli & Yan, 2018; Meerstein-Kessel *et al*., 2018; Prajapati *et al*., 2020; Tibúrcio *et al*., 2015). The NASBA and RT-qPCR methods have high sensitivity compared to microscopy (Cordray & Richards-Kortum, 2012; Leski *et al.*, 2020; Pett *et al*., 2016; Schneider *et al*., 2005). These methods require mRNA for gametocyte detection. In the process of RNA extraction, traces of DNA are co-extracted with the RNA, and this result in false transcripts amplification signal. For accurate quantification of gametocyte, DNAse or N-acetylglucosamine is added during extraction process to remove the asexual parasites i.e., traces of DNA.

2.8 Asymptomatic malaria

Asymptomatic malaria is widely defined as malarial parasitemia in the absence of malaria related symptoms in individuals who have not received antimalarial treatment within the past one week and no reported fever within 48 hours of sampling. Following repeated malaria exposure, there occurs elevated production of anti-inflammatory cytokine (e.g., IL-10) and downregulation of pro-inflammatory cytokines (e.g., interferon-γ, tumor necrosis factor-α) and immune cells (e.g., Natural Killer cells, regulatory T cells). This leads to manifestation of asymptomatic infection (Kimenyi *et al*., 2019). Collectively, multiple studies have reported high asymptomatic malaria prevalence among individuals living in malaria endemic areas (Bousema *et al.*, 2014; Chen *et al*., 2016; Lindblade *et al*., 2013). Malaria Atlas data show trends in proportion of asymptomatic malaria reservoirs across countries. However, there is limited data on trends of proportion of asymptomatic malaria reservoirs across small geographical scales.

Gametocyte density is an important measure for estimating the contribution of infectious reservoirs to onward transmission of malaria. The success of transmission of the malaria parasite from human to mosquito is heavily dependent on density of both male and female mature gametocytes in human peripheral blood circulation (Ahmad *et al*., 2021; Bradley *et al*., 2018; Da *et al*., 2015; Morlais *et al*., 2015). At higher gametocyte density, likelihood of transmission success is increased until a threshold is reached, beyond which infectivity is saturated. Likewise, at lower gametocyte densities, male gametocyte become limiting factor for transmission (Bradley *et al.*, 2018). Asymptomatic malaria individuals in malaria endemic areas may harbor a parasite for extended periods, spanning into couple of months in the presence of treatment. During this infection duration, density of parasite may fluctuate several folds, and some clonal infections are cleared while others persist, or new infections may also set in (Ashley & White, 2014; Bretscher *et al*., 2011; Felger *et al*., 2012; Nguyen *et al*., 2018; Roe *et al.*, 2022). In natural infections, *P. falciparum* parasitemia moderately correlate with gametocyte density (Koepfli *et al*., 2015).

Mosquitoes can be infected by asymptomatic malaria reservoirs (Chaumeau *et al*., 2019; Gouagna *et al.*, 2004; Sumner *et al*., 2021; Tadesse *et al*., 2018). During malaria surveillance in the health centers, the asymptomatic malaria reservoirs escape detection since most of them do not seek treatment as they do not have symptoms that would otherwise prompt them to seek treatment. Therefore, they are considered "hidden enemy" in the community in war against malaria elimination (Agaba *et al*., 2022; Doum *et al*., 2023; Melese *et al*., 2022). In community surveys, field deployable tools for screening for malaria parasite such as microscopy and RDTs are used. These tools cannot detect low density infections (i.e., submicroscopic infections) which are equally present in malaria endemic regions and are potential reservoirs for transmission, constituting 20-50% of infections in pre-elimination and elimination settings (Beshir *et al.*, 2013; Okell *et al.*, 2012; Tadesse *et al*., 2015; Wu *et al*., 2015).

2.9 Life cycle of *Anopheles* species mosquito

Mosquitos go through four distinct stages of development e.g., egg, larva, pupa and adult (Figure 3). The first three stages would remain in a larval habitat while the adult mosquito would fly to seek blood meal or nectar. Female adult mosquitos take blood meals as they are needed for the egg

laying process. The development cycle from egg to adult stages takes about 10-14 days but may vary depending on conditions such as temperature, food source (CDC, 2022).

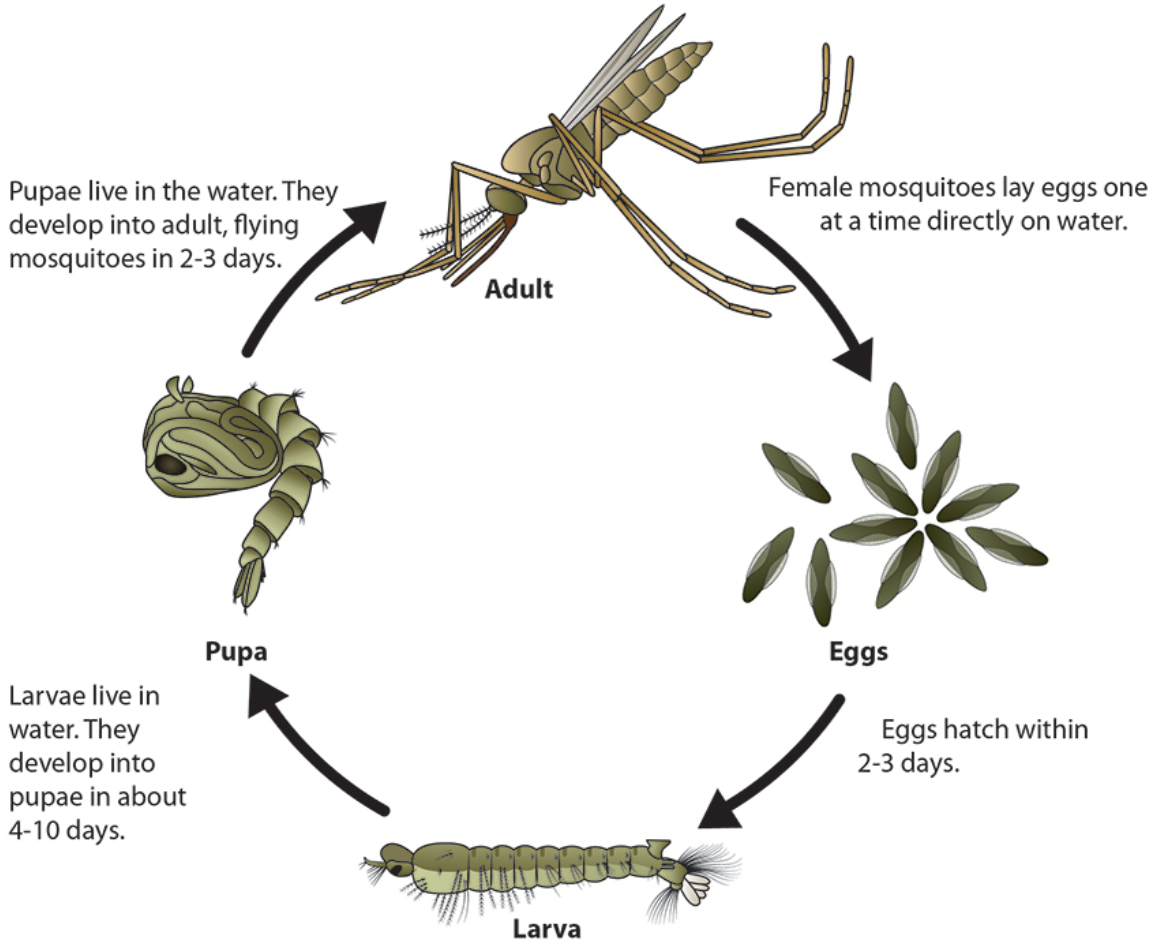

Figure 3. Life cycle of *Anopheles* species mosquito. Adapted from Centers for Disease Control and Prevention (2022). This figure shows the four mosquito developmental stages. An adult female mosquito lay eggs that hatch into larva then pupa. The pupa then develops to adult flying mosquitoes.

2.9.1 Mosquito larval habitats

The number of adult mosquitoes is dependent on the presence and suitability of larval breeding habitats to support developmental stages of the vector. The *Anopheles gambiae* complex, also known as *An. gambiae* sensu lato (s.l), comprises of *An. gambiae* sensu stricto (s.s) and *An. arabiensis*. These vectors prefer to breed in shallow waters exposed to sunlight, and habitats may be temporary or permanent, fresh or saline (Machault *et al*., 2009; Ondiba *et al*., 2019). *An.*

funestus, in contrast prefers a wide range of aquatic habitats including waterbodies that are mostly shady, permanent or semi-permanent, with floating or thick vegetation and algae such as in swamps, marshes, and edges of streams (Emidi *et al*., 2017; Gimnig *et al.*, 2001; Minakawa *et al*., 2012). To identify potential larval habitats of main mosquito vectors in western Kenya, we mapped features such as swamps, marshes, drainage ditches, pond, dams, water-filled sand pit sand, forests and sugarcane plantations. Sugarcane plantations are characterized by water features that provide suitable breeding ground for survival and fecundity of *An. gambiae* and *An. arabiensis* mosquitoes (Demissew *et al*., 2020; Hawaria *et al*., 2021). Also, sugarcane pollen-associated volatiles attract gravid *An.arabiensis* (Wondwosen *et al.*, 2018).

2.10 Seasonal malaria transmission

In many countries in the sub-Saharan region, malaria transmission occurs year-round, and the transmission is characterized by temporal variability i.e., seasonality, with peaks in transmission occurring during and shortly after the rainy season (Cairns *et al.*, 2015; Carneiro *et al.*, 2010; Idris *et al*., 2016; Ikeda *et al*., 2017; Reiner *et al*., 2015; Selvaraj *et al*., 2018). Changes in seasonality impact on mosquitoes' abundance. During the rainy season, mosquitoes become plentiful because the change in climate favor their breeding, development and survival (Abiodun *et al*., 2016; Afrane *et al*., 2012; Lyons *et al*., 2013). Few studies have investigated impact of climate change on seasonality of malaria transmission, and they found contrasting findings in trends e.g., a decline or an increase or no substantial change in proportion of clinical malaria cases between seasons (Lubinda *et al*., 2021; Nigussie *et al*., 2023). This points to a complex relationship that is impacted by ecology, topography of study sites among other stressors.

According to Kenya Metereological Department, over the past several years, Kenya has been experiencing three seasons within a year namely: dry season, long rains season and short rains season. Generally, the timing for dry season occurs between the months of mid-December and March while long rains season occurs between the months of April and June. This is followed by little precipitation until the month of August. Short rains occur in between the months of late September and November. Variations in the rainfall amounts have been recorded between the years, however, the timing for seasonality in most regions of the country including western Kenya has been largely stable over the years. A higher clinical malaria cases are reported during and shortly after rainy season in areas largely classified as endemic regions i.e., western Kenya and

coastal area. In the context of this work, a term “dry season” was used to refer to the mid December to March low transmission period, and “rainy season” to refer to the April to July high transmission period.

2.11 Risk factors for malaria transmission

Factors associated with malaria transmission may arise at the individual, household or regional (small scale such as a village) level. Transmission success is hinged on availability of parasites, vectors, and environmental conditions. Parasitic factors that influence transmission include their presence, density, and resistance to drug treatment. A previous study reported high probability of detecting gametocyte from individuals with high parasite density. Further, the study found that parasite density was a significant predictor of gametocyte density (Koepfli *et al*., 2015). Gametocyte densities are a strong determinant of parasite transmission potential to mosquitoes (Bradley *et al*., 2018; Da *et al*., 2015). Mosquitoes are more attracted to individuals who harbor gametocytes in blood circulation (Busula *et al.*, 2017), thus having gametocytemia poses an increased risk for mosquito bites. *P. falciparum* infected erythrocytes secretes a metabolite, (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate, that triggers an increase in the production of carbon (iv) oxide, aldehydes and monoterpenes by the infected cells, that were found to enhance vector attraction and feeding on the infected blood (Emami *et al*., 2017). Other mosquito attractants include exhaled carbon (iv) oxide, body sweat, skin odors and body heat.

Blood stage malaria parasites that are resistance to some of the most currently used antimalarial drugs including artemisinin based combination therapy have been observed (Ariey *et al*., 2014; Conrad & Rosenthal, 2019; Uwimana *et al*., 2021). Failure to clear the parasites in circulation poses significant risk to the population as these individuals can be infectious to mosquitoes, thus continuing the transmission cycle.

Other factors such as behavior, suboptimal medical adherence, lack of previous exposure to infection, migration or travel, age and sex also influence transmission. In regions where there are ongoing malaria transmission, activities that enhance exposure to mosquito vectors such as spending most of time in the fields and in play grounds, or laxity to use vector control interventions such as bed-net or vaccine increases individual’s chance of having malaria (Guelbéogo *et al*., 2018; Soma *et al*., 2021). Likewise, people who do not adhere to drug prescription are at higher risk of

disease progression or spreading the infections to others (Bruxvoort *et al*., 2015; Challenger *et al*., 2017; Cohen & Saran, 2018; Mace *et al*., 2011).

Younger children under the age of 5 years have a high likelihood of being sick of clinical or symptomatic malaria compared to older children and adults, thus they form the largest age group diagnosed with clinical malaria in health facilities (Gonzales *et al*., 2020; Long & Zavala, 2017). Immune responses against malaria parasite helps to clear infection and/or the disease or suppress density of parasites in circulation (Barua *et al*., 2019; Doolan *et al*., 2009; Gonzales *et al*., 2020; Rodriguez-Barraquer *et al*., 2018). Repeated exposure to malaria parasties, potentially corresponding to the frequency of infectious mosquito bites received over time, is associated with the development and subsequent enhancement of immune response to malaria (Barua *et al.*, 2019; Doolan *et al*., 2009; Rodriguez-Barraquer *et al*., 2018). As opposed to adults and older children who mount strong or sufficient immune response against malaria parasites following repeated exposure over time (Crompton *et al*., 2014; Kurup *et al*., 2019; Rochford & Kazura, 2020; Rogers *et al*., 2021), younger children lack or mount insufficient immune response to malaria parasite thus the parasite can replicate and continue and invading erythrocytes, causing clinical malaria. Pregnant women also have increased chances of malaria infections women (Almaw *et al*., 2022; Chua *et al*., 2021; Jain *et al*., 2022; Lingani *et al*., 2022).

Persons living in area of intense transmission are more exposed to multiple potential infectious mosquito bites than those living in areas where transmission has greatly reduced, and infectious mosquito vector population is low. In regions where transmission has substantially reduced, most infections would become symptomatic, prompting health care seeking behavior.

In many community surveys across settings of differing transmission intensities, older children were found to harbor the majority of non-febrile *Plasmodium* infections (Kamau *et al*., 2020; Mensah *et al*., 2021; Salgado *et al.*, 2021; Walldorf *et al*., 2015; Worku *et al*., 2014). In longitudinal studies involving asymptomatic malaria persons followed over several weeks, higher probability of clinical malaria among previously asymptomatic malaria individuals was observed (Le-Port *et al*., 2008; Njama-Meya *et al*., 2004; Sumner, *et al*., 2021).

Multiple studies found higher prevalence of malaria infections or clinical cases reported in health facilities in males than females across all ages (Abdalla *et al*., 2007; Awosolu *et al*., 2021; Chaparro-Narváez *et al*., 2016; Houngbedji *et al.*, 2015; Mulu *et al*., 2013; Pathak *et al*., 2012). In contrast, few studies found higher malaria incidences or prevalence among females compared to males (Okiring *et al.*, 2022; Quaresima *et al*., 2021). In a longitudinal study involving individuals harboring malaria parasite over several weeks without being treated for malaria, a shorter time-to-clear malaria parasite was observed in females compared to male counterparts (Briggs *et al*., 2020). The differences in risk of malaria transmission in males versus females are attributed to various reasons such as behavior (Finda *et al*., 2019; Moon & Cho, 2001; Pathak *et al*., 2012), sex-based immunological responses to malaria infections (Delić *et al*., 2011; Klein *et al*., 2016; Kurtis *et al*., 2001; Leenstra *et al*., 2003).

Household factors that may pose higher risk to infections include living in areas that are close to places conducive for mosquito breeding. These include but not limited to rivers (Bhondoekhan *et al.*, 2020; Oesterholt *et al*., 2006; Sluydts *et al*., 2014; Zhou *et al*., 2021), swamps and marshes (Haque *et al.*, 2011; Mwandagalirwa *et al*., 2017), forests (Haque *et al*., 2011; Rosas-Aguirre *et al*., 2021), drainage ditches and trenches (Haque *et al*., 2011; Nicholas *et al*., 2021), rice fields (Musiime *et al*., 2020), grasses cover (Kweka *et al*., 2012), ponds and pools (Nambunga *et al.*, 2020), sugarcane (Demissew *et al.*, 2020; Wondwosen *et al*., 2018). Further, infection risk is increased when living in houses built in low-lying altitudes where climate favor vector reproduction, parasite development and survival in the mosquito vector (Fouque & Reeder, 2019), or living in house structures that offer suitable resting and hiding place for mosquitos, or that allow massive entry or exit of mosquitos into or out of household (Ondiba *et al*., 2018; Yaro *et al*., 2021).

A change in climate can have impacts on malaria transmission. Climatic factors including temperature, rainfall and humidity are the key parameters that directly influence transmission (Alemu *et al*., 2011; Nyasa *et al*., 2022; Yamba *et al*., 2023). Moderate-to-higher temperatures in the range 21℃ to 34 ℃ promote development of mosquito larvae in their habitats and development of *P. falciparum* parasites in the vector. An increase in altitude results in a decrease in temperature, and this impact on malaria transmission success. At lower altitude, vector numbers and proportion of infective mosquitoes become more (Asgarian *et al*., 2021; Asigau *et al.*, 2017; Bødker *et al*.,

2003; Eisen *et al*., 2008). Also, compared to higher altitude regions, increasing land use activities in low altitude areas may increase number of mosquito breeding habitats (Kweka *et al*., 2016; Steiger *et al*., 2016; Paul *et al*., 2018; Shah *et al*., 2022; Sheela *et al*., 2017; Zahouli *et al*., 2017).

Anthropogenic greenhouse gases emissions results in rapid warming of the earth (Lelieveld *et al*., 2019; Nunes, 2023; Ruddiman, 2003). Changes in climate among other factors affect transmission dynamics, and may lead to spread or re-emergence of vector-borne diseases thus posing major threat to the control and prevention of vector-borne diseases. In general, an increase in temperature results in plentiful vectors, longer survival and higher feeding activity, and enhanced pathogen development in the vector thus potentiating transmission (Fischer *et al*., 2020; Nyawanda *et al*., 2023).

Natural water sources including rainfall largely influence the establishment, quality, and abundance of potential breeding sites for mosquitoes. As a result of the availability of many water collection spots during the rainy season, more breeding sites are observed compared with when there is little or no rainfall. Different *Anopheline* species prefer various water bodies e.g transparent vs. turbid or sunlit vs. dark, saline vs. fresh, having vegetation vs. lacking. Relative humidity (i.e., amount of moisture in the air) in the range 60-80% promote mosquito survival, flight activity, host-seeking behavior (Drakou *et al*., 2020; Garg *et al*., 2009; Haque *et al*., 2010; Santos-Vega *et al*., 2022) and their biting activity (Li *et al*., 2013). Mosquitos are highly susceptible to desiccation. Thus, adequate humidity is vital for their survival. Mosquitoes become more active and bite often when humidity is high e.g during the night.

2.12 Heterogeneity in malaria transmission

Heterogeneity in malaria transmission may be temporal (i.e., vary in time) or spatial (i.e., vary in space or geographical location). The variation in space can be across continents, countries, regions, or within villages in a community, whereas variation in time can be across seasons or across specific duration following roll-out of malaria interventions. Factors explaining temporal heterogeneity can be directly ascertained including roll-out of intervention aimed at reducing mosquito vector population such as indoor residual spraying, bed-net use for a period (Alhassan *et al*., 2022; Dulacha *et al*., 2022; Hamusse *et al*., 2012) or strategies aimed at reducing parasite infection rate such as increasing testing capacity followed by treatment (Froeschl *et al*., 2018;

Ndong *et al*., 2019; Otten *et al*., 2009). However, identifying factors explaining spatial heterogeneity is complex as transmission may be impacted by plethora of factors. It is not known whether transmission vary across small geographical scales in regions of western Kenya, and likely factors explaining potential varitions in infection prevalence. Malaria control and elimination strategies applied at general level in areas where malaria transmission differs over small geographical scales did not achieve optimal utility or effectiveness (Gari & Lindtjørn, 2018; Hemingway *et al.*, 2016; Kaehler *et al*., 2019; Tizifa *et al*., 2018). Thus, understanding the factors contributing to small scale variation in malaria prevalence are critical for designing programs tailored to the local environment.

2.13 Metrics for malaria transmission

Malaria transmission intensity can be measured by determining exposure to mosquito vector and/or parasite infection. Metrics for exposure to parasite infections include prevalence (Amoah *et al.*, 2021; Koepfli *et al*., 2015; Sultana *et al*., 2017) , seroprevalence and incidence rate or malaria cases (Dulacha *et al*., 2022; Nahum *et al.*, 2010). Whereas metrics for exposure to mosquito infections include entomological inoculation rate (EIR) (Kilama *et al.*, 2014; Shaukat *et al*., 2010), mosquito vector abundance, their composition, and oocyst densities (Amek *et al.*, 2012; Epopa *et al*., 2019; Gil *et al*., 2003), human biting rate (HBR), sporozoite positivity rate or sporozoite infectivity rate (SIR) (Abraham *et al.*, 2017; Moreno *et al*., 2009).

Prevalence refers to the proportion of individuals in sampled population who test positive for malaria parasite using microscopy, RDT or PCR at a given time e.g in a cross-sectional survey. Seroprevalence is determined by measuring circulating parasite antigen or antibodies produced by human in response to exposure, using immunological techniques including enzyme-linked immunosorbent assay (ELISA), magnetic bead-based immunoassay or RDT. The incidence rate is determined by calculating the proportion of individuals who get newly infected with malaria parasites or are newly clinically diagnosed with malaria at health facility over a period. HBR is determined from the number of mosquito bites received by a person per day. SIR is determined through examining proportion of mosquitos that are infectious to human e.g mosquitoes having sporozoite. EIR is determined by counting the number of infectious mosquito bites received by a person over time. The EIR is also determined by the product of SIR and HBR.

2.13.1 Levels of malaria transmission intensity

Malaria transmission can be classified into three different levels such as “low”, “moderate” or “high” transmission intensity based on the metrics for malaria transmission. Different studies use varying approaches to measure metrics for transmission. Given lack of standardization of the methods and limitations of each method, and considering variations in local climatic, topographical and human factors, comparison across studies becomes very challenging. Therefore, the terms (i.e., “low”, “moderate” or “high”) used in this study may not be ganeralized. In Kenya, malaria transmission is categorized into four epidemiological zones (i.e., highland epidemic prone, endemic, semi-arid and seasonal malaria transmission, and low risk malaria areas) by Kenya Malaria Indicator Survey Council. All the study sites for this work are found within the endemic zone where malaria burden and population at risk of infection is highest. In the context of this work, we have used terms e.g., “low-”, “moderate-” or “high transmission” to describe levels of transmission in the study sites chosen.

2.14 Study sites description

Three sites in western Kenya namely Chulaimbo in Kisumu County, Kimira-Oluch in Homa Bay County and Teso South constituency in Busia County were chosen as study sites for this work. Malaria prevalence data from Kenya Malaria Indicator Survey (2015) conducted among school age children reported an estimated malaria parasite prevalence (by microscopy) of 22% in Kimira-Oluch in Homa Bay County, 24% in Chulaimbo in Kisumu County and 37% in Teso South in Busia County. Guided by this data, this study purposively chose Teso South as a proxy for “high transmission” site and Chulaimbo as a proxy for “moderate transmission” site. Two-rounds of mosquito vectors control program such as indoor residual spraying (IRS) was implemented in Homa Bay County including in Kimira-Oluch region during the period 2017-2018. According to the Kenya “End of Spray” Report (2018), deployment of the IRS program resulted in significant reduction of mosquitoes densities and sporozoites rates, which indicate a reduction in malaria transmission (Kelly-Hope & McKenzie, 2009; Kilama *et al*., 2014; Kulkarni *et al*., 2006). Therefore, this study chose Kimira-Oluch as a proxy for “low transmission” site.

According to Kenya National Bureau of Statistics, the Chulaimbo subcounty region of Kisumu County where this study was conducted has a population of approximately 12,500 persons in an

area of 25 km^2 (i.e., 500 persons per square kilometer) The region experiences seasonal rains in April-July and October – November. The average annual rainfall is 1350 mm. The mean annual temperature ranges from 12°C to 34°C. The average relative humidity ranges from 66 to 83%. The average altitude is 1381 meters above the sea level. *Anopheles arabiensis* is the predominant mosquito vector species followed by *Anopheles gambiae* sensu stricto (Machani *et al*., 2020).

Kimira-Oluch regionin Homa Bay County has a population of about 17,200 persons, and an area of 28 km^2 (i.e., 615 persons per square kilometer). The region experiences seasonal rains in April-August and October - November, and an annual rainfall of 1200 mm. The mean annual temperature ranges from 18°C to 29 °C. The average relative humidity ranges from 64 to 78%. The average altitude is 1330 meters above the sea level. *Anopheles funestus* is the predominant mosquito vector species (McCann *et al*., 2014).

Teso South constituency has a population of approximately 168,116 persons, and a land area of 302.9 km^2(i.e., 555 persons per square kilometer). The average altitude is 1162 meters above the sea level. The region experiences seasonal rains in April-June and September-November, and an annual rainfall between 760 mm and 2000 mm. The maximum temperature ranges between 26°C and 30°C and minimum temperature is between 14°C and 22 °C. The average relative humidity ranges from 60% to 77%. The main malaria vectors population in this region is *Anopheles gambiae s.s* and *An. funestus* (Githinji *et al*., 2020).

2.15 Rationale and research gaps summary

Plasmodium parasite like other pathogens have various strategies for enhancing their survival in host or maximizing pathogenicity. One among many strategies depicted by *Plasmodium* parasite is the ability to change its phenotype expression to remain relevant in continuing transmission. Transmission success depends on the availability of enough gametocytes in blood circulation and presence of plentiful mosquito vectors. Strategy to increase gametocyte production is of benefit to the parasite in maximizing transmission potential when mosquitoes are plentiful. In malaria endemic areas where transmission is seasonal, higher infections are anticipated in the rainy season, but underlying reasons remain undeciphered. It is not known whether parasite increases its commitment to gametocyte production among parasite reservoirs during rainy season to coincide

with the plentiful mosquitoes. Identifying the factors contributing to increased infections in the rainy season will help design control measures to cover up the factors.

In regions where there is ongoing malaria transmission, mounting evidence shows the existence of individuals carrying *Plasmodium* parasites yet do not have clinical symptoms of malaria. These individuals are considered as transmission reservoirs and are critical in driving transmission in the community, thus are obstacle to malaria elimination. There is limited data on how parasite and gametocyte prevalence and densities, and proportion of individuals that would miss detection by field-deployable diagnostics vary across regions of differential transmission intensities. Knowledge gain on the relationship between parasitological indices and spatial variation in transmission is important to guide malaria control and elimination strategies.

Transmission may differ over fine scales and this impacts malaria control strategies. The extent of a possible decline in transmission across villages in western Kenya is not known. Also, factors contributing to variation in transmission over small scales in the region remain speculative. Identifying factors associated with malaria transmission over small scales is key to guide targeted malaria control and elimination measures.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study sites

The current study was conducted in three malaria endemic regions in western Kenya where *P. falciparum* is the primary malaria parasite species (Akala *et al*., 2021). The sites were Chulaimbo in Kisumu County, Kimira-Oluch in Homa Bay County and Teso South constituency in Busia County (Figure 4). The sites were chosen based on their considered differences in malaria transmission intensities. Kimira-Oluch was chosen as proxy for low transmission, Chulaimbo as moderate transmission and Teso South as high transmission.

Further, in Teso South site, 20 clusters (Figure 5) were purposively chosen based on their differences in topography and spatial distribution for a wider coverage to study impact of potential larval habitats on malaria transmission between the clusters. The clusters were defined as villages with approximately 150 households each. The region has undulating terrain traversed by features including rivers, swamps, marshlands, drainage ditches, forests, pond, excavation pits among others that promote the establishment of larval habitats, thus making it suitable for studying impact of larval habitats on malaria transmission in the region.

3.2 Study populations

Across all the sites, blood samples were collected from males and females from age 6 months to less than 100 years who were present in the community during the time of sampling. The participants were sampled irrespective of clinical symptoms of malaria. Data on recent history of antimalarial drug treatment and presence of fever within 48 hours prior to blood collection was collected.

In Teso South site, participants' socio-demographic and clinical characteristics were captured using CommCare data collection tool. This included age, sex, bed net use, period of residency in study area, previous involvement in malaria vaccine trials, history of antimalarial treatment within the past 2 weeks and 1 month, and presence of malaria related clinical symptoms 2 days prior to sample collection. Household information such as village location, level of education of household head, Global Positioning System (GPS), kitchen location, altitude above sea level, roof material,

eave type, number of participants in the household and presence of screen on window were also collected using the CommCare data collection tool.

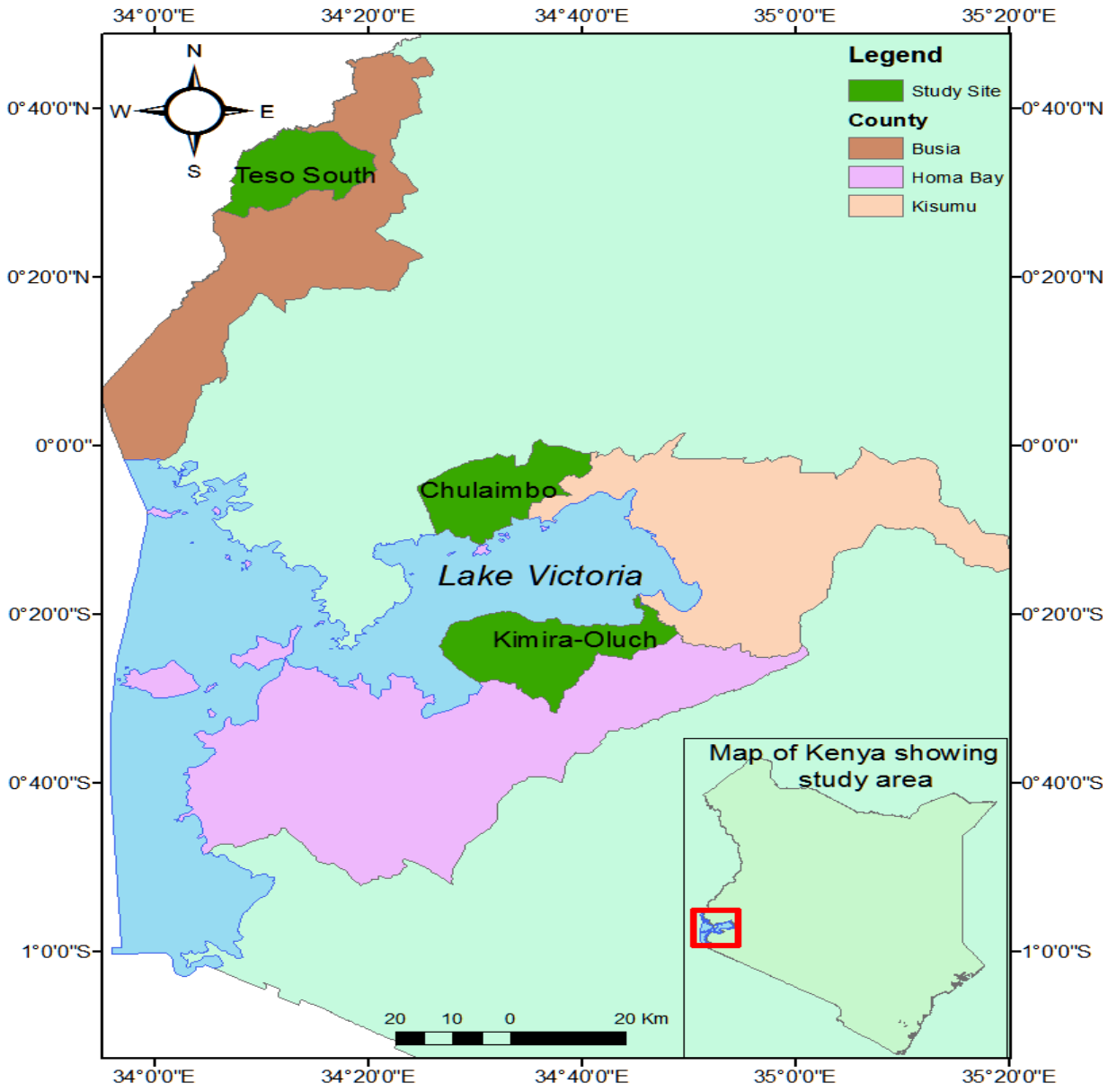

Figure 4. Map showing Chulaimbo, Kimira-Oluch and Teso South study sites. Teso South, Chulaimbo and Kimira-Oluch are proxies for a "high", "moderate" and "low" malaria transmission site respectively.

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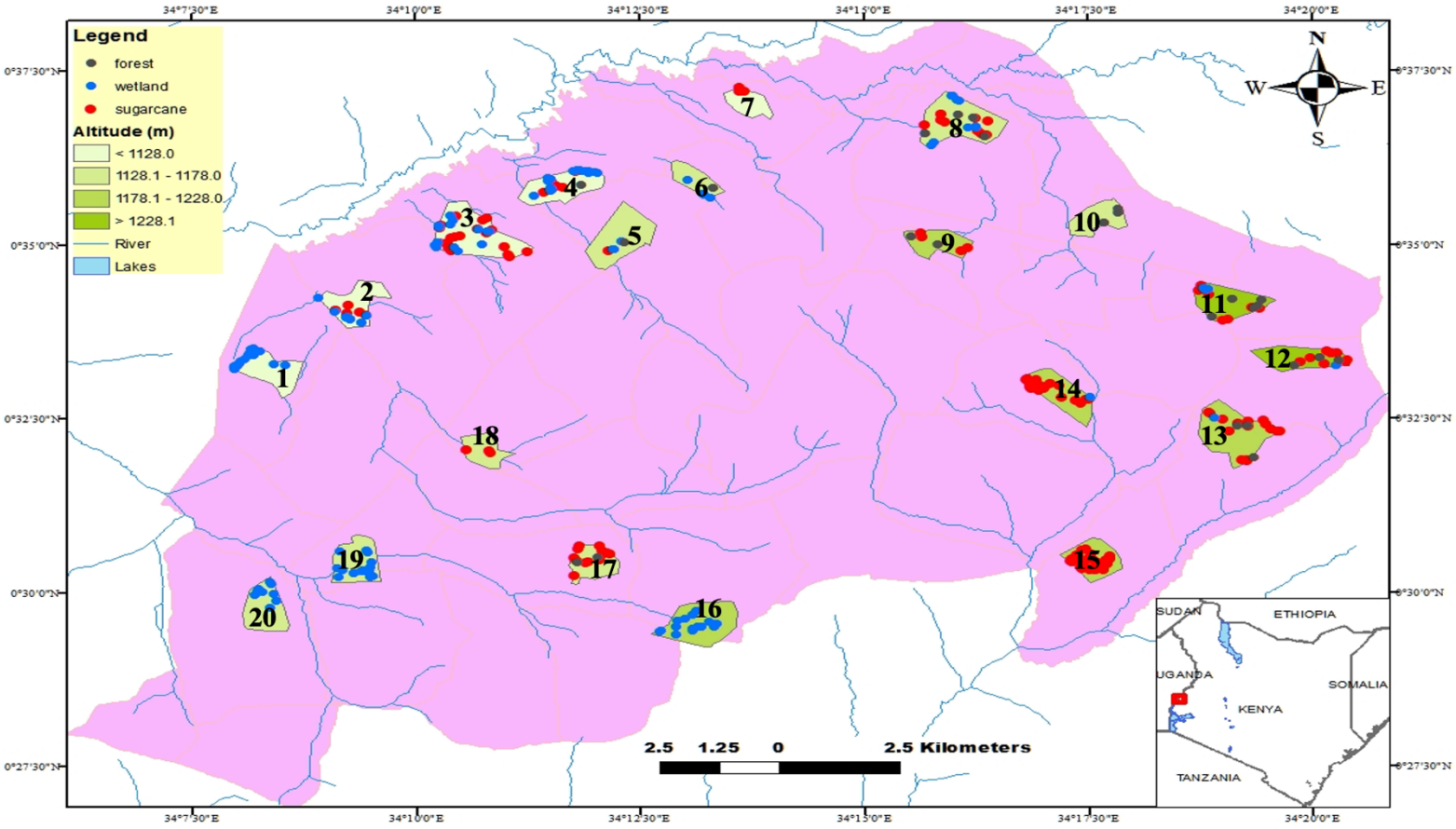

Figure 5. Map showing location of the 20 clusters in Teso South, Busia County in western Kenya. The clusters vary in altitudinal transects and they collectively span an altitudinal range of 207 metres (1077-1284 metres). Various habitats such forest plantation (shown in black dot), wetland (shown in blue dot) and sugarcane plantation (shown in red dot) were identified within the clusters. The study area is traversed by rivers (blue line).

3.3 Ethical consideration

All adults study participants aged 18 years and above gave informed consent prior to obtaining clinical and socio-demographic information and drawing a blood sample. Participants aged 13-17 years gave informed assent prior to participation in the study. Each assent was accompanied by a consent provided by parent or legal guardian of the minor. A parent or guardian was allowed to give consent on behalf of a child aged less than 13 years. Versions of the local dialects of the consent and assent forms were availed to the participants to choose from so as to make informed decision. A participant was asked to choose preferred version. Each participant was given a copy of signed assent or consent form (see Appendices A-J).

Ethical approvals to conduct the studies were granted by Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Unit (approval no. 3931) (see Appendix K), Maseno University Ethics Review Committee (MUERC protocol number 00456) (see Appendix L) and National Commission for Science Technology and Innovation (see Appendix M). Approval to ship the samples from Kenya to University of Notre Dame in the USA where some laboratory procedures were performed was granted by KEMRI - Scientific and Ethics Review Unit.

3.4 Research design

A cross sectional study design was employed in Teso South site. The sampling was done at a single time point, i.e., July 2021, shortly after the long rains season. In Chulaimbo and Kimira-Oluch, repeated cross sectional study design was employed. The sampling was done at two time-points, i.e., in the months of January-March 2019, which is during the dry season, and in the months of June-July 2019, which is shortly after the long rains season.

3.4.1 Sampling and sample size

Simple random sampling method was used. Fisher's formula (Fisher, 1998) was used to determine the minimum number of persons to be sampled in the sites.

$$\text{Fisher's formula,} \quad n = \frac{Z^2\,PQ}{D^2}$$

Where n = sample size

Z = Standard error for mean at 1.96

P = prevalence

Q = 1-prevalence

D = Absolute precision at 5%

To determine the minimum number of study participants to be sampled in Chulaimbo and Kimira-Oluch per season, this study used a 20% *P. falciparum* infection prevalence from Kenya Malaria Indicator Survey reported in these regions (Bashir *et al*., 2019). Substituting the values into the formula, the minimum number of persons required per season in each site was 246 i.e.,

$$n = \frac{Z^2\,PQ}{D^2} \quad \text{..... (1)}$$

$$n = \frac{1.96^2\ 0.20x0.80}{0.05^2} \quad \text{..... (2)}$$

$$n = 246$$

The current study sampled 262 and 419 individuals in Chulaimbo during the dry and rainy season respectively. Given the higher population density in Kimira-Oluch compared to Chulaimbo, the sample size was further increased in Kimira-Oluch, thus a total of 854 and 1324 individuals were sampled during the dry and rainy season respectively.

In Teso South site, this study used 37% *P. falciparum* infection prevalence from Kenya Malaria Indicator Survey data (Bashir *et al*., 2019). Substituting the values into the formula, the minimum number of persons required was 359 i.e.,

$$n = \frac{Z^2\,PQ}{D^2} \quad \text{..... (1)}$$

$$n = \frac{1.96^2\ 0.37x0.63}{0.05^2} \quad \text{..... (2)}$$

$$n = 359$$

This study equally aimed to investigate variation in transmission risk among clusters in the Teso South site, thus we purposively chose 20 clusters based on their differences in altitude. From each cluster, we collected blood samples from over 150 individuals using convenience sampling. The >150 persons per cluster was sufficient to give a high power (>80%) to detect ≥10% change in infection prevalence across the clusters. A total of 3061 individuals were sampled in Teso South.

3.4.2 Inclusion criteria

Across all the three sites, blood sampling was done for male and female residents from age 6 months to 99 years, and whose consent or assent was voluntarily granted.

3.4.3 Exclusion criteria

Individuals who declined to give consent or were enrolled in an ongoing antimalarial drug intervention program were excluded from the current study.

3.5 Mosquito habitat selection and sampling in Teso South

Potential mosquito breeding habitats within a 250 m radius of the households were identified during the dry season and their GPS captured. The chosen radius represents a potentially feasible area for targeted or reactive vector control. While we are not aware of trials of focal larval source management, other reactive vector control interventions applied similar geographical scales (Aidoo *et al*., 2018; Hsiang *et al*., 2020; Sturrock *et al*., 2013; Vilakati *et al*., 2021). During the dry season, larval habitats are few, fixed, and findable. Compared with rainy season, sampling potential larval habitats in the dry season is more informative about potential benefit of larval source management in reducing malaria transmission (Maheu-Giroux & Castro, 2013; WHO, 2019).

The habitats were classified as swamp, marshland, shallow well, drainage ditch, forest, pond and sugarcane plantation. The following definitions were used in the context of this study. “Swamp” was defined as waterlogged soil having trees and other woody plants as dominant vegetation. “Marshland” was defined as waterlogged soil having non-woody plants as dominant vegetation. “Shallow well” was defined as an enlarged surface water collection hole. “Sand pit” was defined as a water ground with excavation activities including for sand, murram, stone and mud. “Drainage ditch” was defined as a watercourse in lower lying area off the side of a road or in farmland. “Pond” was defined as small-medium size approximately ≤ 50 m x 30 m inland body of standing water. “River” was defined as natural body of water that flows downstream. “Forest” was defined using UNESCO standards as area of land > 0.5 ha with trees as dominant form of life and canopy cover comprising > 10% with a tree height of ≥5 m (Figure 6). A minimal interval of >5 m radius was considered between neighboring habitats.

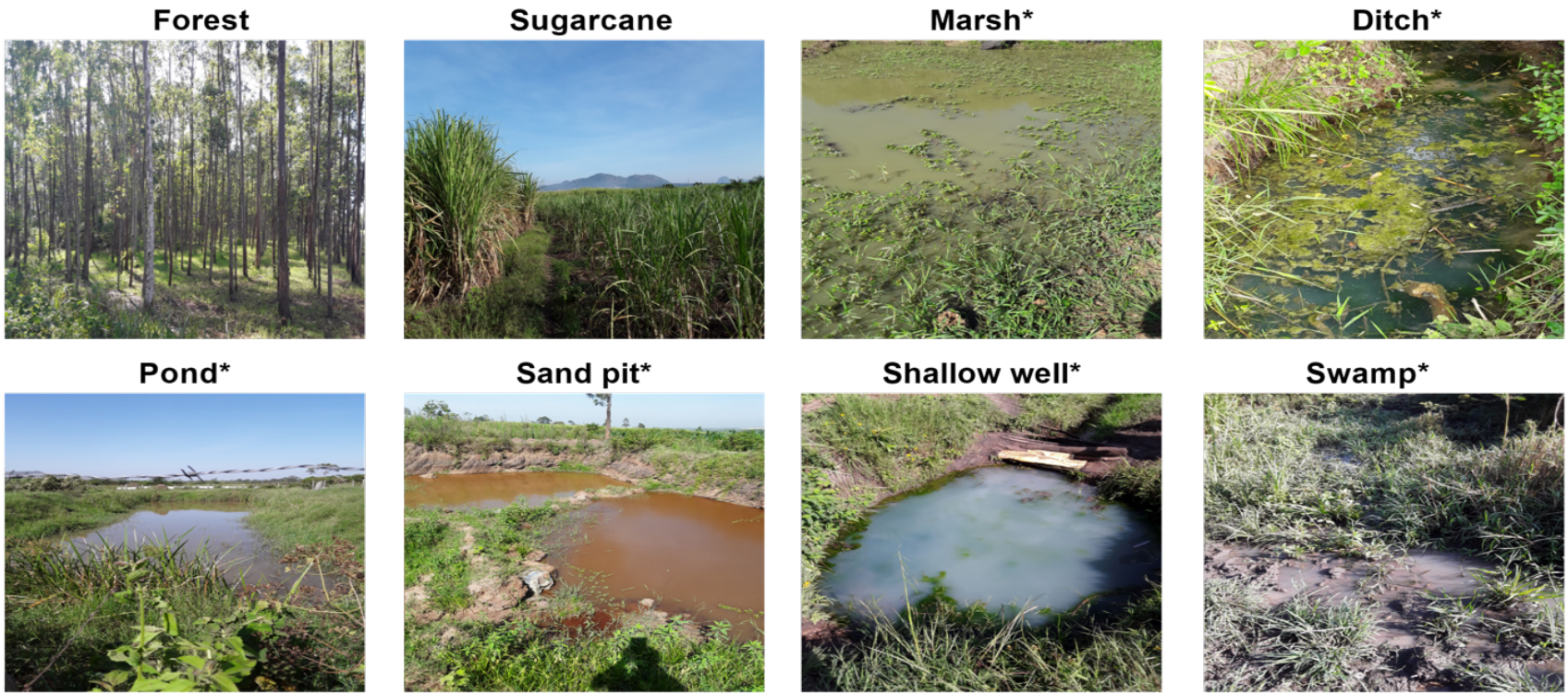

Figure 6. Habitats identified in Busia site during the dry season. Habitats marked with asterisk (*) collectively are referred to as wetlands in this article.

3.6. Blood collection, processing and storage

For each participant, 350-500 µL of finger-prick blood was collected using contact-activated lancet into Ethylene diamine tetraacetic acid (EDTA) microtainer tube (Becton Dickinson, New Jersey, United States) and transported to KEMRI laboratory in Kisumu, Kenya. Aliquots of 100 µL blood from the EDTA microtainer tube was transferred to a tube containing 500 µL of RNAprotect stabilizing reagent (Qiagen) and stored at -80℃ freezer until ribonucleic acid (RNA) extraction. The remaining blood was stored at -20℃ freezer until deoxy-ribonucleic acid (DNA) extraction.

For safety concerns, single self-lock lancets were used. Used lancets were disposed in lockable sharps containers with tight lids. Once full, the containers were disposed in incinerator. Measures to ensure the current study samples did not mix with other samples in the laboratory were put in place including storing in designated freezer(s) in specific towers. Sample storage boxes were labeled with unique identifier denoting study site name and period of collection. Inventory for all the samples in the towers were prepared to help track the samples whenever necessary, and this was important in minimizing on thawing and freezing of unintended samples.

3.7 Parasite screening and quantification by microscopy

For each participant, 6 µL and 2 µL of blood were used to prepare thick and thin blood smear slides for microscopy. Slides were stained and read by certified microscopists according to World Health Organization standard. Briefly, thin smears were fixed with methanol. Thick and thin blood smears were then stained in 10% Giemsa for 15 min, dried and examined microscopically with a high-power objective.

A slide was identified as either negative or positive for *Plasmodium* species. Asexual parasite density was calculated whenever a slide was reported as positive. Each slide was read by two independent microscopists. A third independent reader was involved in case of discrepancy in identifying a positive slide, species, and whenever differences in asexual parasite density exceeded acceptable range between the readers. Parasite density was determined by averaging the densities reported by the readers.

Briefly, the malaria blood slides were read from known blood volume in a known surface area. For thick film, 40 microscopic high-power fields were examined for asexual parasites, and parasites density determined, assuming 8000 white blood cells counts per µl of blood. For thin film, parasite density was determined from the product of parasites counts per 2000 red blood cells (RBC) and assumed RBC density of 5,000,000 cells per µl of blood. A slide was declared negative after examining 100 microscopic fields.

3.8 Parasite's genetic material extraction

Plasmodium parasites' genomic DNA was extracted for detection and quantification of *P. falciparum* asexual parasite, and RNA extracted for detection and quantification of gametocytes.

3.8.1 Parasite genomic DNA extraction

Parasite genomic DNA was extracted from 100 µl of blood using the Genomic DNA Extraction kit (Macherey-Nagel, Düren, Germany) and eluted in an equivalent volume of elution buffer. Briefly, 100 µl blood was put in sample well. Then 10 µl of proteinase K and 40 µl of lysis buffer were added to allow protein digestion and cell membrane lysis respectively. The mixture was then mixed at 1200 revolution per minute (rpm) for 10 minutes to allow homogenization. The purposes of Proteinase K and lysis buffer were to digest proteins and lyse cell membrane respectively. 12.5 µl of magnetic beads and 150 µl binding buffer were added and the mixture span at 1200 rpm for 5 minutes. The binding buffer facilitate coupling of genomic DNA in the solution to the beads. The mixture was then placed in a magnet block for 2 minutes to allow separation of bound DNA-beads complex from other materials in the solution (i.e., supernatant). Supernatant was then removed. 500 µl of wash buffer then added, mixed at 1200 rpm for 5 minutes and placed in a magnet block for 2 minutes then supernatant removed. 300 µl of second wash buffer then added, span at 1200 rpm for 5 minutes and placed in a magnet block for 2 minutes then supernatant removed. 300 µl of 80% alcohol then added, mixed at 1200 rpm for 5 minutes and placed in a magnet block for 2 minutes then supernatant removed. The alcohol precipitated the DNA. The DNA was then air-dried for 15 minutes. 100 µl of elution buffer then added, mixed at 1200 rpm for 5 minutes and placed in a magnet block for 2 minutes then DNA solution transferred into storage tube or plate. The elution buffer detaches DNA from the beads by weakening the magnetic field. The extracted DNA was stored at -20 ℃ freezer.

3.8.2 Parasite RNA extraction

For all the gametocytes assays, ribonucleic acid (RNA) was extracted from 100 µl of blood using the pathogen Nucleic Acid Extraction kit (Macherey-Nagel, Düren, Germany) and eluted in 50 µl volume of elution buffer, i.e., RNA was concentrated two-folds during extraction. Briefly, tube containing 100 µl of blood preserved in 500 µl of RNAprotect buffer was span at 8500 rpm for 10 minutes, and supernatant removed This was followed by addition of 1 ml of phosphate buffered saline (PBS) then spinning at 8500 rpm for 10 minutes, and supernatant removed. 50 µl of PBS was then added to the pellet then transferred to sample well. 10 µl of proteinase K, 90 µl of lysis buffer and 2 µl of carrier RNA were added, then mixed at 1200 rpm for 15 minutes. 10 µl of magnetic beads and 300 µl of binding buffer were added and mixed at 1200 rpm for 2 minutes. The mixture was then placed in a magnet block for 2 minutes then supernatant removed. 450 µl of wash buffer then added, mixed at 1200 rpm for 2 minutes and placed in a magnet block for 2 minutes then supernatant removed. 300 µl of second wash buffer then added, span at 1200 rpm for 2 minutes and placed in a magnet block for 2 minutes then supernatant removed. 300 µl of 80% alcohol then added, mixed at 1200 rpm for 2 minutes and placed in a magnet block for 2 minutes then supernatant removed. The RNA was then air-dried for 10 minutes. 50 µl of elution buffer then added, mixed at 1200 rpm for 2 minutes and placed in a magnet block for 2 minutes then RNA solution transferred into storage tube or plate. The extracted RNA was stored at -80 ℃ freezer.

3.8.3 DNase treatment

The RNA is often co-extracted with DNA (i.e., asexual parasite). This leads to false amplification signal and over quantification of gametocyte densities (Meerstein-Kessel *et al*., 2018). Therefore, the DNA has to be removed through digestion. Various approaches that digest the DNA genetic material include addition of DNase enzyme or N-Acetyl-glucosamine. In this study, we used rDNase enzyme treatment kit (Macherey-Nagel, Düren, Germany).

Briefly, 25 µl of extracted RNA was transferred to sample well. 2.5 µl of rDNase mix reconstituted from 1 volume of DNase enzyme for every 10 volumes of enzyme buffer, then mixed at 1200 rpm for 2 minutes followed by incubation for 15 minutes at ambient temperature. 10 µl of magnetic beads and 300 µl of binding buffer were added and mixed at 1200 rpm for 10 minutes. The mixture was then placed in a magnet block for 2 minutes then supernatant removed. 3000 µl of wash buffer then added, mixed at 1200 rpm for 2 minutes and placed in a magnet block for 2 minutes then

supernatant removed. 300 µl of second wash buffer then added, span at 1200 rpm for 1 minutes and placed in a magnet block for 2 minutes then supernatant removed. 300 µl of 80% alcohol then added, mixed at 1200 rpm for 2 minutes and placed in a magnet block for 2 minutes then supernatant removed. The RNA was then air-dried for 10 minutes. 25 µl of elution buffer then added, mixed at 1200 rpm for 2 minutes and placed in a magnet block for 2 minutes then RNA solution transferred into storage tube or plate. The treated RNA was stored at -80 ℃ freezer.

3.9 Molecular asexual parasite and gametocyte screening and quantification

Asexual parasite (hereafter refers to parasite) and gametocytes were screened using quantitative polymerase chain reaction (qPCR) and quantitative reverse transcription PCR (RT-qPCR) respectively.

3.9.1 Molecular parasite screening and quantification

Extracted DNA was screened for *P. falciparum* parasite infection using *var* gene acidic terminal sequence (*var*ATS) qPCR assay according to previously published protocol (Hofmann *et al*., 2015). Briefly, amplification of the target *var*ATS was undertaken in a single step 12 µl reaction mix composed of 4 µl of genomic DNA template, 6 µl of 2X Fast Advanced master mix, 0.48 µl of 10 µM probe, 0.48 µl of 10 µM primers, and 1.04 µl of nuclease-free water. The DNA was amplified using forward primer (5'CCCATACACAACCAAYTGGA3') and reverse primer (5'TCGCACATATCTCTATGTCTATCT3') and probe (5'6-FAM-TRTTCCATAAATGGT-NFQ-MGB3'). Each cycle entailed initial denaturation at 50 ℃ for 2 minutes, second denaturation at 95 ℃ for 2 minutes, 45 cycles of primer annealing at 95 ℃ for 10 seconds and extension at 55 ℃ for 30 seconds. The qPCR results were converted to *var*ATS copies/µL using external standard curve of ten-fold serial dilutions (5-steps) of 3D7 *P. falciparum* parasites. The parasites *var*ATS copies/µL were quantified using digital PCR (dPCR).

Briefly, the dPCR assay targeting *var*ATS was undertaken in a single step 22 µl reaction mix composed of 2 µl of genomic DNA template, 11 µl of 1X probe supermix without deoxyuridine triphosphate (dNTP), 0.55 µl of 10 µM probe, 1.98 µl of 10 µM primers, and 6.47 µl of nuclease-free water using primers (5'CCCATACACAACCAAYTGGA3') and (5'TTCGCACATATCTCTATGTCTATCT3') and probe (5'6-FAM-TRTTCCATAAATGGT-NFQ-MGB3'). Each cycle entailed initial denaturation at 95 ℃ for 10 minutes, second denaturation at 94 ℃ for 30 seconds, 45 cycles of primer annealing at 55 ℃ for 1 minute and

extension at 98 ℃ for 10 minutes. Parasite density was determined by dividing *var*ATS copies/µL by 20, reflecting the approximate *var*ATS copy numbers per genome (Hofmann *et al*., 2015). The *var*ATS assays were specific to *P. falciparum* parasite detection.

3.9.2 Molecular gametocyte screening and quantification

For gametocyte detection by quantitative reverse - transcription PCR (RT-qPCR), only *P. falciparum* qPCR-positive samples were screened. Gametocytes were quantified by targeting female *pfs25* messenger ribonucleic acid (mRNA) transcripts using one-step RT-qPCR assays (Alkali Scientific, Florida, United States) with slight modifications.

Briefly, amplification of the target *Pfs25* was undertaken in a single step 12 µl reaction mix composed of 2 µl of genomic RNA template, 6 µl of1X Radiant SYBR Green 1-step Lo-Rox mix, 0.6 µl of reverse transcriptase, 1 µl of 10 µM primers, and 2.4 µl of nuclease-free water. RNA was amplified using *pfs25* forward primer (5'CGTTTCATA CGCTTGTAAATG3') and *pfs25* reverse primer (5'TTAACAGGATTGCTTGTATCTAA3'). Each cycle entailed initial denaturation at 45 ℃ for 10 minutes, second denaturation at 95 ℃ for 2 minutes, 45 cycles of primer annealing at 95 ℃ for 10 seconds and extension at 58 ℃ for 30 seconds. The *pfs25* RT-qPCR results were converted to pfs25 transcript copies/µL using external standard curve of ten-fold serial dilutions (5-steps) of DNA of 3D7 culture parasites quantified by dPCR. About 10–20 *pfs25* transcripts are detected per gametocyte (reported as 90 transcripts/gametocyte when measured against a circular plasmid, which reflects a 5 to 10-folds overestimate) (Koepfli *et al*., 2016; Wampfler *et al*., 2013). The DNAse treatment was successful as subset of RNA samples tested for genomic DNA by *var*ATS qPCR were all negative.

Briefly, the dPCR assay targeting *pfs25* was undertaken in a single step 22 µl reaction mix composed of 2 µl of genomic DNA template, 11 µl of 1X probe supermix, 0.22 µl of 10 µM probe, 0.66 µl of 10 µM primers, 8.12 µl of nuclease-free water, *pfs25* forward and reverse primers and probe (5'-5HEX/TGTAAGAAT/ZEN/GTAACTTGTGGTAACGGT/3IABkFQ3'). Each cycle entailed initial denaturation at 95 ℃ for 10 minutes, second denaturation at 94 ℃ for 30 seconds, 45 cycles of primer annealing at 54 ℃ for 1 minute and extension at 98 ℃ for 10 minutes.

3.10 Data protection

Confidentiality was maintained on all the information obtained in the course of the study. Data was maintained in password protected personal laptop computer and backed up in protected Dropbox and hard drive kept in lockable cabinet. Data on any other encrypted computers did not include participant names. All hard copies materials were coded using unique personal identifiers. As it is required in general practice that persons doing research involving human must have knowledge about protection of human subjects in research, prior to the start of the study, I was trained in principles of research involving human subjects.

3.11 Data collection and processing

Data was collected using a soft copy and hard copy of a pre-formatted and pre-tested questionnaire. For soft-copy, CommCare data collection tool was used. The data collected were downloaded in excel sheet format for cleaning, validation and subsequent analysis.

3.12 Data analyses

Data was statistically analyzed using R v.4.1.0 and GraphPad Prism v.9.0 softwares. To achieve objective 1, parasite densities and gametocyte densities were $\log_{10}$ transformed and geometric means per µL blood calculated whenever densities were reported. The Shapiro-Wilk test was employed to test normality of data following log transformation. Differences in parasite and gametocyte densities between seasons were compared using unpaired *t*-test. Differences in prevalence, proportions of gametocyte positive infections, or proportion of submicroscopic infections between seasons were compared using the Chi-squared (χ^2) test or Fisher's exact test. Multiple logistic regression analysis was used to determine predictors of gametocyte positive infections. Predictors for gametocyte density were determined by multiple linear regression analysis.

To achieve objective 2, differences in parasite densities across the low, moderate and high transmission sites were determined by analysis of variance (ANOVA) test. Tukey's test was employed to determined differences between the sites, whenever mean densities differed across the sites. Differences in gametocyte densities between low and moderate transmission sites were determined by unpaired *t*-test. Differences in parasite prevalence, or proportion of submicroscopic infections across the three sites were determined by χ^2 test for trends in proportions. Differences

in gametocyte prevalence, or proportion of gametocyte positive infections between low and moderate transmission sites were determined by χ^2 test or Fisher's exact test.

To achieve objective 3, association between altitude and prevalence, habitat number and prevalence, and habitat number and altitude were tested by Spearman correlations. Heterogeneity in parasite prevalence, or proportion of submicroscopic infections across the clusters were determined using the χ^2 test for trends in proportions. Determination of distance from the households to the nearest river or other habitats were done in ArcMap version 10.3.

A generalized linear mixed effect model (GLMM) with the logit link was used to analyze the dichotomous data on malaria infection detection by PCR and microscopy, respectively. Specifically, the model is formulated as logit (μ) = log (μ/(1−μ)) = $\boldsymbol{X\beta}$ + $\boldsymbol{Zb}$, where $\boldsymbol{\beta}$ is the vector of beta coefficients corresponding to the fixed effects in the design matrix $\boldsymbol{X}$, $\boldsymbol{b}$ is the vector of beta coefficients for unknown random effects with corresponding design matrix $\boldsymbol{Z}$, and $\boldsymbol{\mu}$ is the probability of malaria infection given $\boldsymbol{X}$ and $\boldsymbol{Z}$. $\boldsymbol{X}$ includes age category, sex, altitude, education level of household head, kitchen location, roof material, screens window, eave type, household population, bed net coverage percentage, distance to the nearest river, distance to the nearest habitat within cluster, habitat number per cluster, and habitat number within 250 m of household as fixed effects, and $\boldsymbol{Z}$ includes two random effects of cluster and household nested within cluster. The following numerical attributes were standardized before model fitting: altitude, household population, distance to the nearest river, distance to the nearest habitat within cluster, habitat number per cluster, and habitat number within 250 m of household. The results were transformed back to the original scale for reporting.

The maximum likelihood estimates exp ($\hat{\beta}$) for the odds ratio (OR) of malaria infection associated with each covariate was obtained, along with the 95% confidence interval, via the Laplace approximation of the marginal likelihood function. The raw p-value on the effect of each covariate was calculated, as well as the False Discovery Rate (FDR) -adjusted p-value (or q-value). The FDR-adjusted p-value is calculated by $q_{(i)} = \min_{\mathrm{k=i,\ldots m}} \{\min (p_{(i)} m/k, 1)\}$ for $i = 1, \ldots, m$, where $p_{(i)}$ is the i-th smallest p-value among the m p-values associated with m multiple tests.

From the GLMM, the clustering effects at the cluster, and household within cluster levels was estimated after adjusting for the model covariates $\boldsymbol{X}$. Median odds ratio method (MOR) was used to estimate the clustering effects (Larsen & Merlo, 2005). The MOR value is always ≥ 1. When there is no clustering effect, MOR is 1; the further way MOR is from 1, the stronger the clustering effect. Specifically, the MOR for cluster in this study is exp $(\sqrt{2\sigma_1}\Phi^{-1}(0.75))$ and the MOR for household nested within cluster is exp $(\sqrt{2\sigma_2}\Phi^{-1}(0.75))$, where σ_1^2 is the variance of the random effect of cluster and σ_2^2 is the variance of the random effect of household, respectively.

CHAPTER FOUR

RESULTS

4.1 Impact of seasonality on *Plasmodium falciparum* parasite transmission potential

None of the participants in the study had received the "RTS,S" malaria vaccine that was administered to children who participated in an implementation trial in neighboring counties in western Kenya (Otieno *et al*., 2016). Across Kimira-Oluch (hereafter refered to as Homa Bay) and Chulaimbo (hereafter refered to as Kisumu), 2859 samples with age distribution representative of the population were analyzed in the rainy and dry seasons. The distribution of ages and sex of the study participants sampled in Homa Bay and Kisumu across the seasons are summarized in Table 1.

Table 1. Demographic characteristics across seasons per site

	Kisumu		Homa Bay	
	dry	rainy	dry	rainy
	N=262	N=419	N=854	N=1324
Demographic data				
Age group in years	N (%)	N (%)	N (%)	N (%)
<5	45 (17.2)	62 (14.8)	158 (18.5)	227 (17.1)
5-14	75 (28.6)	173 (41.3)	154 (18.0)	419 (31.6)
15-39	73 (27.9)	120 (28.6)	276 (32.3)	377 (28.5)
40-59	43 (16.4)	41 (9.8)	150 (17.6)	182 (13.7)
>59	26 (9.9)	23 (5.5)	116 (13.6)	119 (9.0)
Female	155 (59.2)	234 (55.8)	578 (67.7)	840 (63.4)

In both seasons, the prevalence of *P. falciparum* infection by qPCR was significantly higher in Kisumu than Homa Bay (rainy: 48.2% vs. 7.8%, $P < 0.001$, dry: 27.1% vs. 9.4%, $P < 0.001$, Figure 7A). In Kisumu, the parasite prevalence was significantly higher in the rainy season ($P < 0.001$), but it did not differ between seasons in Homa Bay ($P = 0.192$, Figure 7A). A sensitivity of 100 blood stage parasites/µL (corresponding to limit of detection for *Plasmodium* parasite detection

by conventional methods) was assumed to determine proportion of infections that would be detected by light microscopy. Similar trend in prevalence between the seasons per site was observed when infections were diagnosed by microscopy (Kisumu: dry vs rainy, 8.8% vs 16.0%, *P*=0.010), Homa Bay: dry vs. rainy, 2.0% vs. 1.5%, *P*=0.500)).

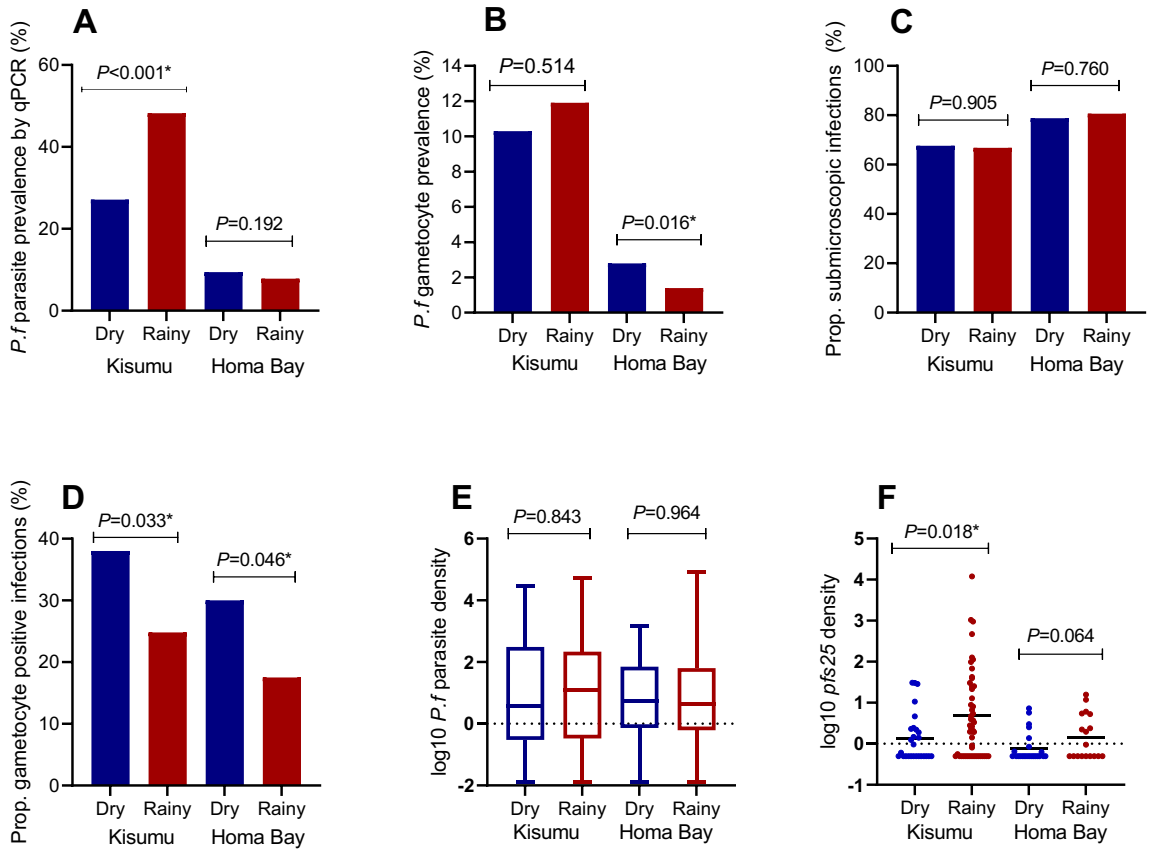

Figure 7. Trends of *P. falciparum* parasitological indices across dry and rainy seasons in Kisumu and Homa Bay. A: Bar graphs showing parasite prevalence across seasons per site. B: Bar graphs showing gametocyte prevalence across seasons per site. C: Bar graphs showing proportion of submicroscopic infections across seasons per site. D: Bar graphs showing proportion of gametocyte positive infections across seasons per site. E: Box plots showing mean parasite density across seasons per site. F: Scatter plots showing mean *pfs25* density across seasons per site. *Pfs25* density is proxy for gametocyte density. Asterisk (*) indicate significant at *P*<0.05. Parasite density was determined by qPCR, and gametocyte density by RT-qPCR. Differences in densities

were measured using *t*-test, and differences in proportions measured using Chi-squared test. qPCR: quantitative real time polymerase chain reaction. RT-qPCR: reverse transcription qPCR.

The prevalence of gametocytes in the population did not differ between the seasons in Kisumu but was significantly higher in the dry than in the rainy season in Homa Bay (Figure 7B). Proportion of submicroscopic infections (i.e., infections of < 100 parasites/µL relative to all infections) did not differ between seasons in both sites (Figure 7C). The proportion of all individuals with blood stage parasites detected by qPCR who were positive for gametocytes (the proportion of gametocyte positive infections) was significantly higher in the dry season than in rainy season in both sites (Figure 7D). Parasite densities by qPCR did not differ between seasons in both sites (Figure 7E). In constrast, mean gametocyte densities were 3.5-fold significantly higher in the rainy than dry season in Kisumu. Similar trends were observed in Homa Bay, where mean gametocyte densities were 1.8-fold higher in the rainy season than in the dry season (Figure 7F).

Given the similarity of seasonal patterns in parasitological indices in Homa Bay and Kisumu, data was pooled by season thus the subsequent results across the seasons are presented for both sites combined. Parasite prevalence was significantly higher in the rainy season (17.5%, 305/1743) compared to the dry season (13.5%, 151/1116, P=0.005, Figure 8A). Prevalence of gametocytes did not differ between the seasons (dry: 4.6% (51/1116), rainy: 3.9% (68/1743), Figure 8B). No difference in the proportion of submicroscopic infections was observed between seasons (dry: 73.5% (111/151), rainy: 71.5% (218/305), P = 0.648, Figure 8C). 52.9% (63/119) of all infections with gametocytes detected by RT-qPCR were submicroscopic across all surveys, with equal proportions in the dry (52.9% (27/51)) and rainy season (52.9% (36/68)). The proportion of gametocyte positive infections was significantly higher in the dry season (33.8%, 51/151) compared to the rainy season (22.3%, 68/305, P = 0.009, Figure 8D). Parasite densities did not differ across the seasons (Figure 8E). In contrast, mean gametocyte densities were 3-folds significantly higher in the rainy season compared to dry season (Figure 8F). Mean *pfs25* densities were 3-folds lower in submicroscopic infections compared to patent infections (1.26 vs. 3.64 transcripts/µL, P = 0.003).

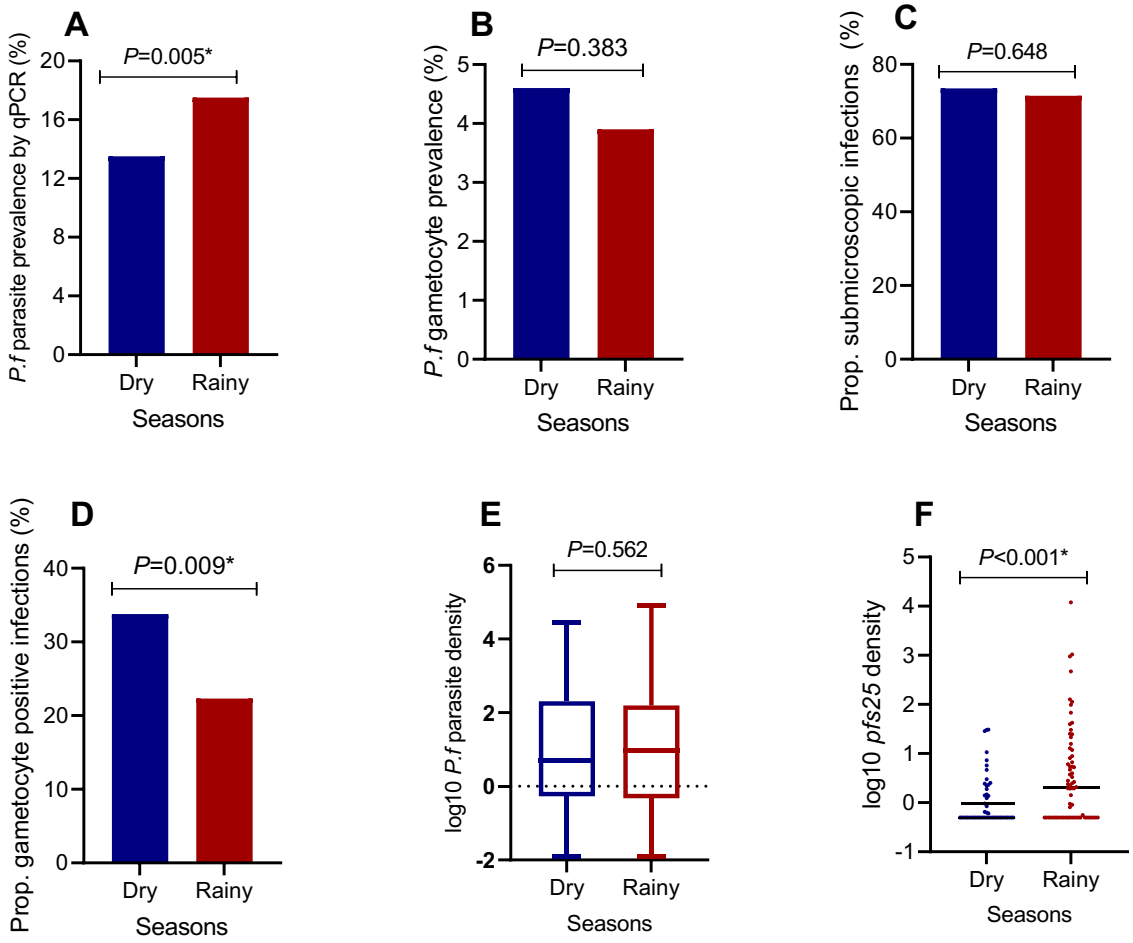

Figure 8. Trends of *P. falciparum* parasitological indices across dry and rainy seasons in Homa Bay and Kisumu combined. A: Bar graphs showing parasite prevalence across seasons. B: Bar graphs showing gametocyte prevalence across seasons. C: Bar graphs showing proportion of submicroscopic infections across seasons. D: Bar graphs showing proportion of gametocyte positive infections across seasons. E: Box plots showing mean parasite density across seasons. F: Scatter plots showing mean *pfs25* density across seasons. *Pfs25* density is proxy for gametocyte density. Asterisk (*) indicate significant at $P<0.05$. Parasite density was determined by qPCR, and gametocyte density by RT-qPCR. Differences in densities were measured using *t*-test, and differences in proportions measured using Chi-squared test. qPCR: quantitative real time polymerase chain reaction. RT-qPCR: reverse transcription qPCR.

4.1.1 Predictors of gametocyte infections

The probability to detect gametocytes was correlated with parasite density. Each 10-folds increase in parasite genome copies resulted in 3.23-folds higher odds in carrying *pfs25* transcripts. In multivariable analysis, only parasite density and season were found to be significantly associated with the probability that an individual was gametocyte positive (Table 2). Age group ($P = 0.195$), sex ($P=0.214$), and site ($P=0.364$) were not associated. Likewise, gametocyte density was only significantly associated with parasite density and season, but not site ($P = 0.063$), age group ($P = 0.733$), or sex ($P = 0.611$) (Table 2).

Pronounced variation in infections having gametocyte was observed, with many medium- or high-density infections not carrying any detectable gametocytes. Among infections with a density of >2000 *var*ATS copies/µL (corresponding to >100 parasites/µL), in the rainy season 60.0% (24/40) carried gametocytes versus 36.8% (32/87) in the dry season ($P = 0.014$).

Table 2. Multivariable predictors of gametocyte positivity and density

p*fs25* positivity	**aOR**	***P* value**
log10 *Pf* copies	0.53	<0.001
Rainy season	-0.716	0.002
Constant	-1.198	0.005
log10 *pfs25* density	**Coefficient**	***P* value**
log10 *Pf* copies	0.246	<0.001
Rainy season	0.42	0.004
Constant	-1.098	<0.001

aOR: adjusted odds ratio. Predictors of gametocyte positivity is determined by multiple logistic regression while for gametocyte density by multiple linear regression.

Parasite prevalence differed significantly across age groups and was highest among school-age children i.e., 5-14 years (P<0.001, Figure 9A). The proportion of gametocyte carriers was substantially higher among 5-14 years and 15-39 years (Figure 9B). Parasite prevalence was higher in males than females (21.4% vs. 12.8%, $P < 0.001$). Parasite densities differed significantly across age groups ($P < 0.001$, Figure 9C), and the densities peaked in children aged 5-14 years (Figure 9C). Similarly, gametocyte densities differed significantly across age groups ($P < 0.012$, Figure 9D), and the densities peaked in children aged 5-14 years (Figure 9D)

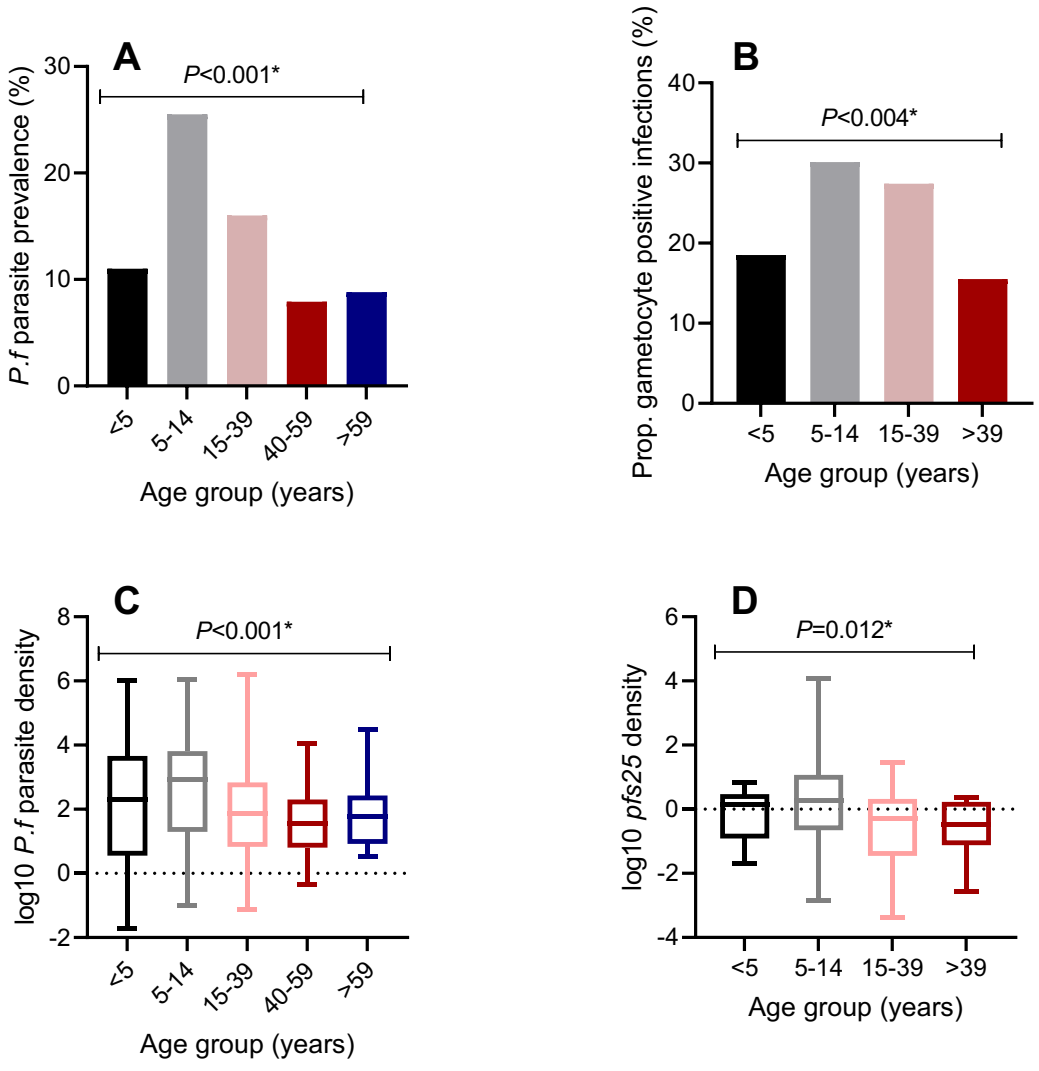

Figure 9. Age trends of parasite and gametocyte carriers and densities. A: Bar graphs showing parasite prevalence across age groups. B: Bar graphs showing proportions of gametocyte positive

infections across age groups. C: Box plots showing mean parasite density across age groups. D: Box plots showing mean *pfs25* density across age groups. *Pfs25* density is proxy for gametocyte density. Asterisk (*) indicate significant at $P<0.05$. Parasite density was determined by qPCR, and gametocyte density by RT-qPCR. Differences in densities were measured using analysis of variance test, and differences in proportions measured using Chi-squared test of trends in proportions. qPCR: quantitative real time polymerase chain reaction. RT-qPCR: reverse transcription qPCR.

4.2 Trends in parasitological indices across regions of varying transmission intensities

Parasitological indices were compared across regions of differential transmission during similar transmission periods.

4.2.1 Trends in parasitological indices in the dry season

Two sites e.g., Homa Bay (low transmission), Kisumu (moderate transmission) were sampled during the dry season. Participants demographic data is provided in Table 1. Parasite prevalence by qPCR differed significantly between Homa Bay and Kisumu, and was 3-folds higher in Kisumu compared to Homa Bay ($P<0.001$, Figure 10A). Similar trend was observed by microscopy, whereby a 4-folds increase in prevalence was recorded in Kisumu than in Homa Bay. Population gametocyte prevalence was significantly higher in Kisumu compared to Homa Bay ($P<0.001$, Figure 10B).

Proportion of submicroscopic infections (i.e., infections of <100 parasites/µL relative to all infections) did not differ between the sites ($P=0.121$, Figure 10C). Also, proportion gametocyte positive infections (i.e., proportion of blood stage parasites reservoirs detected by qPCR who carried gametocytes) did not differ between Kisumu and Homa Bay ($P=0.385$, Figure 10D). Geometric mean parasite density by qPCR did not differ between Homa Bay and Kisumu ($P = 0.825$, Figure 10E). Geometric mean gametocyte density (i.e., mean *pfs25* density) was nearly 2-folds higher in Kisumu than Homa Bay, however, the difference was not statistically significant ($P=0.082$, Figure 10F).

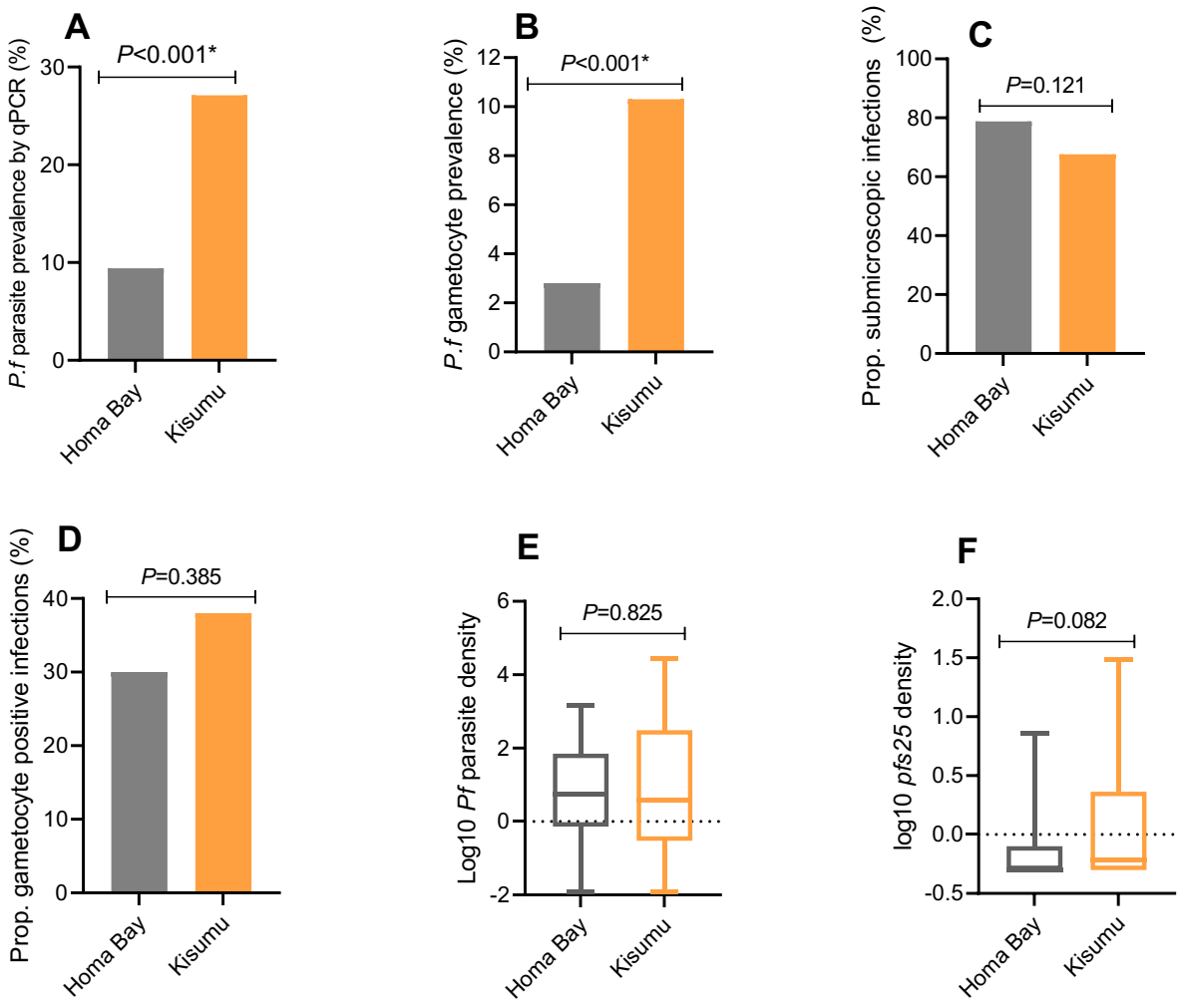

Figure 10. Trends of parasitological indices in Homa Bay and Kisumu in the dry season. A: Bar graphs showing parasite prevalence. B: Bar graphs showing gametocyte prevalence. C: Bar graphs showing proportion of submicroscopic infections. D: Bar graphs showing proportion of gametocyte positive infections. E: Box plots showing mean parasite density. F: Box plots showing mean *pfs25* density. *Pfs25* density is proxy for gametocyte density. Asterisk (*) indicate significant at $P<0.05$. Parasite density was determined by qPCR, and gametocyte density by RT-qPCR. Differences in densities were measured using *t*-test, and differences in proportions measured using Chi-squared test. qPCR: quantitative real time polymerase chain reaction. RT-qPCR: reverse transcription qPCR.

4.2.2 Trends in parasitological indices in the rainy season

In the rainy season, participants from three sites e.g Homa Bay (low transmission), Kisumu (moderate transmission) and Busia (high transmission) were sampled. The participants demographic data from Homa Bay and Kisumu is provided in Table 1, and from Busia is provided in Appendix N.

Parasite prevalence by qPCR was significantly higher in Kisumu and Busia compared to Homa Bay (P<0.001, Figure 11A), Prevalence was similar in Kisumu and Busia, but was 6-folds lower in Homa Bay compared to either Kisumu or Busia. By microscopy, prevalence was 10-folds and 13-folds lower in Homa bay compared to Kisumu and Busia respectively.

Geometric mean parasite density differed significantly across the three sites, and was highest in Busia and lowest in Homa Bay (P<0.001, Figure 11B). The densities were nearly 2-folds and 7-folds higher in Kisumu and Busia respectively compared to Homa Bay (Figure 11B). In contrast, proportion of submicroscopic infections differed significantly across the sites, and was highest in Homa Bay and lowest in Busia (P<0.001, Figure 11C).

Proportion gametocyte positive infections did not differ between Kisumu and Homa Bay (P=0.194, Figure 11D). In contrast, population gametocyte prevalence was significantly higher in Kisumu compared to Homa Bay (P<0.001, Figure 11E). Geometric mean gametocyte density (i.e., mean *pfs25* density) was 3-folds higher in Kisumu than Homa Bay, with a borderline significance (P=0.052, Figure 11F).

Taken together, trends in parasitological indices across the sites were similar in both dry and rainy seasons but were more pronounced during the rainy season. Higher parasite prevalence, gametocyte prevalence, parasite density, and gametocyte density were observed with increasing transmission intensity. In contrast, an inverse trend in proportion of submicroscopic infections was observed with increasing transmission intensity. Proportion of gametocyte positive infections did not differ between the sites.

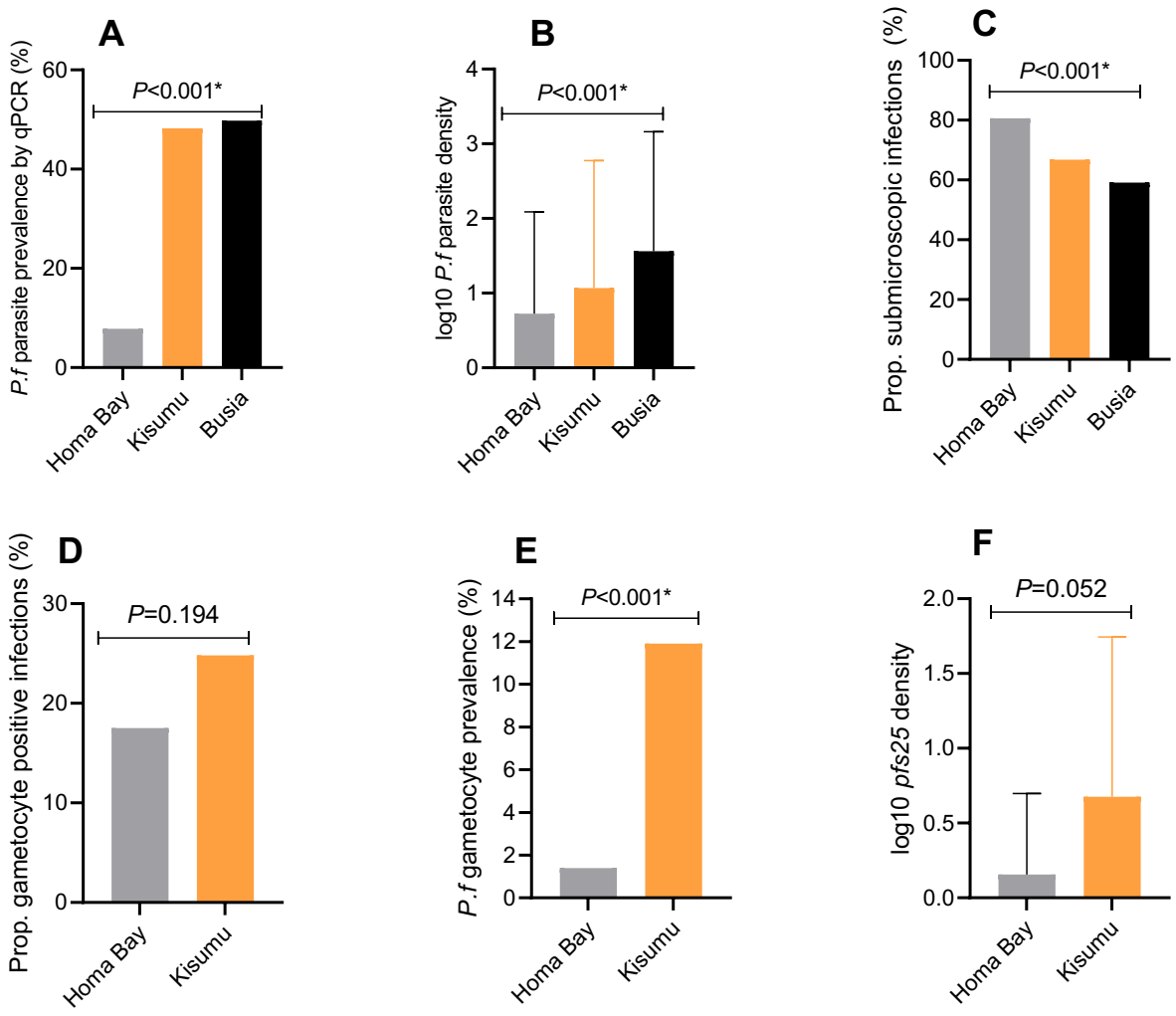

Figure 11. Trends of parasitological indices in Homa Bay, Kisumu and Busia in the rainy season

A: Bar graphs showing parasite prevalence. B: Box plots showing mean parasite density. C: Bar graphs showing proportion of submicroscopic infections. D: Bar graphs showing proportion of gametocyte positive infections. E: Bar graphs showing gametocyte prevalence F: Box plots showing mean *pfs25* density. *Pfs25* density is proxy for gametocyte density. Asterisk (*) indicate significant at $P<0.05$. Parasite density was determined by qPCR, and gametocyte density by RT-qPCR. Differences in parasite densities were measured using analysis of variance test, and differences in gametocyte densties by *t*-test. Differences in proportions measured using Chi-squared test. qPCR: quantitative real time polymerase chain reaction. RT-qPCR: reverse transcription qPCR.

4.3 Small scale variation in malaria transmission risk and predictors for transmission

To explore changes in prevalence across small geographic scales, over 150 participants from each of 20 clusters, age 1 to 99 years representative of the general population, were sampled in Busia. Parameters including participants, households and cluster level covariates are shown in Appendix N. Most of the participants (94.9%, 2906/3061) did not report clinical symptoms of malaria within two days prior to blood draw.

4.3.1. Parasitological data in all clusters combined

Across all clusters, *P. falciparum* parasites were detected in 19.5% (596/3061) of individuals by microscopy and in 49.8% (1524/3061) of individuals by qPCR. Submicroscopic infections (i.e., infections detected by qPCR but not by microscopy) accounted for 60.9% (928/1524) of the infections. The geometric mean parasite density by qPCR was 34.12 parasites/µL, with a 95% confidence interval (CI95) of 24.80 to 46.81 parasites/µL. Densities in submicroscopic infections (4.61 parasites/µL (CI95: 3.72, 5.70)) were statistically significantly lower (P<0.001) than those in microscopy-positive (1018.19 parasites/µL (CI95: 794.50 1304.87)).

Across all clusters, 746 households were sampled, with 1 to 16 individuals per household (median = 5). Majority of the households (98.3%, 733/746) had at least one bed net. 77.2% (576/746) of the households had at least one *P. falciparum* infected individual by qPCR, and 45.0% (336/746) of the households had a least one infection by microscopy. Detecting an infection by microscopy in a household was not a predictor for the presence of submicroscopic infections. Among 336 households with at least one microscopy positive individual, submicroscopic infections were detected in 29.8% (514/1722) of household members. Among 410 households with no microscopy positive infections, submicroscopic infections were detected in 32.0% (429/1339) of household members.

4.3.2 Variation in infection prevalence across clusters

Prevalence by microscopy and qPCR varied significantly across the clusters. By microscopy, prevalence per cluster ranged from 7.8% (12/153) to 32.7% (50/153, P<0.001). By qPCR, prevalence per cluster ranged from 26.1% (40/153) to 70.6% (108/153, P<0.001). Prevalence per cluster by microscopy and qPCR were strongly correlated (R=0.758, P<0.001, Figure 12A).

The proportion of submicroscopic infections (defined as percentage submicroscopic infection relative to infections identified by qPCR) differed significantly across clusters, ranging from 47.7% to 77.1% (P<0.002). The proportion of submicroscopic infections and parasite prevalence by microscopy exhibited a strong and significant inverse correlation (R=-0.840, P<0.001, Figure 12B). In four clusters with highest prevalence, 47.7-53.3% of infections were submicroscopic, while in four clusters with lowest prevalence, 72.2-77.1% were submicroscopic. In contrast, parasite prevalence by qPCR and the proportion of submicroscopic infections were not correlated (P=0.105, Figure 12C). The bed net usage varied across clusters (74%-100%, P<0.001), with 16/20 clusters having over 90% of participants using bed nets.

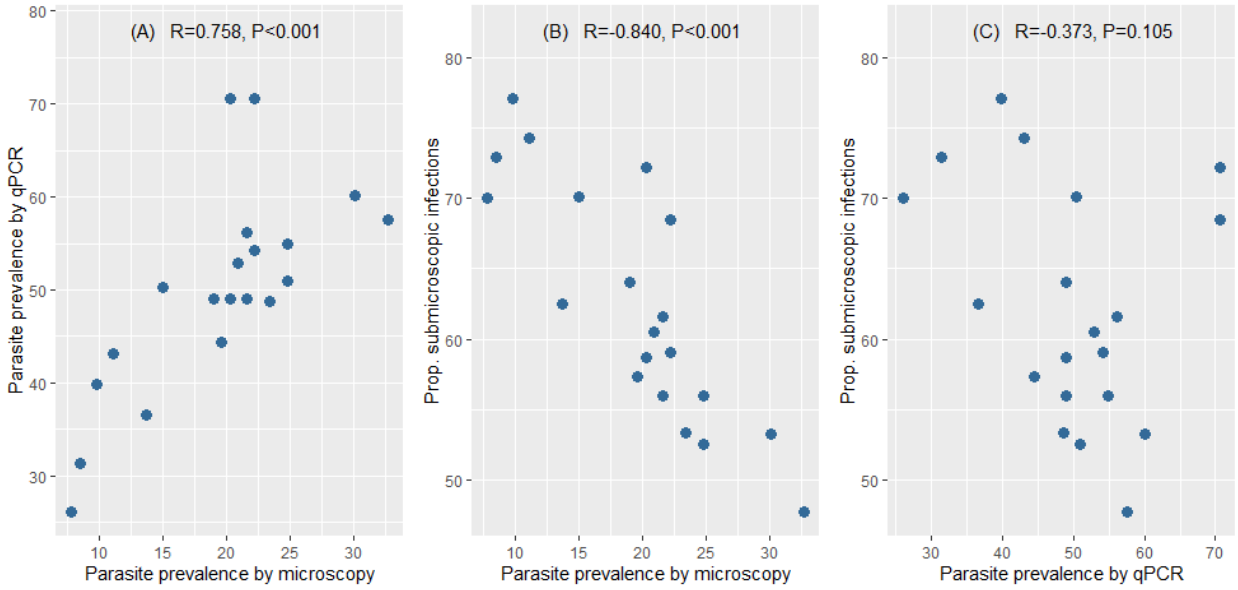

Figure 12. Scatter plots showing relationships between prevalence determined by microscopy and qPCR per cluster (panel A), prevalence by microscopy and proportion of submicroscopic infections (panel B) and prevelance by qPCR and proportion of submicroscopic infection per cluster (C) in all the 20 clusters sampled. Each dot represents a cluster. Prevalence was determined as percentage of individuals having parasite in the blood relative to the total number of individuals sampled in a cluster. Submicroscopic infections were defined as infections that were detected by qPCR method and not by microscopy. Proportion submicroscopic infections was determined by calculating percentage of submicroscopic infections relative to infections identified by qPCR method. Correlation coefficient (R) and P-value (P) were calculated using Spearmann correlation test.

4.3.3 Predictors for variation in infection prevalence across clusters

For a possible explanation of the pronounced differences in infection prevalence across clusters, the abundance and types of potential larval habitats were mapped. Four types of habitats were differentiated: (i) Wetland habitats i.e., water sources such as swamps, ponds, marshes, shallow wells, sand pits and ditches, (ii) rivers (the distance from each household to the closest river was calculated), (iii) forests, and (iv) sugarcane plantations (Figure 5 and 6). Baseline data of the habitats is provided in Appendix N.

A total of 309 potential habitats were identified across the twenty clusters (Figure 5). Three to 34 potential habitats were identified per cluster (Appendix N). The most frequent potential habitats across all the clusters combined were sugarcane plantations (n=145, 46.9%) and wetlands (n=139, 45.0%). The rivers intersecting the study area were widely distributed without clustering (Figure 5). None to 17 habitats were identified within a 250 m radius around each house (Appendix N).

In univariate analysis, the number of habitats (wetland, sugarcane and forest combined) per cluster did not predict prevalence (qPCR: P= 0.955, Figure 13A, microscopy: P=0.951, Figure 13B). The number of habitats stratified by type per cluster also did not predict prevalence (Figure 14A-11F). The clusters spanned an altitude range of 207 meters, ranging from 1077 to 1284m. There was a moderate significant inverse correlation between prevalence per cluster by qPCR and altitude (R=-0.462, P=0.040, Figure 13C), but no correlation between prevalence per cluster by microscopy and altitude (P=0.444, Figure 13D). The number of habitats (wetland, sugarcane, forest combined) per cluster did not differ with increasing altitude (P=0.672, Figure 13E). Likewise, the number of each habitat type did not vary with increasing altitude (Wetland: P=0.074, sugarcane: P=0.373, forest: P=0.093, Figure 14G-11I).

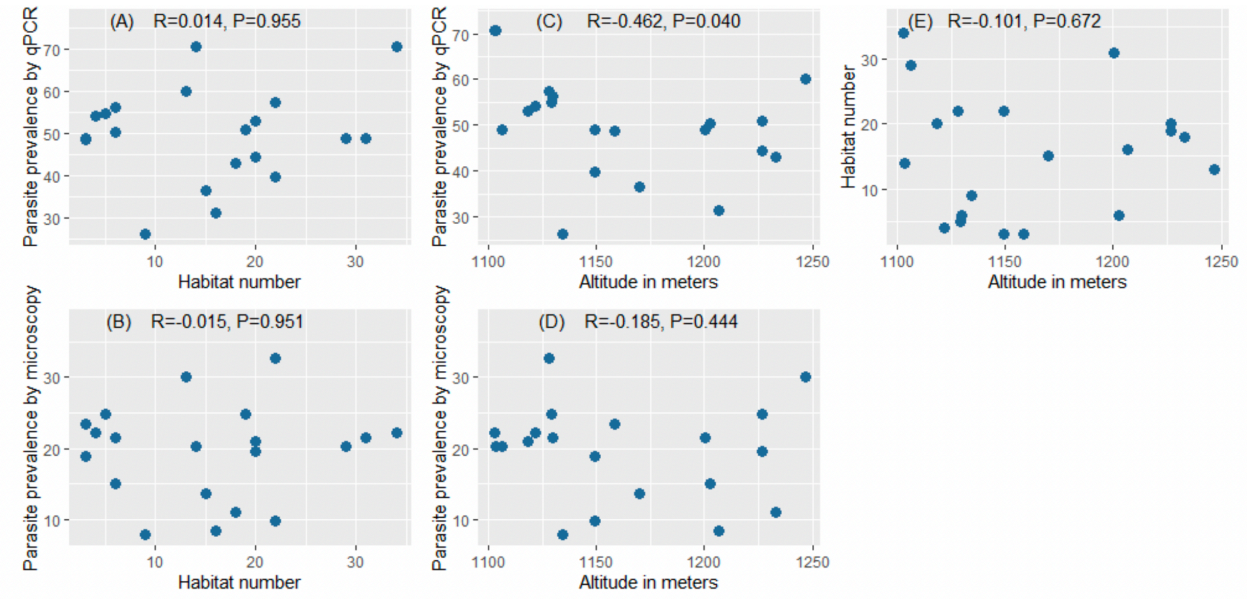

Figure 13. Scatter splots showing relationships of prevalence and habitat number per cluster (panels A and B), prevalence and and altitude per cluster (panels C and D) and habitat number and altitude per cluster (panel E) for all the 20 clusters sampled represented by blue dots. In panels A and C, prevelance is determined by qPCR while in panels B and D prevelance is by microscopy. In panel C, two clusters have identical prevalence of 70.6% by qPCR and similar average altitude thus dots overlap. The habitat number is counts of forest, wetland and sugarcane combined. Prevalence per cluster was determined as percentage of individuals having parasite in the blood relative to the total number of individuals sampled in a cluster. Altitude measurement per cluster was calculated by averaging altitude values of households sampled in a cluster. Correlation coefficient (R) and *P* values (P) are calculated by Spearman correlation test.

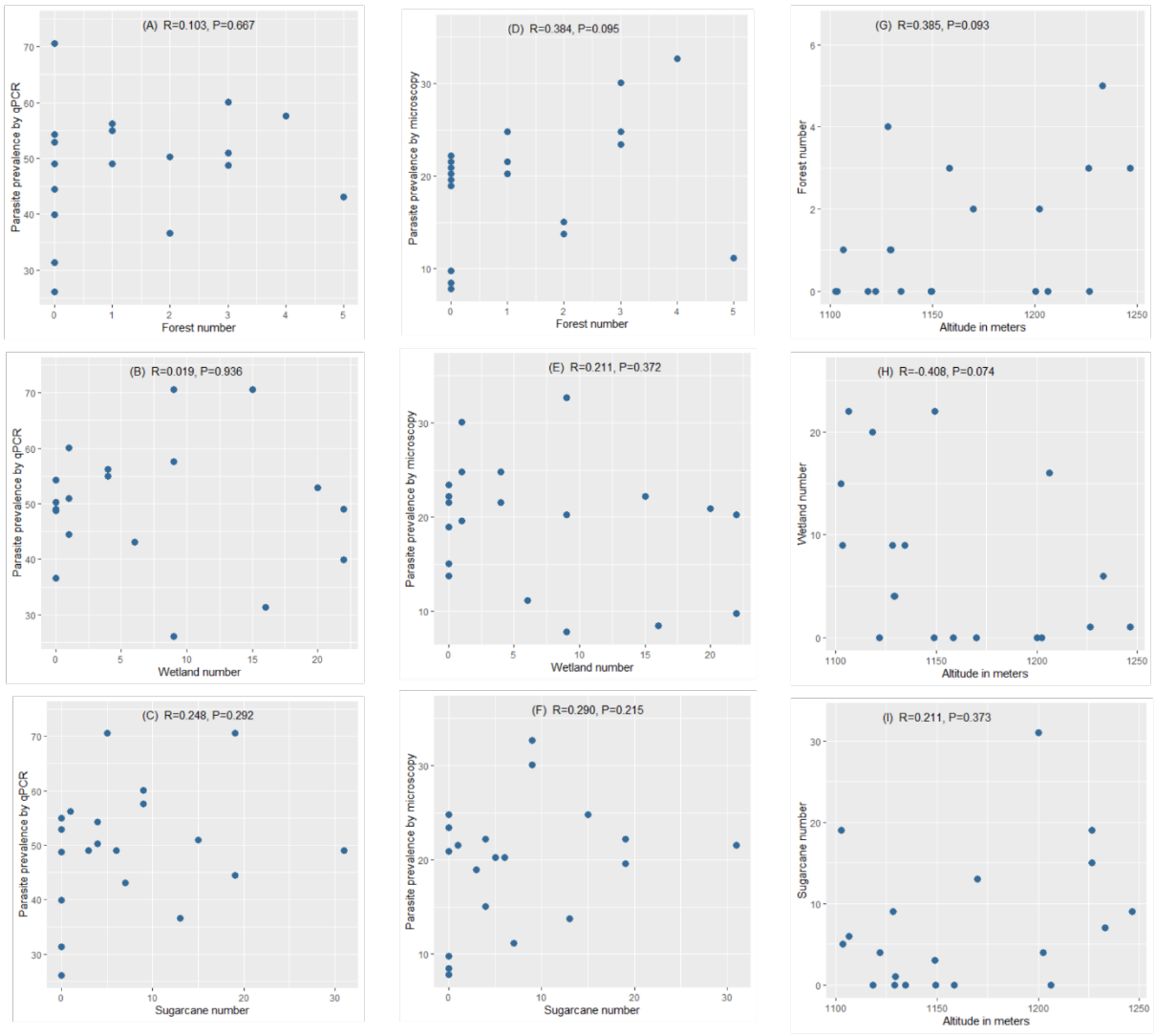

Figure 14. Scatter plots showing relationships of prevalence and habitat type numbers identified per cluster (panels A-F), and altitude and habitat type number identified per cluster (panels G-I) for all the 20 clusters sampled represented by blue dots. Panels A-C show pattern of prevalence determined by qPCR vs. habitat type numbers, panels D-F show pattern of prevalence by microscopy vs. habitat type numbers, panels G-I show pattern of number of habitat type vs. altitude. In panel A, two clusters have identical prevalence of 70.6% by qPCR and similar habitat number thus dots overlap. Habitat number is total counts of the habitats identified. Prevalence per cluster was determined as percentage of individuals having parasite in the blood relative to the total number of individuals sampled in a cluster. Altitude measurement per cluster was calculated by averaging altitude values of households sampled in a cluster. Correlation coefficient (R) and *P* values (P) are calculated by Spearman correlation.

A generalized linear mixed effect model (GLMM) was used to identify risk factors associated with prevalence of infection and to identify covariates that are correlated with pronounced variation in infection prevalence across the clusters. All covariates (Appendix N) were included in the model except those of extreme skewness that would cause difficulties in model fitting, i.e., residency, symptoms, and history of treatment.

Age, sex, eave type, education level of household head, kitchen location and altitude were statistically significantly associated with the likelihood of *Plasmodium* infection by qPCR. In contrast, number of habitats per cluster, the number of habitats within a 250 m radius around the household, roof material, household population, and screens on windows had no significant impact on prevalence (Table 3). The distance to the nearest river and distance to the nearest habitat within cluster had no significant impact on prevalence (Table 3).

Every 10m increase in altitude resulted in a 5.1% reduction in the odds of infection by qPCR. Having the kitchen located outdoors resulted in a 31.0% decrease in the odds of infection by qPCR. If the head of the household completed secondary education, the odds of infection by qPCR decreased by 36.7% compared to households where the head did not attend secondary school. Having an open eave resulted in a 53.4% increase in the odds of infection by qPCR compared to house with closed eaves. Having a male household member resulted in a 62.3% increase in the odds of infection by qPCR. Being 5-15 years resulted in a 153.3% increase in the odds of infection by qPCR compared to being younger than 5 years (Table 3).

Similar trends in predictors of infections were observed by microscopy. School aged children, males, open eaves, and a low education level of the head of household was associated with higher odds of infection. In contrast, altitude, total number of larval habitats per cluster, the number of habitats within 250 m of a household, kitchen location, population size of the household and the distance to the nearest river had no significant impact on prevalence by microscopy (Table 3).

Table 3. Estimated effects of risk factors for *P. falciparum* infection from Generalized Linear Mixed effect Model

	qPCR			**Microscopy**		
Variable	OR (CI95)	*P*-value	*Q*-value	OR (CI95)	*P-value*	*Q-value*
Individual-level factor						
Age (5-15 vs <5)	2.533 (1.939, 3.307)	<0.001*	<0.001*	2.712 (1.987, 3.703)	<0.001*	<0.001*
Age (≥15 vs <5)	1.713 (1.355, 2.165)	<0.001*	<0.001*	1.077 (0.800, 1.450)	0.626	0.752
Sex (male vs female)	1.623 (1.366, 1.928)	<0.001*	<0.001*	1.604 (1.310, 1.964)	<0.001*	<0.001*
Household-level factor						
Eave type (open vs closed)	1.534 (1.220, 1.930)	<0.001*	<0.001*	1.560 (1.197, 2.033)	<0.001*	0.005*
Education of household head (completed secondary vs below secondary)	0.633 (0.493, 0.813)	<0.001*	0.001*	0.636 (0.472, 0.857)	0.003*	0.011*
Kitchen location (Outdoor vs Indoor)	0.690 (0.526, 0.903)	0.007*	0.017*	0.720 (0.541, 0.959)	0.025*	0.074
Altitude (every 10-unit increase)	0.949 (0.912, 0.987)	0.009*	0.019*	0.972 (0.935, 1.011)	0.159	0.261

Distance to the nearest river (every 100-meter increase)	1.038 (0.997, 1.081)	0.068	0.127	1.043 (1.002, 1.087)	0.041*	0.089
Roof (non-corrugated iron vs corrugated iron)	1.267 (0.941, 1.707)	0.118	0.197	0.949 (0.685, 1.316)	0.754	0.808
Household population (increase by 1)	1.031 (0.989, 1.075)	0.147	0.220	1.048 (1.002, 1.097)	0.040*	0.089
Distance to the nearest habitat within cluster (every 100-metre increase)	0.929 (0.829, 1.042)	0.210	0.263	1.002 (0.885, 1.135)	0.975	0.974
Habitat number within 250m of household (increase by 1)	1.028 (0.975, 1.085)	0.307	0.355	1.041 (0.982, 1.103)	0.174	0.261
Screens window (Yes vs No)	0.964 (0.515,1.803)	0.907	0.972	0.537 (0.226, 1.273)	0.158	0.261
Cluster-level factor						
Bed net (coverage % per cluster)	0.144 (0.007, 2.898)	0.206	0.263	0.258 (0.015, 4.403)	0.350	0.477
Habitat number per cluster (increase by 1)	1.000 (0.978, 1.022)	0.984	0.984	0.995 (0.975, 1.016)	0.651	0.752

OR: odds ratio. CI95: 95% confidence interval. Asterisk (*) indicates significant at 5% level. Numbers in parenthesis () show CI95. FDR: False Discovery Rate. *Q*-value is the FDR adjusted *P*-value. The *Q*-value shows proportion of false positive results.

Having the kitchen located outdoors resulted in a 28.0% decrease in the odds of infection by microscopy. Having a male household member resulted in a 60.4% increase in the odds of infection by microscopy. Being 5-15 years resulted in a 171.2% increase in the odds of infection by microscopy compared to being younger than 5 years. If the head of the household completed secondary education, the odds of infection by microscopy decreased by 36.4% compared to households where the head did not attend secondary school. Having an open eave resulted in a 56.0% increase in the odds of infection by microscopy compared to household with closed eaves (Table 3).

4.3.4 Clustering of *Plasmodium falciparum* infections

The median odds ratio (MOR) for households was 2.94 based on the qPCR-detected infections and 2.44 based on the microscopy-detected infections. The MOR for clusters was 1.60 based on the qPCR-detected infections and 1.51 based on the microscopy-detected infections data. The further away an MOR is from 1, the higher the odds a person would be infected with malaria if there is a positive case within the same household or the same cluster. Therefore, the MOR results suggest that there were clustering effects at both household and cluster levels, and that the clustering effect across households is stronger than that across clusters.

CHAPTER FIVE

DISCUSSION

5.1 Parasite transmission potential across seasons

This study found a contrasting pattern of gametocyte densities and proportion of gametocyte positive infections between the dry and the rainy season among afebrile individuals residing in malaria endemic area of western Kenya. In the rainy season, when most transmission is expected to occur, fewer infections harbored gametocytes. Among gametocyte-positive infections, however, gametocyte densities were higher in the rainy season, as was the proportion of infections harboring gametocytes at densities that could likely infect mosquitos was higher. The higher gametocyte densities in the rainy season are particularly noteworthy as parasite densities did not differ between seasons. Thus, the proportion of gametocytes among total blood stage parasites was higher in the rainy season compared to the dry season. These results imply that parasites increase their investment in gametocytes production in the high transmission period to be synchronized with increased vector abundance in the rainy season.

However, the adjustment was not uniform across all infections. Less than a quarter of infections carried detectable gametocytes in the rainy season. This is line with previous studies, where a majority of infections did not carry gametocytes detected by RT-qPCR (Koepfli *et al*., 2017; Tadesse *et al*., 2017). In some cases gametocytes might be present below the limit of detection even by RT-qPCR (Koepfli & Yan, 2018). Yet, even among medium-to-high density infections (above 100 parasites/μL), more than half did not carry gametocytes. Given the high sensitivity of our RT-qPCR, limited detectability cannot explain this result. During the first two weeks of infections, gametocytes sequester in inner organs such as bone marrow and are not available for detection in the blood (Eichner *et al.*, 2001; Farfour *et al*., 2012). This might explain the absence of gametocytes among medium-to-high density infections as observed in this study.

Presence of *pfs25* transcripts detected by RT-qPCR does not necessarily imply infectivity. Molecular methods detect the transcripts at densities below the limit for successful mosquito infections (Tadesse *et al.*, 2019), and the proportion of infections with detectable transcripts depends on the limit of detection of the molecular assay (Hofmann *et al*., 2015; Koepfli & Yan,

2018). Gametocyte density, and the proportion of infections with gametocytes at a density that could infect mosquitos appear to be more informative measures (Churcher *et al.*, 2013; Gonçalves *et al*., 2017). At low-to-moderate gametocyte densities, mosquito infectivity increases with increase in gametocyte density. At high densities of several hundred gametocytes per uL blood, infectivity reaches saturation (Churcher *et al*., 2013), yet very few infections in the present study were in this range.

While our quantification of *pfs25* transcripts is a good marker of infectivity at time of sample collection (Bradley *et al.*, 2018; Churcher *et al.*, 2013; Gonçalves *et al*., 2017), it is only an indirect measure of commitment to transmission. Asexual parasite densities are expected to peak early in the infection when mature gametocytes are not yet circulating. Likely, some of the high-density infections observed in our study were recently acquired and carried sequestered gametocytes that appeared in the blood a few days after sample collection. Among infections with above average proportions of gametocytes, asexual densities might have been higher two weeks prior when gametocyte development was initiated. Alternatively, the pattern might reflect true differences in gametocyte conversion. Few studies have measured the conversion rate directly on field isolates, and those who did found pronounced variation among *P. falciparum* isolates (Poran *et al*., 2017; Smalley *et al*., 1981; Usui *et al*., 2019). The factors underlying these differences remain poorly understood.

Our findings of higher gametocyte densities in the rainy season are in line with xenodiagnostic surveys (i.e., mosquito feeding assays) conducted from asymptomatic residents of Burkina Faso and Kilifi, Kenya. Gametocyte densities determined by molecular assays targeting *pfs25* transcripts and infectivity were substantially higher in the wet compared to the dry season (Gonçalves *et al*., 2017; Lin *et al*., 2016). Similarly, the present study corroborates previous work on asymptomatic individuals in eastern Sudan (Gadalla *et al.*, 2016). These adjustments to seasonality have important implications for programs that aim to detect asymptomatic infections through population screening.

At molecular level, gametocytes production occurs when *P. falciparum* apetala-2 gene (*ap2-g*) is activated. The gene is essential for the differentiation of committed schizonts into early

gametocytes (Kafsack *et al*., 2014). The *ap2-g* is activated when a repressor protein, e.g., heterochromatin protein 1, is removed from *ap2-g* by nuclear protein gametocyte development protein 1 (GDV1) and transcription factor AP2-G3 in sites of low lysophosphatidylcholine concentration (Eksi *et al*., 2012; Filarsky *et al*., 2018). The higher gametocytes levels in the rainy season relative to the dry season imply that there is over expression of *ap2-g* in the rainy season. The stimuli that cause the over expression of *ap2-g* in the rainy season are poorly understood. Whether this is due to frequency of mosquito bites or changes in physiological factors of the human body in response to change in environment and climatic condition is uknown.

Approximately half of all individuals that had gametocyte detected by RT-qPCR carried infections at densities below the limit of detection of microscopy or rapid diagnostic test (i.e., <100 parasites/μL). They thus would escape screening of asymptomatic individuals using field-deployable diagnostics. Gametocyte densities were 3-fold lower in submicroscopic individuals, yet among the 30 infections with moderate to high gametocyte densities, out of which 11 were submicroscopic. Among them, 8 were sampled in the rainy season. Thus, population screening would miss a much larger proportion of infections likely infective in the wet season compared to the dry season. Considering seasonal change in gametocytemia will reinvigorate malaria control and elimination strategies.

As opposed to Kisumu where parasite prevalence doubled in the rainy season, in Homa Bay the prevalence did not change. Sample collection in Homa Bay was done after two rounds of indoor residual sparying, while in Homa Bay indoor residual spraying had not been implemented. The indoor residual spraying might have resulted in the observed pattern in seasonal parasite prevalence in Homa Bay. Also, partly, the variations in seasonal parasite prevalence pattern between Kisumu and Homa Bay may be due to differences in species composition of local vector populations (Ayanful-Torgby *et al*., 2018). In Kisumu, *An. Arabiensis* forms the predominant mosquito vector species followed by *Anopheles gambiae s.s.* (Machani *et al*., 2020), whereas in Homa Bay *An. funestus* is the predominant mosquito vector species (McCann *et al*., 2014). *An. funestus* prefers permanent bodies of water that last beyond the rainy seasons, while *An. arabiensis* prefers temporary holes and pools that dry out once the rainy season ends (Fillinger *et al.*, 2004; Kweka *et al*., 2012; Mala & Irungu, 2011; Ndenga *et al*., 2011).

5.2 Parasite and gametocyte reservoirs across regions of varying transmission intensities

An obvious trend in parasite and gametocyte reservoirs was observed across differing transmission intensities. A higher parasite prevalence by microscopy and qPCR, and parasite density by qPCR was observed in much higher transmission region relative to lower transmission region. Equally, gametocyte prevalence and density by RT-qPCR increased with increasing transmission intensity. A contrasting patter was observed with proportion of submicroscopic infections, where a higher proportion of submicroscopic infections was observed with decreasing transmission intensity. No significant change in proportion of gametocyte positive infections was observed across regions of differing transmission intensity.

Higher parasite density in moderate-to-high transmission paralleled higher gametocyte density, and gametocyte prevalence. Previous work found parasite density as a strong predictor for gametocyte density and probability to detect gametocyte in blood (Koepfli *et al.*, 2015). The higher parasite densities in high malaria transmission region, and the higher proportion of submicroscopic infections observed in low transmission intensity region are in line with past findings (Okell *et al*., 2009, 2012; Slater *et al*., 2019). Arguably, in high transmission regions, reinfections from frequent bites of infected mosquitoes likely result in higher parasitemia that are readily detected by conventional tools thus explaining the relatively lower proportion of submicroscopic infections detected in such regions compared to low transmission regions. Also, due to attenuated or waned host immunity response among individuals in low transmission areas, most infections are maintained at relatively lower levels (Doolan *et al*., 2009; Mugyenyi *et al*., 2017).

In high transmission regions, majority of infections are polyclonal (Abukari *et al*., 2019; Lopez & Koepfli, 2021). There are contrasting reports on the relationship between multiplicity of infections and parasite densities since the relationship is influenced by several factors including clinical symptom status, days after or before treatment and duration of infection (Lerch *et al*., 2019; Mueller *et al.*, 2012; Nkhoma *et al*., 2018; Sondo *et al.*, 2020). It is postulated that in the high transmission regions, there is likely high competition from more virulent parasite strains that might result in increased parasite densities.

Overall, 8-50% of the population sampled per site carried parasites. This reservoir presents a major roadblock for improved malaria control and elimination (Cheaveau *et al*., 2019; Chen *et al*., 2016; Lindblade *et al*., 2013). In addition to improved vector control, programs to actively clear these infections from the population are needed. About 61-80% of infections were submicroscopic across the sites. If left undetected and untreated, some of the these infections would develop into higher density infections over time (Nguyen *et al*., 2018) and contribute to onward transmission of malaria (Barry *et al*., 2021; Sumner *et al*., 2021; Tadesse *et al*., 2018). The submicroscopic infections might not be detected by conventional methods employed in mass screen-and-treat or reactive case detection campaigns. A novel generation of highly sensitive rapid diagnostic tests (RDTs) is able to detect more low-density infections (Niyukuri *et al*., 2022; Slater *et al*., 2022). Whether the sensitivity of these tests is sufficient to reduce transmission if used in reactive case detection campaigns remains to be shown.

5.3 Small scales variations in infection prevalence across clusters in Busia

In Busia County in western Kenya, the prevalence of *P. falciparum* infection by qPCR differed nearly 3-folds across 20 clusters. Overall, malaria prevalence was high at 50% by qPCR. By microscopy, the infection prevalence was 20% and differed 4-folds across the clusters. Prevalence by qPCR per cluster paralleled prevalence by microscopy. Thus, screening by microscopy might inform interventions to reduce the asymptomatic prevalence, such as through targeted mass drug administration.

Nearly two thirds of infections in all clusters were submicroscopic. Prevalence by microscopy and submicroscopic prevalence per cluster were inversely correlated, suggesting that a larger proportion of very low-density infections existed in low transmission clusters. Given that most of these low-density infections would be missed by field deployable conventional screening methods, variations in detection capacity across the high and low transmission clusters need to be considered by malaria intervention programs.

Clustering of infections was also observed at the household level. Yet, the presence of microscopy-positive infections was not a predictor for submicroscopic infections at household level, and thus cannot guide control strategies towards further targeted treatment at household level. This contrasts with the observations in Bioko Island, where prevalence is lower, and presence of RDT-positive

individuals increases the odds of submicroscopic infections in same household by 2.6-fold (Hergott *et al*., 2021) and with numerous studies where prevalence of asymptomatic and/or submicroscopic infections was higher in household members of clinical cases, a pattern exploited by reactive case detection programs (Bhondoekhan *et al*., 2020; Parker *et al*., 2016; Stuck *et al*., 2020; Sturrock *et al*., 2013; Zemene *et al.*, 2018). The contrasting findings between this study and other studies might be explained by differences in transmission intensity. Reactive case detection is widely employed and evaluated in low transmission or elimination areas where clustering of infections becomes more pronounced (Mogeni *et al*., 2017; Stresman *et al.*, 2020), while the current study was carried out in a moderate-to-high transmission area.

5.3.1 Factors explaining small scales variation in infection prevalence in Busia

Three to 34 potential larval habitats were found per cluster, and none to 17 found within a 250 m radius around households. Despite the pronounced variation in the habitat number across the clusters, there was no impact of habitat number on infection prevalence. Multiple environmental factors including temperature, light, salinity, vegetation, hydrology and geomorphology determine the establishment of larval habitats (Grech *et al.*, 2019; Hamid *et al*., 2019; Rejmánková *et al*., 2013). The habitats identified in the present study, e.g. drainage ditches, forests, and swamps were found elsewhere to contain mosquito larvae (Hinne *et al.*, 2021; Kweka *et al.*, 2011; Musiime *et al*., 2020; Nambunga *et al*., 2020; Nicholas *et al.*, 2021). Analysis of the impact of proximity to rivers was hampered by low variation in this parameter, as most of the clusters were evenly transected by rivers. This points to a complex relationship between proximity to potential larval habitats and risk of infection. Multiple previous studies reported an increased risk for prevalence or incidence among residents in close proximity to larval habitats (Bhondoekhan *et al*., 2020; Haque *et al*., 2011; Mwandagalirwa *et al*., 2017; Oesterholt *et al*., 2006; Rosas-Aguirre *et al*., 2021; Zhou *et al*., 2021), while others did not (Musiime *et al*., 2020; Sluydts *et al*., 2014).

Altitude was a key predictor for prevalence of infection within a narrow altitudinal range of 207 m. A 5% decrease in the odds of infection was reported per every 10 m increase in altitude. This finding corroborates previous studies across much larger altitude ranges (i.e., 600-1400 m), where significantly fewer clinical cases (Reyburn *et al*., 2005; Siya *et al*., 2020), lower prevalence (Baidjoe *et al*., 2016; Dabaro *et al*., 2021; Githeko *et al.*, 2006; Maxwell *et al*., 2003; Shililu *et al*., 1998; Zhou *et al*., 2021), and reductions in the proportion of *P. falciparum* infected mosquitoes

and *Anopheles* vector abundance were observed with increasing altitude (Asigau *et al*., 2017; Attenborough *et al*., 1997; Bødker *et al*., 2003; Daygena *et al*., 2017; Eyong *et al*., 2016; Hast *et al*., 2019). Lower temperatures at higher altitudes, which directly impact development and survival of mosquitos and *Plasmodium* parasites (Beck-Johnson *et al*., 2013; Christiansen-Jucht *et al*., 2014) might cause the observed pattern (Fouque & Reeder, 2019).

In addition, individual and household factors were predictors for transmission. Infection prevalence was higher among individuals living in households with open eaves and with the kitchen located indoors. The impact of kitchen location might be explained by local vector population behavior. The main mosquito vectors in this region are *Anopheles gambiae s.s.* and *An. funestus* (Githinji *et al*., 2020) that exhibit endophilic and endophagic behaviors (Githeko *et al*., 1996; Wamae *et al*., 2015). Spending more time indoors thus increases the risk of infection. The endophagic behavior of the mosquitos can also explain the impact of open eaves, which was associated with increased risk of infection. Prevalence was also higher among males, school age children, and in households where the head had not completed secondary school, corroborating previous findings (Briggs *et al*., 2020; Coalson *et al*., 2016; Karim *et al*., 2021; Mohan *et al.*, 2021; Obsie & Gondol, 2021; Walldorf *et al*., 2015). Females were found to clear malaria parasite faster in the absence of antimalarial treatment compared to male counterparts (Briggs *et al*., 2020).

5.4 Limitations

The current study did not assess whether the potential habitats carried larvae. However, the features we mapped are known breeding sites for *Anopheles* mosquitoes (Hinne *et al.*, 2021; Kweka *et al.*, 2011; Musiime *et al*., 2020; Nambunga *et al*., 2020; Nicholas *et al.*, 2021). Therefore, we believe they accurately reflect the transmission dynamics in the region. Notably, given the relatively shorter duration of mosquito's egg development to larva (approximately 1-2 weeks), and the single time point sampling nature of this study, we believe mapping persistent potential mosquito habitats like the case presented here as opposed to inspecting for larvae was more informative in studying ongoing transmission across seasons in the study area. Finally, we did not conduct gel electrophoresis assays to confirm the positive amplification signals generated by the real time qPCR.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study presents three major conclusions, each from a specific objective. From the first specific objective, we conclude that *Plasmodium falciparum* parasites increase gametocyte production in the rainy season compared to dry season. Given that the increase in gametocytemia is not paralleled by increased parasite density, screening using conventional methods such as malaria rapid diagnostic test or light microscopy in the rainy season would miss a larger proportion of the infectious reservoir than in the dry season.

From the second specific objective, we conclude that malaria transmission risk differs by several magnitude across regions in western Kenya, and that the variations are more pronounced in the rainy season (i.e., up to 6-fold difference) compared to during the dry season (i.e., up to 3-fold difference). This finding is important to guide the National Malaria Control and Advisory Council on resources allocation and choice of interventions programs.

From the third specific objective, we conclude that pronounced variation in infection prevalence exist across small georgraphic scales spanning villages in Teso South sub-county. Among the determinants of infection, altitude was a key predictor for prevalence of infection at household. Other factors associated with higher infection prevalence included a kitchen located indoors, open eaves, a lower level of education of head of household, school aged children, and being male. In contrast, potential larval habitat frequency had no direct impact on prevalence.

Findings from the current work resulted in three publications (see Appendices O-Q). This work advances the existing field of knowledge in the following ways:

i. Has established that *P. falciparum* parasites exhibit plasticity in response to changes in seasonality to maximize its transmission potential when mosquitoes are plentiful. Thus, there is a need to target the increased gametocytemia during transmission period.
ii. Has highlighted that there is substantial variation in magnitude of transmission reservoirs across regions in western Kenya. In low transmission region, a substantially higher

proportion of parasite reservoirs are missed when screened using conventional diagnostic tool e.g microscopy.

iii. Has established that risk for malaria transmission varies over a narrow scale in western Kenya, and that altitude is the main predictor of transmission in this region thus is among key factors to consider when undertaking malaria risk mapping. Further, present study has identified the determinants of transmission that could be targeted to control and eliminate malaria.

iv. Has highlighted the need for active surveillance and treatment of asymptomatic malaria reservoirs to subvert transmission.

6.2 Recommendations

Based on the results obtained from specific objective one of this study, the following recommendations are made:

i. There is need to consider seasonal changes of gametocytemia among asymptomatic malaria reservoirs when formulating malaria prevention strategies.

ii. Screening for gametocytemia by microscopy method need to be considered alongside the standard malaria parasite diagnosis.

iii. Administering fast-acting gametocytocidal drug in addition to asexual blood-stage treatment during transmission period will be important in reducing transmission.

iv. Further research should be carried out to understand the stimuli that cause parasites to increase gametocyte density in the rainy season. Whether this is due to frequency of uninfected mosquito bites or changes in physiological factors of the human body in response to seasonality.

Based on the results obtained from specific objective two of this study, the following recommendations are made:

i. National malaria Control and Advisory Council need to take into consideration differences in relative abundance of parasite reservoirs across regions in western Kenya to inform on best policies and strategies.

ii. There is need for continued active surveillance of transmission reservoirs using conventional methods and molecular tools in all transmission settings to inform on intervention measures.

Based on the results obtained from specific objective three of this study, the following recommendations are made:

i. Malaria intervention and control programs need to consider pronounced variation in infection prevalence at small geographical scales when designing malaria control strategies and policies.
ii. There is need to consider roll-out of complementary programs to scale up malaria elimination efforts, such as treatment of school aged children at the start of transmission season, and household structures modification.
v. Larval source management need to target all potential habitats across a wider geographical area in Busia County.
vi. Further research is needed to determine whether small-scale spatial variation in prevalence is maintained throughout seasons, and whether habitat density and prevalence correlate during some times of the year.

This work makes significant contributions to Sustainable Develop Goal on good health and well-being by improving understanding of malaria transmission in space and time thus informing approaches to control malaria transmission to achieve improved human health. Also, the current work contributes to the Sustainable Develop Goal on partneships for the goals. Processes leading to implementation of this work brought together many players from various institutions both internationally and locally including Universities, County Governenment, research institutions and community units. The various players were key in ensuring financial support, training, technical capacities, space for the work, facilitating entry into the community and offering intellectual capital. In addition, this work makes contribution to Science Technology and Innovation pillar of Kenya Vision 2030 through conducting research, education and training. Finally, the current work contributes to health theme of the County Integrated Development Plan by highlighting approaches to control and prevent malaria transmission.

Fingings from this work will be diseminated to various institutions and regulatory bodies including County Government Department of Health, Ministry of Health, Kenya Malaria Indicator Survey and World Health Organization.

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APPENDICES

Appendix A: Informed consent for study participation (English version-Busia site)

The Impact of Novel Vector Control Tools on Asymptomatic *Plasmodium falciparum* Infection Prevalence

Investigators and Institutions:

Cristian Koepfli (University of Notre Dame), Eric Ochomo (KEMRI), Colins Oduma (Egerton University) Tiffany Huwe (University of Notre Dame), John Gimnig (CDC), Winnie Chebore (KEMRI), Kephas Otieno (KEMRI), Simon Kariuki (KEMRI), John Gimnig (CDC), Achuyt Bhattarai (CDC), Aaron Samuels (CDC)

KEMRI/CDC- Kenya Medical Research Institute and CDC, Kisumu Kenya,
CDC- Centers for Disease Control and Prevention, Atlanta USA,
KEMRI- Kenya Medical Research Institute, Kisumu Kenya

PART I.

We are asking you/your child to join a study "**The Impact of Novel Vector Control Tools on Asymptomatic *Plasmodium falciparum* Infection Prevalence**". Your village has been selected to participate in a trial on the effect of spatial repellents for malaria control. We would like to study whether spatial repellents have an impact on asymptomatic malaria infections. Even though you or your child might not feel sick, it is possible that you are infected, and if bitten by a mosquito, you could transmit the parasite. Joining this study is voluntary.

Why is this research study being done?

Malaria, spread by a mosquito, continues to be a major health problem in many parts of the world, including here. If mosquitoes were repelled away from people and did not bite them, malaria would decrease. This study will measure whether spatial repellent reduce the number of asymptomatic malaria infections.

Who is doing this study?

This study is being done by the Kenya Medical Research Institute (KEMRI) with the US Centers for Disease Control and Prevention (CDC) and the University of Notre Dame. The protocol has been reviewed and approved by both the KEMRI and CDC Ethical Review Committee.

What will happen to me/my child in this study?

We will ask you a few questions about your/your child's health and behaviour. We will then take a finger prick blood sample to test for malaria. The amount of blood taken will be approximately 200 microliters. We will again collect the same amount of blood 12 and 24 months later but only if you will consent during that time.

Will blood samples be stored?

We will store your/your child's blood because we cannot do all the tests immediately. We would like to store any left-over blood samples to do tests in the future to answer important public health questions. This is referred to as "long term storage" of samples. These samples will not be used for human genetic testing but only for tests on the malaria parasites. Before doing any tests on the blood samples in long term storage, we will ask the Ethics Committee for approval. You can choose not to have your/your child's blood in long term storage and still participate in this study.

What is the risk to me/my child by participating?

When blood is taken, there is usually some pain at the site of pricking and there is a small risk of infection or bruising. Usually, the pain is felt for no longer than 15 minutes.

What is the benefit of participating?

There is no direct benefit for you or your child. We hope, however, to find novel ways to reduce malaria transmission. If so, this will benefit your community and others in Kenya and other countries that have malaria.

Compensation

There will be no payment given for participation in this study. The study will cover the costs of all research blood tests.

Can I withdraw from the study?

Yes. You/your child is free to leave the study at any time. The study and/or local Ethics Committees have the right to stop the study at any time for any legitimate reason.

What happens if I decline participation or change my mind later on?

This is OK. The doctors will respect your decision because you/your child's participation in this study is voluntary. You may discontinue your participation in this study at any time you choose and there will be no penalty.

Confidentiality

All information about you/your child will be kept confidential and will not be shared with anyone outside of the study. Your/your child's name will not be used in any way in the reporting of information learned in this study. All the research documents and blood samples will be labeled using your/your child's study number. Information from the study which are in computers will be protected using passwords only known to a few members of the research team. The information collected from this trial, without names or personal identifiers, will be shared with investigators at Notre Dame in the USA. All forms, logbooks etc. will be stored in locked filing cabinets. Your name will not appear on any study documents or on stored specimens.

Who to contact if you have questions

If you have questions about this study, or if you feel your child has been harmed, please get in touch with the **Dr. Eric Ochomo, P.O. Box 1578 Kisumu or on 0723845457**, if you have any questions on your/your child's rights as a study participant or you would like to get in touch with someone outside of the study please communicate with **secretary or chairperson of KEMRI SERU P.O. Box 54840 00200, telephone (057) 2722541 or 0717719477 or 0722205901 or 0733400003 email address: SERU@kemri.org**

These phone numbers are not for emergencies. If you or your child is sick, go immediately to the nearest health facility or hospital.

PART II.

Consent

I, ..., have been informed of the aims of the project entitled "**The Impact of Novel Vector Control Tools on Asymptomatic *Plasmodium falciparum* Infection Prevalence**" and I agree to participate /allow my child to participate in the study. I have had the opportunity to ask questions and my concerns have been addressed to my satisfaction. I voluntarily consent to participate /for my child to participate in this study and understand that I, or my child, have the right to withdraw from the study at any time without in any way affecting future medical care provided.

Name: Participant: (participant, parent, or witness writes)

_____

_____ _____

Participant or parent/guardian signature Date (dd-mm-yyyy)

_____ Thumbprint if unable to sign

Participant or parent/guardian printed name

If participant is illiterate:

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should appose their thumb-print as well.

(Example: I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.)

Witness Name: (*witness writes*):

_____

Witness Signature (*witness signs and dates*):

_____ Date:_____(dd/mm/yyyy)

Consenter's Name (*consenter writes*)

_____

Date _____(dd/mm/yyyy)

Consent for the storage of blood for future testing

☐ **I do NOT** consent for long term storage of my/my child's blood (stop here)

☐ **I do** Consent to allow my/my child's blood to be stored in long term storage for future testing for public health use.

_____ _____

Participant or parent/guardian signature Date (dd-mm-yyyy)

_____ Thumbprint if unable to sign

_____

Participant or parent/guardian printed name

If participant is illiterate:

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should appose their thumb-print as well.

(Example: I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.)

Witness Name: (*witness writes*):

_____

Witness Signature (*witness signs and dates*):

_____Date:_____(dd/mm/yyyy)

Consenter's Name (*consenter writes*)

_____. Date _____(dd/mm/yyyy)

Consent for the shipment of blood

☐ I **do NOT** consent to allow my / my child's blood to be shipped for additional testing outside the country.

☐ I **do** Consent to allow my / my child's blood to be shipped for additional testing outside the country.

Name: Parent or Legal Guardian (*Parent or witness writes)*

_____

Signature: Parent or Legal Guardian (*Parent signs and dates, or provides thumbprint and witness dates)*

_____ Date:_____(dd/mm/yyyy)

Illiterate Participant

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should appose their thumb-print as well.

(Example: I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.)

Witness Name (*witness writes*)

_____

Witness Signature (*Witness signs and dates)*

Date :_____(dd/mm/yyyy)

Consenter's Name (*consenter writes)*

_____. Date :_____(dd/mm/yyyy)

Appendix B: Informed consent for study participation (Kiswahili version-Busia site)

Athari za zana za kudhibiti vekta za riwaya juu ya kuenea kwa maambukizi ya *plasmodium falciparum* yasiyokua na dalili

Wachunguzi na taasisi:

Cristian Koepfli (University of Notre Dame), Eric Ochomo (KEMRI/CDC), Colins Oduma (Egerton University), Tiffany Huwe (University of Notre Dame), John Gimnig (CDC), Winnie Chebore (KEMRI), Kephas Otieno (KEMRI), Simon Kariuki (KEMRI), Achuyt Bhattarai (CDC), Aaron Samuels (CDC)

KEMRI/CDC-taasisi ya utafiti wa matibabu ya Kenya na CDC, Kisumu Kenya,
CDC-vituo kwa ajili ya kudhibiti magonjwa na kuzuia, Atlanta USA,
KEMRI-taasisi ya utafiti wa matibabu Kenya, Kisumu Kenya

SEHEMU YA I.

Tunakuomba wewe/mtoto wako kujiunga katika utafiti "**athari za riwaya za udhibiti wa vekta juu ya kuenea kwa maambukizi ya *Plasmodium falciparum* yasiyokua na dalili".** Kijiji chako kimechaguliwa kushiriki katika jaribio juu ya athari za viwinga vya anga kwa udhibiti wa malaria. Tungependa kujifunza kama viwinga vya anga vina athari kwa maambukizo ya malaria isiyokua na dalili. Ijapokua wewe au mtoto wako hamwezi hisi kuwa wagonjwa, inawezekana kwamba wewe umeambukizwa, na kama umeumwa na mbu, unaweza kusambaza vimelea. Kujiunga na utafiti huu ni kwa hiari.

Kwa nini utafiti huu wa somo lifanywa?

Malaria, inayosambazwa na mbu, inaendelea kuwa tatizo kubwa la afya katika sehemu nyingi duniani, ikiwa ni pamoja na hapa. Iwapo mbu wangetenwa watu na kutowauma, malaria ingepungua. Utafiti huu utapima hali ya hewa ya anga ya kupunguza idadi ya maambukizo ya malaria yasiokua na dalili.

Ni nani anayefanya utafiti huu?

Utafiti huu unafanywa na taasisi ya utafiti ya matibabu ya Kenya (KEMRI) na Marekani Vituo vya kudhibiti magonjwa na uzuiaji (CDC) na Chuo Kikuu cha Notre Dame. Itifaki imepitiwa na kuidhinishwa na kamati ya tathmini ya maadili ya KEMRI na CDC.

Nini kitatokea kwangu/mtoto wangu katika utafiti huu?

Tutakuuliza maswali machache kuhusu afya na tabia ya mtoto wako. Kisha tutachukua sampuli ya damu ya kidole ili kupima malaria. Kiasi cha damu kitakachochukuliwa kitakuwa karibu na mikro lita 200. Tutakusanya tena kiwango sawa cha damu miezi 12 na 24 baadaye lakini ikiwa utakubali wakati huo.

Je, sampuli za damu zitahifadhiwa?

Tutahifadhi damu yako/mtoto wako kwa sababu hatuwezi kufanya majaribio yote mara moja. Tungependa kuweka akiba yoyote ya sampuli ya damu ili kufanyia vipimo katika siku zijazo ili kujibu maswali muhimu ya afya ya umma. Hii inajulikana kama "Hifadhi ya muda mrefu" ya sampuli. Sampuli hizi hazitatumika kwa ajili ya kupima maumbile ya binadamu lakini tu kwa ajili ya vipimo vya vimelea vya malaria. Kabla ya kufanya majaribio yoyote kwenye sampuli ya damu katika hifadhi ya muda mrefu, tutauliza kamati ya maadili ya kuidhinishwa. Unaweza kuchagua kutokuwa na damu yako/mtoto wako kwa hifadhi wa muda mrefu na bado kushiriki katika utafiti huu.

Je, kuna hatari gani kwangu/mtoto wangu kwa kushiriki?

Wakati damu ni itakapochukuliwa, kuna kawaida baadhi ya maumivu katika eneo lililochomwa na kuna hatari ndogo ya maambukizi au kukwaruzwa. Kawaida, maumivu hayasalii kwa muda mrefu zaidi ya dakika 15.

Nini faida ya kushiriki?

Hakuna manufaa ya moja kwa moja kwa wewe au mtoto wako. Hata hivyo, tunatarajia kupata njia za riwaya za kupunguza maambukizi ya malaria. Ikiwa hivyo, hii itafaidi jamii yako na wengine nchini Kenya na nchi nyingine ambazo zina malaria.

Fidia

Hakutakuwa na malipo yaliyotolewa kwa ajili ya ushiriki katika utafiti huu. Utafiti itakuwa inagharamia gharama za uchunguzi wote wa damu ya utafiti.

Je! Naweza kujiondoa kutoka kwa utafiti?

Ndiyo. Wewe/mtoto wako mko huru kuondoka katika masomo wakati wowote. Utafiti na/au kamati ya maadili ya ndani ina haki ya kukomesha utafiti wakati wowote kwa sababu yoyote halali.

Ni nini kinatokea kama nitakataa kushiriki au kubadili mawazo yangu baadaye?

Hii ni sawa. Madaktari wataheshimu uamuzi wako kwa sababu ushiriki wako/mtoto wako katika hili utafiti ni wa kujitolea. Unaweza kuvunja ushiriki wako katika masomo haya wakati wowote unachagua, hakutakuwa na adhabu.

Usiri

Habari zote kuhusu wewe/mtoto wako litahifadhiwa siri na haitangawizwa na mtu yeyote nje ya utafiti. Jina lako/mtoto wako halitatumika kwa njia yoyote katika kuripoti habari kutoka kwa utafiti huu. Nyaraka zote za utafiti na sampuli za damu itaandikwa kwa kutumia nambari yako/ya mtoto wako wa utafiti. Maelezo kutoka kwa utafiti ambayo yapo katika kompyuta itakuwa salama kwa kutumia nywila inayojulikana tu kwa wanachama wachache wa timu ya utafiti. Habari zilizokusanywa kutoka kwa jaribio hili, bila majina au vitambulishi binafsi, itatumika pamoja na wachunguzi katika Notre Dame katika USA. Aina zote, vitabu vya logi na kadhalika vitahifadhiwa kwenye makabati ya faili iliyofungwa. Jina lako halitaonekana kwenye nyaraka zozote za utafiti au kwenye vielelezo vilivyohifadhiwa.

Nani wa kuwasiliana kama una maswali

Ikiwa una maswali kuhusu utafiti huu, au ukihisi mtoto wako ameumizwa, tafadhali wasiliana **na Dk Eric Ochomo, S.L.P. Box 1578 Kisumu au 0723845457**, kama una maswali yoyote juu ya haki za mtoto wako kama mshiriki wa masomo au ungependa kuwasiliana na na mtu nje ya utafiti Tafadhali kuwasiliana na Katibu au mwenyekiti wa **KEMRI SERU P.O. Box 54840 00200, telephone (057) 2722541 or 0717719477 or 0722205901 or 0733400003 email address: SERU@kemri.org**. Nambari hizi za simu sio za dharura. Kama wewe au mtoto wako ni mgonjwa, kwenda mara moja kwa kituo cha afya kilicho karibu au hospitali.

SEHEMU YA II.

Ridhaa

Mimi, ..., nimeaarifiwa kuhusu malengo ya mradi ijulikanayo kama “Athari ya zana za kudhibiti vekta za riwaya juu ya maambukizi ya Plasmodium falciparum yasiyokua na dalili "na Ninakubaliana kushiriki/Ruhusu mtoto wangu kushiriki katika utafiti. Nimekuwa na fursa ya kuuliza maswali na wasiwasi wangu umeshughulikiwa kwa kuridhika kwangu. Ninahusika kwa hiari kushiriki/kwa mtoto wangu kushiriki katika utafiti huu na kuelewa kwamba mimi, au mtoto wangu, nina haki ya kujitoa kutoka kwa mafunzo wakati wowote bila kwa njia yoyote yanayoathiri huduma ya matibabu ya baadaye iliyotolewa.

Jina: mshiriki: (mshiriki, mzazi, au shahidi anaandika)

_____

_____ _____

Saini ya mshiriki au mzazi/mlezi Tarehe (Siku//Mwezi/Mwaka)

_____ alama za vidole ikiwa hawezi kupiga sahihi

Ikiwa mshiriki hawezi kusoma na kuandika:

Shahidi anayejua kusoma na kuandika lazima asaini (kama inawezekana, mtu huyu anapaswa kuchaguliwa na mshiriki na haipaswi kuwa na uhusiano na timu ya utafiti). Washiriki ambao hawajui kusoma na kuandika wachapishe alama za vidole.

(Mfano: nimeishuhudia usomaji sahihi wa fomu ya ridhaa kwa uwezo wa mshiriki, na mtu binafsi alikuwa na nafasi ya kuuliza maswali. Nathibitisha kwamba mtu binafsi ametoa kibali kwa uhuru.)

Jina la shahidi: (shahidi anaandika):

_____

Sahihi ya mshahida (mshahidi atie sahihi na tarehe):

_____Tarehe: (Siku//Mwezi/Mwaka)

Jina la mwenye kupeana igizo (andika)

_____ Tarehe: (Siku//Mwezi/Mwaka)

Ridhaa ya uhifadhi wa damu kwa ajili ya kupima baadaye

☐ **Mimi sijakubali** kwa muda mrefu uhifadhi wa damu ya mtoto wangu (wacha hapa)

☐ **Ninakubali kupeana idhini ya kuruhusu** damu ya mtoto wangu kuwa kuhifadhiwa katika hifadhi ya muda mrefu kwa ajili ya kupima baadaye kwa matumizi ya afya ya umma.

_____ _____

Sahii ya mshiriki au mzazi/mlezi Tarehe: (Siku//Mwezi/Mwaka

_____chapaa gumba kama hawezi kupiga sahihi

Jina la kuchapishwa la mshiriki au mzazi/mlezi

_____ _____

Sahihi ya mshiriki au mzazi/mlezi Tarehe: (Siku//Mwezi/Mwaka)

_____Chapa gumba kama hawezi kupiga sahihi

Ikiwa mshiriki hawezi kusoma na kuandika:

Shahidi anayejua kusoma na kuandika lazima asaini (kama inawezekana, mtu huyu anapaswa kuchaguliwa na mshiriki na haipaswi kuwa na uhusiano na timu ya utafiti). Washiriki ambao hawajui kusoma na kuandika wachapishe alama za vidole.

(Mfano: nimeishuhudia usomaji sahihi wa fomu ya ridhaa kwa uwezo wa mshiriki, na mtu binafsi alikuwa na nafasi ya kuuliza maswali. Nathibitisha kwamba mtu binafsi ametoa kibali kwa uhuru.)

Jina la shahidi: (shahidi anaandika):

_____

Sahihi ya mshahida (mshahidi atie sahihi na tarehe):

_____Tarehe: (Siku//Mwezi/Mwaka)

Jina la mwenye kupeana igizo (andika)

_____ Tarehe: (Siku//Mwezi/Mwaka)

Ridhaa ya uhifadhi wa damu kwa ajili ya kupima baadaye

☐ **Mimi sijakubali** kuruhusu damu yangu / damu ya mtoto yangu kusafirishwa kwa majaribio zaidi nje ya nchi.

☐ **Ninakubali kupeana idhini ya kuruhusu** damu yangu / damu ya mtoto yangu kusafirishwa kwa majaribio zaidi nje ya nchi.

_____ _____

Sahii ya mshiriki au mzazi/mlezi Tarehe: (Siku//Mwezi/Mwaka)

_____chapaa gumba kama hawezi kupiga sahihi

Jina la kuchapishwa la mshiriki au mzazi/mlezi

_____ _____

Sahihi ya mshiriki au mzazi/mlezi Tarehe: (Siku//Mwezi/Mwaka)

_____ Chapa gumba kama hawezi kupiga sahihi

Ikiwa mshiriki hawezi kusoma na kuandika:

Shahidi anayejua kusoma na kuandika lazima asaini (kama inawezekana, mtu huyu anapaswa kuchaguliwa na mshiriki na haipaswi kuwa na uhusiano na timu ya utafiti). Washiriki ambao hawajui kusoma na kuandika wachapishe alama za vidole.

(Mfano: nimeishuhudia usomaji sahihi wa fomu ya ridhaa kwa uwezo wa mshiriki, na mtu binafsi alikuwa na nafasi ya kuuliza maswali. Nathibitisha kwamba mtu binafsi ametoa kibali kwa uhuru.)

Jina la shahidi: (shahidi anaandika):

_____

Sahihi ya mshahida (mshahidi atie sahihi na tarehe):

_____Tarehe: (Siku//Mwezi/Mwaka)

Jina la mwenye kupeana igizo (andika)

_____ Tarehe: (Siku//Mwezi/Mwaka)

Appendix C**:** Informed consent for study participation (Ateso version - Busia site)

The Impact of Novel Vector Control Tools on Asymptomatic *Plasmodium falciparum* Infection Prevalence

Investigators and Institutions:

Cristian Koepfli (University of Notre Dame), Eric Ochomo (KEMRI/CDC), Colins Oduma (Egerton University), Tiffany Huwe (University of Notre Dame), Winnie Chebore (KEMRI), Kephas Otieno (KEMRI), Simon Kariuki (KEMRI), John Gimnig (CDC), Achuyt Bhattarai (CDC), Aaron Samuels (CDC)

KEMRI/CDC- Kenya Medical Research Institute and CDC, Kisumu Kenya,
CDC- Centers for Disease Control and Prevention, Atlanta USA,
KEMRI- Kenya Medical Research Institute, Kisumu Kenya

ATUTUBET NASODIT.

Elipito siong' ijo/ikoku kon akilom akisisia naka "**The Impact of novel vector control tools on asymptomatic *plasmodium falciparum* infection prevalence". A**gelun atutubet kon ajausu akisisia kana kanuka atamitere ajokus ka "Spatial Repellents akirebokin adeka nakekimidi. Akotosi siong' akisisiaun kejei ationus apede kuju ka ng'ul lu emam kitodiarito ekimidi ka Kwaana kese. Ata kere kemam ijo/ikoku kon kedekakina, ebeikiit adeka ajaus akwaana, naarae kikonyo ijo esirut ebeikini ijo ayekakin itwaan ice ekurut loka adeka nakekimidi. Mam kekurakiite ijo ajaus akisisia na.

Inyobo asomaete ooni aking'ic na?

Adeka nakekimidi, na itolosi esirut etolosit arausu icen kang'aleu kitung'a ilarisinoi nu egelagela ka kwap atutubet kon ejei toma. Kerebokin isiru luka adeka ajaus arir itung'a kakikonyo kesi, editaari adeka nakekimidi. Akisisia na eng'arakin ajenun abeite kebeikito iboro luka Spatial Repellent apyang'ar ajaus kadekasia kama ka ng'ul lu emam ketodiarito adeka nakekimidi ka kwaana kese.

Ng’ai esomae aking’ic na?

Kenya Medical Research Institute (KEMRI) nesi esomaete aking’ic na kiton Luka US Centers for Disease Control and Prevention (CDC) kiton a university of Notre Dame. Ikisila luka king'ici etenunete katutubor ikisila ng'ul kalu egwoikito ko KEMRI kiton CDC.

Inyena ikokin kamaang’i/ikoku ang’i kakisisia kana?

Ang’isete siong’ ijo kere ikoku kon ang’itasia adisi ekamasi kang'aleu kon kiton nakikoku kon. Adaete siong' ijo esindani acilun iikot kobokorit koni kanuka akinuok ekimidi. Alemarete siong' ikoto idisi mam kedepari imiana 200 microlitres. Enyuto siong’ bobo akilipa alemun aakot kama kon kolopia 12 kiton 24 lu egwapakinet kicamaki ijo.

Ing’adakinete siong' aakot?

Ang’adakinete siong' aakot koni/ nu kikoku koni naarai mam siong' kebeikinete akinuok aakoto kijokis kakolong' kape. Elipito siong’ aking’adakin aakot nuedeunosi akisoma aking’ici apaaran ace nakoking’aren tetere ebeikinete siong' anyakuni ang'itasia acee nukaang'aleu. Na enyarite “akolong' nakaujan nakaking'adakin aakot". Emam asomisio ace nu etosomaere aakot kon akidepe akinuok ekurut lokekimidi. Enyute aking'it lu egwoikitosi ikisila lukaking'ici eroko kegeuna akitosoma aakot kon nu eng'adakite. Ibeikin ijo ing'eri kogwai aakot kon kere nu kikoku kon konye itolosit ijo ajausu akisisia.

Inyena aronus kama ang’i/ikoku ang’i kacamu ong’?

Kedaai ijo alemari aakot ejauni apipilu nama edaio kitoni ebeikin nama edao atenyari. Ducu, mamu apipilu kedepari idakikana itomon kikanyi (15 mins).

Inyena ajokus kajaus ang’i akisisia na?

Emam ang’arasit idumuni ijo kere ikoku koni kajaus kakisisia kana. Konye atamitos siong' irotin lukajokak kanukakitideuni adeka nakekimidi. Kerau kong'in, ebuni atutubete kon kiton itung'a Lucee kokenya kitoni akwapin acee nu ejasi kekimidi akiing'arakin kakisisia kana.

Etacenit

Emam etacenit kama ka lu ecamunete ajausu akisisia na. Enyuti akisisia agwoikin etacenit kijokis nakakinuok aakot.

Abekini ong' alomari kakisisia kana?

Ebo. Ebeikini Ijo/ikoku koni alomari kakisisia kana esaa kijokis. Ebeikinete lu egwoikito ikisila lukaking'ici (Ethics Committee)" akitogwo akisisia akolong' kijokis na anyunata kesi ebee itemit.

Inyana bo ikokin kibeloki ijo akiitunun kitoni alomari kakisisia kana?

Emam ibore ikokin. Enyuto imuruok akiyong' atiakunete kon nibarai ijo kere ikoku kon akilomo akisisia erai nakalemun.

Nuigwaite

Akiro kere nuikamuuto ijo kere ikoku koni mam kelimokino ituani ni ejei king'a kakisisia. Mam kenyute akitosom ekiror kon kere lokikoku kon akolong' kijokis na epudunere king'a akiro nukakisisia. Mamu kenyute akitosoma ekirori kon kere lokikoku koni kopone kere kakisisia kana akidepe enamba lo enyute ainakin ng'ini itwani. Enyute akidar akiro kon etosomao elacatait (password) lo ejena kama kaluedishika lu ejasi akisisia. Nu emouno kakisisia kana enyute akimoro kalukang'icaka kaluce alomuni ko Notre Dame Amerika. Akigireta kere ing'adakino mamu ekirori ketakanuni kalaro kapede.

Kejaasi aking'itasia ng'aibo ing'isio?

Kejai aking'isetait ikamuut akisisia na kere adumu ikoku kon ationus, kodoloki emuruon E**ric Ochomo P.O. BOX 1578 KISUMU** kere **0723845457.** Kejai aking'isetait ikamunit abeit kikoku koni ekamuuti akisisia na kitodiki lokegirgiran **kere loke kicolong loka KEMRI SERU P.O. Box 54840 00200, telephone (057) 2722541 or 0717719477 or 0722205901 or 0733400003 email address: SERU@kemri.org.** Asimunu nu mamu king'arakinete akolong' nakatipite. Kedekakin ikoku kere ijo elope, komatar katipete adekis na apiete kajo.

ATUTUBET NAKIYAREIT (PART 2)

Acamakinet

Ong'…….....Atodikin ong' atenikina kakisisia kokirori "**The Impact of Novel Vector Control Tools on Asymptomatic *Plasmodium falciparum* Infection Prevalence**" Acamu ong' / ikoku angi akilomo akisisia na. Ainakin eong' apak naka king'it ang'itasia kadumun anyakunet kese kakileskina. Atiaku eong' / ikoku ang'i akilomo akisisia na ajeni eong' ebee eong' kere ikoku ang'i ebeikinete alomari kakisisia akolong' kijokis komam kebeikit amunari nu anyuti eong' akiisit kadekis apak acee koking'aren

Ekiror kelopet etogo, (Participant, Parent or witness writes)

_____

_____ _____

Participant or parent / guardian signature Date (dd- mm- yy)

_____kodokoki ebokorit kemamu kejeni akigir

Kemamu elope etogo kejeni akigir:

Lokitee lo ejeni akigiri ng'esi igiri (kebeikina, koraete lo aseuni elope etogo komamu kejenuna kalukasisiaka). Lu ejasi akisisia komam kejenete akigiri, itemit kesi de akidokokin akan.
(Bala : Atee eong' isomakino lo eleskina akilomo akisisia epapula lokacamakinet aso konye ajeikin nesi kapak nakaking'it ang'itasia, kalemun mam kesikurakite.

Ekirori kalo kitee (Egiri lokitee)

_____

Akani kalokitee (Edokokin lokitee akan kaparasia)

_____Apaaran _____(dd/mm/yyyy)

Ekiror kalokang’itan (*consenter writes*)

_____

Akan_____Apaaran _____(dd/mm/yyyy)

Acamakinet kanuka akigwaa aakot konuoki kapak kace

MAM eong' kacamakit kogwai aakot ang'i / kere nukikoku (stop here)

EBO, acamaki eong' aakot ang'i/ nukikoku ang'i kogwai konuoki kapak kace koking'aren

Ekiror kelopet etogo, (Participant, Parent or witness writes)

_____

_____ _____

Participant or parent / guardian signature Date (dd- mm- yy)

_____kodokoki ebokorit kemamu kejeni akigir

Kemamu elope etogo kejeni akigir:

Lokitee lo ejeni akigiri ng'esi igiri (kebeikina, koraete lo aseuni elope etogo komamu kejenuna kalukasisiaka). Lu ejasi akisisia komam kejenete akigiri, itemit kesi de akidokokin akan.

(Bala : Atee eong' isomakino lo eleskina akilomo akisisia epapula lokacamakinet aso konye ajeikin nesi kapak nakaking'it ang'itasia, kalemun mam kesikurakite.

Ekirori kalo kitee (Egiri lokitee)

_____

Akani kalokitee (Edokokin lokitee akan kaparasia)

_____Apaaran _____(dd/mm/yyyy)

Ekiror kalokang’itan (*consenter writes*)

_____

Akan_____Apaaran _____(dd/mm/yyyy)

Acamakinet kanuka adiar aakot akitosoma apaaran ace?

☐ **MAM,** eong' kacamuut ayaar aakot ang'i/ nukikoku angi kakitosoma apaaran ace kakwap kanakoking'a

☐ **EBO**, Acamuut eong aakot ang'i kiton nukikoku ang'i kitosomae kapak kacee kakwap kanakoking'a

Ekiror kelopet etogo, (Participant, Parent or witness writes)

_____

_____ _____

Participant or parent / guardian signature Date (dd- mm- yy)

_____kodokoki ebokorit kemamu kejeni akigir

Kemamu elope etogo kejeni akigir:

Lokitee lo ejeni akigiri ng'esi igiri (kebeikina, koraete lo aseuni elope etogo komamu kejenuna kalukasisiaka). Lu ejasi akisisia komam kejenete akigiri, itemit kesi de akidokokin akan.
(Bala : Atee eong' isomakino lo eleskina akilomo akisisia epapula lokacamakinet aso konye ajeikin nesi kapak nakaking'it ang'itasia, kalemun mam kesikurakite.

Ekirori kalo kitee (Egiri lokitee)

_____

Akani kalokitee (Edokokin lokitee akan kaparasia)

_____Apaaran _____(dd/mm/yyyy)

Ekiror kalokang'itan (*consenter writes*)

_____

Akan_____Apaaran _____(dd/mm/yyyy)

Appendix D: Assent form (for children between 13-17 years, English version – Busia site)

Study Title: The Impact of Novel Vector Control Tools on Asymptomatic *Plasmodium falciparum* Infection Prevalence

Investigators and Institutions:

Cristian Koepfli (University of Notre Dame), Eric Ochomo (KEMRI/CDC), Colins Oduma (Egerton University), Tiffany Huwe (University of Notre Dame), John Gimnig (CDC), Winnie Chebore (KEMRI), Kephas Otieno (KEMRI), Simon Kariuki (KEMRI), John Gimnig (CDC), Achuyt Bhattarai (CDC), Aaron Samuels (CDC)

KEMRI/CDC- Kenya Medical Research Institute and CDC, Kisumu Kenya,
CDC- Centers for Disease Control and Prevention, Atlanta USA,
KEMRI- Kenya Medical Research Institute, Kisumu Kenya

Name of Participant: …….. Age: …….. (years)

Each volunteer will be given a copy of the full Informed Assent Form.

Who are we?

My name is ……. from KEMRI. We are studying transmission of malaria and other infections that can make you sick in this region. For this study, we work together with Dr. Eric Ochomo at KEMRI and other researchers at KEMRI, CDC and the University of Notre Dame in the USA.

Why are we meeting with you?

We want to tell you about a study that involves children like yourself. We want to see if you would like to be in this study.

Why are we doing this study?

We want to study whether some people like you are infected with malaria parasites or other infections even if they do not feel sick. We also want to study whether the antibodies in your body

indicate you have been infected in the past. This information will help us determine how to improve malaria control in this area.

What will happen to you if you are in the study?

If you decide to be in the study, we will first check your body temperature and ask you some questions about your health to know how you are feeling. We do not provide treatment of malaria or any other illnesses. If we find any abnormal results in the screening, we will inform you and your parent and encourage you to seek medical treatment. If you are otherwise healthy, you will be asked to allow us to collect 200 uL of blood from a finger or heel. We will again collect the same amount of blood 12 and 24 months later but only if you will consent during that time. We will save the blood sample for studying parasites and testing antibody responses to malaria using the serum. The duration of participation in this study is within one hour.

Will any part of the study hurt?

Blood drawing may cause some discomfort. We will use a sterile needle to collect finger-prick or heel-prick blood, so you will not be exposed to any pathogens from blood drawing. If we have difficulties in drawing your blood, we will stop immediately.

Who will know that you are in the study?

We will not tell anyone except your parents/guardians that we draw blood from you.

Do you have to be in the study?

No, you don't. No one will get angry or upset you if you don't want to do this. Just tell us if you don't want to be in the study. And remember, you can change your mind at any point during your participation in this study if you decide you don't want to be in the study anymore.

What will we do with your blood samples and medical records?

We want to use the blood sample you provide to determine whether you might carry malaria parasites without knowing it, and to study antibodies that might tell us whether you have carried the parasite recently, or have been bitten by mosquitos that can transmit diseases. If we find the malaria parasite, we will do studies on the genes of this parasite to understand where it comes from and the best methods for diagnosis and treatment.

You will give consent for future studies that we may perform in the laboratory. The sample may be shared with other researchers in foreign countries. Your name will not appear on the stored sample. Results from future studies may be reported in medical journals or at scientific conferences or meetings.

The data from your questionnaire we will complete will have coded identifiers instead of your name. Data are stored on computers that are secured with passwords, thus only the researchers with permission will have access to it.

Do you have any questions?

You can ask questions at any time. You can ask now. You can ask later If you have questions about this study, or if you feel your child has been harmed, please get in touch with the **Dr. Eric Ochomo, P.O. Box 1578 Kisumu or on 0723845457,** if you have any questions on your child's rights as a study participant or you would like to get in touch with someone outside of the study please communicate with **secretary or chairperson of KEMRI SERU P.O. Box 54840 00200, telephone (057) 2722541 or 0717719477 or 0722205901 or 0733400003 email address: SERU@kemri.org.** These phone numbers are not for emergencies. If you or your child is sick, go immediately to the nearest health facility or hospital.

Assent

I have read, or it has been read to me, and understands this consent form, and I am willing to participate in the study.

Signature of the Child: _____

Printed Name: _____Date: _____

Signature of the Investigator: _____

Printed Name: _____ ; Date: _____

A copy of this Informed Assent Form has been provided to participant _____ (initialed by the researcher/assistant)

Appendix E: Assent form (Kiswahili version – Busia site)

Kichwa cha somo: Athari za zana za kudhibiti vekta za riwaya juu ya kuenea kwa maambukizi ya *plasmodium falciparum* yasiyokua na dalili

Wachunguzi na taasisi:
Cristian Koepfli (Chuo Kikuu cha Notre Dame), Eric Ochomo (KEMRI/CDC), Colins Oduma (Chuo Kikuu cha Egerton), Tiffany Huwe (Chuo Kikuu cha Notre Dame), John Gimnig (CDC), Winnie Chebore (KEMRI), Kephas Otieno (KEMRI), Simon Kariuki (KEMRI), John Gimnig (CDC), Achuyt Bhattarai (CDC), Aaron Samuels (CDC)

KEMRI/CDC-taasisi ya utafiti wa matibabu ya Kenya na CDC, Kisumu Kenya,
CDC-vituo kwa ajili ya kudhibiti magonjwa na kuzuia, Atlanta USA,
KEMRI-taasisi ya utafiti wa matibabu Kenya, Kisumu Kenya

Jina la mshiriki: ..... **Umri:** Miaka.....
Kila anayejitolea atapewa nakala ya fomu kamili ya ridhaa iliyokubaliwa.

<u>Sisi ni nani?</u>
Jina langu ni..... kutoka KEMRI. Tunafanya masomo ya maambukizi ya malaria na maambukizo mengine yanayoweza kukufanya uwe mgonjwa katika eneo hili. Kwa utafiti huu, tunafanya kazi pamoja na Dkt Eric Ochomo na watafiti wengine katika Chuo Kikuu cha Notre Dame katika USA.

<u>Kwa nini tunakutana na wewe?</u>
Tunataka kukuambia kuhusu utafiti ambao unahusisha watoto kama wewe mwenyewe. Tunataka kuona kama ungependa kuwa katika utafiti huu.

<u>Kwa nini tunafanya utafiti huu?</u>
Tunataka kujifunza kama baadhi ya watu kama wewe wameambukizwa na vimelea vya malaria au maambukizi mengine hata kama hawahisi wagonjwa. Pia tunataka kujifunza kama antibodi

katika mwili wako zinaonyesha kuwa umeambukizwa katika siku za nyuma. Taarifa hii itatusaidia kuamua jinsi ya kuboresha udhibiti wa malaria katika eneo hili.

Nini kitatokea kwako ikiwa uko katika utafiti?

Kama umeamua kuwa katika utafiti, kwanza tutaangalia hali ya joto wa mwili wako. na kukuuliza maswali kadhaa juu ya afya yako kujua jinsi unavyohisi. Hatupeani matibabu ya malaria au magonjwa mengine yoyote. Kama tutapata matokeo yoyote isiyo ya kawaida katika uchunguzi, tutakujulisha wewe na mzazi wako na kuwahimiza kutafuta matibabu. Kama una afya njema, utaulizwa kuturuhusu sisi kukusanya karibu mikro lita 200 ya damu kutoka kidole au kisigino. Tutakusanya tena kiwango sawa cha damu miezi 12 na 24 baadaye lakini ikiwa utakubali wakati huo. Tutahifadhi sampuli ya damu kwa ajili ya kujifunza kuhusu vimelea na kupima majibu ya antibodi kwa malaria kwa kutumia seramu. Muda wa kushiriki katika utafiti ni kwa muda wa saa moja.

Je, sehemu yoyote ya utafiti huo ina madhara?

Kutolewa kwa damu inaweza kusababisha baadhi ya usumbufu. Tutatumia sindano safi kukusanya damu iliyochomwa kidole au kisigino kwa hivyo hautakuwa wazi kwa vimelea vyovyote kutoka kwa kutolewa damu. Ikiwa tutakua na shida katika kutoa damu yako, tutaacha mara moja.

Ni nani atakaye jua kwamba uko katika masomo?

Hatutaambia mtu yeyote ila wazazi wenu au walezi wenu kwamba sisi tunawateka damu kutoka kwenu.

Je, unapaswa kuwa katika utafiti?

Hapana, si sivyo. Hakuna mtu atakuwa na hasira au atakasirishwa na wewe kama hutaki kufanya hivyo. Tuambie kama hutaki kuwa katika utafiti. Na kumbuka, unaweza kubadilisha akili yako wakati wowote wa kushiriki kwako katika utafiti huu ikiwa unaamua hutaki kuwa katika utafiti tena.

Tutafanya nini na sampuli zako za damu na rekodi za tiba?

Tunataka kutumia sampuli ya damu unayotoa kubaini kama unaweza beba vimelea vya malaria bila kujua, na kujifunza antibodi ambayo inaweza kutuambia kama wewe umebeba vimelea hivi karibuni, au umeumwa na mbu ambao wanaweza kueneza magonjwa. Kama tutapata vimelea ugonjwa wa malaria, tutafanya utafiti juu ya jeni ya vimelea hii kuelewa ambapo linatoka na njia bora kwa ajili ya utambuzi na matibabu.

Utatoa kibali kwa masomo ya baadaye ya jeni ili tuweze kufanya katika maabara. Sampuli hiyo itashirikiwa na watafiti wengine katika nchi za kigeni. Jina lako halitaonekana kwenye sampuli iliyohifadhiwa. Matokeo kutoka masomo ya baadaye yanaweza kuripotiwa katika majarida ya matibabu au katika mikutano ya kisayansi au mikutano.

Data kutoka kwenye dodoso lako tutakamilisha itakuwa na vitambulishi vya kodi badala ya jina lako. Data zimehifadhiwa kwenye kompyuta ambazo zimehifadhiwa na nywila, hivyo watafiti walio na idhini tu ndio wana ruhusa wa kuzifikia.

Una maswali yoyote?

Unaweza kuuliza maswali wakati wowote. Unaweza kuuliza sasa. Unaweza kuuliza baadaye kama una maswali kuhusu utafiti huu, au kama unahisi mtoto wako ameumizwa, tafadhali kuwasiliana na **Dkt. Eric Ochomo, S.L.P. Box 1578 Kisumu au katika 0723845457**, kama una maswali yoyote juu ya haki mtoto wako kama mshiriki wa utafiti au ungependa kuwasiliana na mtu nje ya utafiti tafadhali wasiliana na **Katibu au mwenyekiti wa KEMRI SERU S.L.P. Box 54840 00200, simu (057) 2722541 au 0717719477 au 0722205901 au 0733400003 anwani ya barua pepe: SERU@kemri.org.**

Nambari hizi za simu sio za dharura. Iwapo wewe au mtoto wako ni mgonjwa, nenda mara moja kwenye kituo cha afya kilicho karibu au hospitali.

Kibali

Nimesoma, au imesomwa kwangu, na kuelewa fomu hii ya kibali, na niko tayari kushiriki katika utafiti.

Sahihi ya mtoto: _____

Jina la kuchapishwa: _____;Tarehe: _____

Sahihi ya mchunguzi: _____

Jina la kuchapishwa: _____Tarehe: _____

Nakala ya fomu ya kibali hii ya taarifa imetolewa kwa mshiriki _____(kwa kutumia mtafiti/msaidizi)

Appendix F: Assent form (Ateso version – Busia site)

Study Title: The Impact of Novel Vector Control Tools on Asymptomatic *Plasmodium falciparum* Infection Prevalence

Investigators and Institutions:

Cristian Koepfli (University of Notre Dame), Eric Ochomo (KEMRI/CDC), Colins Oduma (Egerton University), Tiffany Huwe (University of Notre Dame), John Gimnig (CDC), Winnie Chebore (KEMRI), Kephas Otieno (KEMRI), Simon Kariuki (KEMRI), Achuyt Bhattarai (CDC), Aaron Samuels (CDC)

KEMRI/CDC- Kenya Medical Research Institute and CDC, Kisumu Kenya,
CDC- Centers for Disease Control and Prevention, Atlanta USA,
KEMRI- Kenya Medical Research Institute, Kisumu Kenya

Ekiror ka lokasisian: ……..... Ikaru: …….....

Edumunete lukasisiak kijokis epapulat lokacamakino

<u>Siong kes ka ng'ai?</u>

Ekirori ang'i erai ……. Asomae ong' katon KEMRI. Asomaete siong' aking'ici kadeka nake kemidi kitoni adekisia acee nu ebeikinete ainakin ijo edekakin katutubet kon kana. Kakisisia kana, asomaete siong' kitoni emuruon Eric Ochomo loko KEMRI kiton lukang'icak icee alomon ko KEMRI, CDC kiton University of Notre Dame luka USA.

<u>Inyobo akotosi siong aanyun ijo?</u>

Akotosi siong' akitodikin ijo ekamasi ka king'ici' na ejaasi idwee bala ijo toma. Akotosi siong' anyuni kibeikin ijo ajausu akisisia na.

<u>Inyobo asomaata siong' aking'ic na?</u>

Akotosi siong' ajenun kebeikini itwaan bala ijo akamun adeka nakekimidi kere adekisia acee ata kemam ijo kedekakina. Akotosi siong de ajenun kebeikini aakot kakwaan kon atodikini kedekakina itwaan sek. Ajenuni akiro ng'unu eng'arakini oni arebokini ekimidi kaduket ook.

Inyena esomakini Kama kon ijei ijo akisisia ?

Kitiaku ijo akilomo akisisia, enyuto siong' akinuok amwanusu kakwana koni beree kaking'it ijo ang'itasia adisi ekamasi kang'aleu kon. Mam siong' kemadete ekimidi kere adekasinoi acee. Kenuokun adeka kijokis ka Kwan kon, enyuto siong akitodikin ijo kiton ekaurian kon teter ibeikinete osi akouni akimado. Kingale ijo, ebuuto siong akilipa alemari akoot kobokorit keree kakeju kon nu edolete 200uL. Enyuto siong' bobo alemun aakot kama kon kolapio 12 kiton 24 lu ebuuto koking'aren kicamaki ijo. Enyuto siong' aking'adakin aakot kon Kanu kakitolosit akinuok ekamasi kiton ekurut lo eyauti ekimidi. Ajausu akisisia na erai akolong' 1 Boni.

Enyuuti alaro apede kakisisia ayauni apipilu?

Ariuni aakot ebeikin eyauni apipilu adiosi. Enyuto siong' akitosom Esindani lo emadite ariuni aakot kobokorit kon loka akan kere akeju teter mam ijo Kelokin akwaana kon adekasinoi. Kecata ariuni aakot ka Kwana kon, mam siong' kitolosiete.

Ng'ai ejenuni eji ijo akisisia na?

Mam siong kalimokinete itwan akidepe ikauriaka kon ebee elemarete siong aakot kama koni.

Ekotokina ijo tar ajaus akisisia na?

Mam, mam ijo kekotokina. Emam itwan kemunauni etau kon emam ijo kekote ajaus akisisia na. kemam ijo kekote kolimoki siong. Koitu ebee ibeikin ijo akibelokin aumusio kon akolong' kijokis kemam ijo kekote ajautu akisisia naa.

Inyena esomakinete siong aakot koni kitoni akiro ekamasi kangaleu koni ?

Abutosi siong akitosoma aakot koni ajenun ijasi ijo kadeka nakekimidi konye mam ijo kejenut. Kiton akinuok isirikari luedarete akuan anyuni kidakit ijo ekurut loke kemidi lo idumunit ijo pacu kere kikonyit ijo Esirut lo ebeikin akisikama adekasinoi. kedumun ekurut lokekimidi, enyuto siong

akisisia ekamasi kekipone kene kiton ajenuni namaelomutere, kajenuni irotini lukajokaka luka kinuoko kitoni akimado.

Enyuti ijo ainakin acamakinet kanuka kisisia na ebeikin isomakin kolabaratori kapak kana kokingaren. Ebeikinete siong imorete nu adumutu siong kolabaratori ekamasi kaakoto kon kitoni lukangicaka alomuni kakwapin nukokinga . Mam ekiror kon kenyut ajausu aakot kon. Nu engicuno konkingaren ebeikino itodiaro kakitosom akitaboi nukangaleu, kere De atukuno nuka lukangicaka.

Ekaratasit lokang'itasia koni epiakino a code mere ekiror koni, kapiakini a computa, kakigwa ke "password" komam itwaan icee kebeikini aanyun.

Ijasi ijo kangitasia?

Ibeikin ijo a kingit angitasia akolong kijokis. Ibeikin ijo akingit kipokona. Ibeikin ijo akingit kakolong kacee Ijasi ijo kangitasia ekamasi kakisisia kere imunit ijo ejasi nukarokok nu adumakinosi nama kikoku, ibeikini ijo adolokini emuruon **Eric Ochomo, P.O. Box 1578 Kisumu or on 0723845457,** ijasi ijo kangitasia kere nu itupasi kajaus koni kere ikote ijo anerakin kitwan ne emam kejii akisisa na kodoloki **lokapoloni KEMRI SERU P.O. Box 54840 00200, telephone (057) 2722541 or 0717719477 or 0722205901 or 0733400003 email addres**.
Inambai lu mere lukatipite. Kidekakin ijo keree ikoku koni, koloto adekisi na apiete kajo katipite.

Akani kikoku:

_____

Ekiror:_____;Aparasia:_____

Akani kaloka ng'itan: _____

Ekiror: _____;Aparasia: _____

Appendix G: Informed consent for study participation (English version- Kisumu and Homa Bay sites)

Title of study: Environmental Modification in Sub-Saharan Africa: Changing Epidemiology, Transmission and pathogenesis of *Plasmodium falciparum* and *P. vivax* Malaria

This assent form will be explained and signed by each study participant

Name of Volunteer _____ Age (years)_____

Investigators conducting the study

Dr. Harrysone Atieli, Maseno University, Kenya

Prof. Guiyun Yan, Program in Public Health, University of Carlifonia, Irvine, USA

Purpose of study

The purpose of this study is to assess the impact of environmental modifications *Plasmodium falciparum* and *P. vivax* Malaria epidemiology and disease burden.

Procedure to be followed

Approximately 250 μL of blood will be collected by finger prick in EDTA microtainers. Thin and thick smears will be made. We will ask you some questions about whether you had malaria in the past two weeks and whether you have taken antimalarial drugs. This information is important for us to determine how active malaria transmission occurs in your village.

Inclusion criteria

We will include all residents located in the study area who are willing to participate in the study regardless of their sex, age and economic status.

Exclusion criteria

Residents who are unwilling to participate in the study or change their willingness to participate at any point in time.

Discomforts and risks

The finger-prick blood collection method causes slight discomfort. Sterile blood lancets followed with sterile ethanol will be used for every single person, the procedures will pose very minimal risk of being infected by other pathogens.

Benefit to participants

You will not receive financial benefit from your participation, however, if you have a fever or ill, you will be referred to the local clinic for care.

Reimbursement for medical treatment

This project will be responsible for diagnosis of malaria and referral to local clinics for evaluation. Competent staff member of the Kenyan Ministry of Health will perform the evaluation and provide appropriate treatment. The project will only cover the costs of the normal standard treatment of uncomplicated malaria approved by Kenyan Ministry of Health. The study will not attend to other diseases unrelated to malaria. If other illness or diseases are identified during the malaria screening, we will provide referral to the appropriate local health authorities. In cases of emergencies, transport, whenever possible, will be provided to the nearest government medical facility.

Confidentiality

Information related to you will be treated in strict confidence to the extent provided by law. Your identity will be coded and will not be associated with any published results. Your code number and identity will be kept in a locked file of the Principal Investigator and Kenyan Investigator.

Freedom to withdraw

Your participation in this study is voluntary and you may discontinue your participation at any time without prejudice and without affecting future health care.

Assent form

The form will be explained to each participant and signed by the Investigator or the leading scientist conducting the study.

New findings

You will be told of any significant new findings developed during the course of study.

Do you have questions?

You can ask questions at any time. You can ask now. You can ask later. You can talk to me or to someone else at any time during the study.

Harrysone Atieli	Maseno	0721347437
Guiyun Yan	UC Irvine	1-9498240175

For any questions pertaining to rights as a research participant, contact person is: **The Secretary, Maseno University Ethics Review Committee, Private Bag, Maseno; Telephone numbers: 057-51622, 0722203411, 0721543976, 0733230878; Email address**
: muerc-secretariate@maseno.ac.ke; muerc-secretariate@gmail.com.

I HAVE READ AND UNDERSTAND THIS CONSENT FORM, AND I AM WILLING TO PARTICIPATE IN THE STUDY

_____	_____	_____
Subject's name	Signature	Date
_____	_____	_____
Witness' name	Signature	Date
_____	_____	_____
Investigator's name	Signature	Date

Each participant will be given a copy of signed consent form

Appendix H: Assent form (English version - Kisumu and Homa Bay sites)

Title of study: Environmental Modification in Sub-Saharan Africa: Changing Epidemiology, Transmission and pathogenesis of *Plasmodium falciparum* and *P. vivax* Malaria

This assent form will be explained and signed by each study participant

Name of Volunteer _____ Age (years)_____

Who are we?

Our names are Dr. Harrysone Atieli and Dr. Guiyun Yan. Dr. Atieli is a lecturer and research scientist in Maseno University. Dr. Yan is an Associate Professor in the Program in Public Health at the University of Carlifonia at Irvine.

Why are we meeting you?

We want to tell you about a study that involves children like yourself. We want to see if you would like to be in this study.

Why are we doing this study?

We are trying to determine malaria vector, transmission and parasite dynamics in East Africa.

What will happen to you if you are in the study?

A tiny amount of blood will be taken from your finger by pricking it to find out whether you are infected with malaria parasites.

Exclusion criteria

Residents who intend to relocate during the study period and are unwilling to participate in the study will be excluded.

Discomforts and risks

The finger-prick blood collection method causes slight discomfort. Sterile blood lancets followed with sterile ethanol will be used for every single person, the procedures will pose very minimal risk of being infected by other pathogens.

Benefit to participants

You will not receive financial benefit from your participation, however, if you have a fever or ill, you will be referred to the local clinic for care.

Reimbursement for medical treatment

This project will be responsible for diagnosis of malaria and referral to local clinics for evaluation. Competent staff member of the Kenyan Ministry of Health will perform the evaluation and provide appropriate treatment. The project will only cover the costs of the normal standard treatment of uncomplicated malaria approved by Kenyan Ministry of Health. The study will not attend to other diseases unrelated to malaria. If other illness or diseases are identified during the malaria screening, we will provide referral to the appropriate local health authorities. In cases of emergencies, transport, whenever possible, will be provided to the nearest government medical facility.

Confidentiality

Information related to you will be treated in strict confidence to the extent provided by law. Your identity will be coded and will not be associated with any published results. Your code number and identity will be kept in a locked file of the Principal Investigator and Kenyan Investigator.

Freedom to withdraw

Your participation in this study is voluntary and you may discontinue your participation at any time without prejudice and without affecting future health care.

Assent form

The form will be explained to each participant and signed by the Investigator or the leading scientist conducting the study.

New findings

You will be told of any significant new findings developed during the course of study.

Do you have questions?

You can ask questions at any time. You can ask now. You can ask later. You can talk to me or to someone else at any time during the study.

Harrysone Atieli	Maseno	0721347437
Guiyun Yan	UC Irvine	1-9498240175

For any questions pertaining to rights as a research participant, contact person is: **The Secretary, Maseno University Ethics Review Committee, Private Bag, Maseno; Telephone numbers: 057-51622, 0722203411, 0721543976, 0733230878; Email address**: muerc-secretariate@maseno.ac.ke; muerc-secretariate@gmail.com.

IF YOU WANT TO BE IN THE STUDY, SIGN YOUR NAME ON THE LINE BELOW

Signature of the child _____

Printed name_____ Date_____

Signature of the parent/guardian_____

Printed name_____ Date_____

Signature of the Investigator _____

Printed name_____ Date_____

(Each participant will be given a copy of this assent form)

Appendix I: Informed consent for study participation (Kiswahili version- Kisumu and Homa Bay sites)

Kichwa cha utafiti: Marekebisho ya Mazingira katika Afrika Kusini mwa Jangwa la Sahara: Kubadilisha Epidemiolojia, Usambazaji na pathogenesis ya *Plasmodium falciparum* na *P. vivax* Malaria

Fomu hii ya idhini itaelezwa na kusainiwa na kila mshiriki wa utafiti

Jina …… Umri (miaka) ……

Wachunguzi wanaofanya utafiti

Dkt. Harrysone Atieli, Chuo Kikuu cha Maseno, Kenya

Prof. Guiyun Yan, Mpango katika Afya ya Umma, Chuo Kikuu cha Carlifonia, Irvine, Marekani

Kusudi la kusoma

Madhumuni ya utafiti huu ni kutathmini athari za marekebisho ya mazingira *Plasmodium falciparum* na *P. vivax* Epidemiology ya Malaria na mzigo wa magonjwa.

Utaratibu wa kufuatwa

Takriban 250 microliter ya damu itakusanywa kwa kuchomwa kidole katika vidude vidogo vya EDTA. Smears nyembamba na nene zitafanywa. Tutakuuliza baadhi ya maswali kuhusu kama ulikuwa na malaria katika wiki mbili zilizopita na kama umetumia dawa za kuzuia malaria. Taarifa hizi ni muhimu kwetu ili kubainisha jinsi maambukizi ya malaria yanavyotokea katika kijiji chako.

Vigezo vya kuingizwa

Tutajumuisha wakaazi wote walio katika eneo la utafiti ambao wako tayari kushiriki katika utafiti bila kujali jinsia zao, umri na hali yao ya kiuchumi.

Vigezo vya kutengwa

Wakazi ambao hawataki kushiriki katika utafiti au kubadilisha nia yao ya kushiriki wakati wowote.

Usumbufu na hatari

Njia ya kukusanya damu ya kidole-chomo husababisha usumbufu kidogo. Lanceti za damu zisizo na tasa zikifuatiwa na ethanoli tasa zitatumika kwa kila mtu, taratibu zitaleta hatari ndogo sana ya kuambukizwa na vimelea vingine vya magonjwa.

Faida kwa washiriki

Hutapokea manufaa ya kifedha kutokana na ushiriki wako, hata hivyo, ikiwa una homa au mgonjwa, utatumwa kwa kliniki ya karibu kwa ajili ya huduma.

Malipo ya matibabu

Mradi huu utakuwa na jukumu la utambuzi wa malaria na rufaa kwa kliniki za mitaa kwa tathmini. Mfanyikazi stadi wa Wizara ya Afya ya Kenya atafanya tathmini na kutoa matibabu yanayofaa. Mradi huo utagharamia tu matibabu ya kawaida ya kawaida ya ugonjwa wa malaria usio ngumu ulioidhinishwa na Wizara ya Afya ya Kenya. Utafiti hautashughulikia magonjwa mengine yasiyohusiana na malaria. Ikiwa magonjwa au magonjwa mengine yatatambuliwa wakati wa uchunguzi wa malaria, tutatoa rufaa kwa mamlaka zinazofaa za afya za mitaa. Katika hali ya dharura, usafiri, wakati wowote iwezekanavyo, utatolewa kwa kituo cha matibabu cha karibu cha serikali.

Usiri

Taarifa zinazohusiana na wewe zitashughulikiwa kwa imani kali kwa kiwango kinachotolewa na sheria. Utambulisho wako utawekewa msimbo na hautahusishwa na matokeo yoyote yaliyochapishwa. Nambari yako ya msimbo na utambulisho vitawekwa katika faili iliyofungwa ya Mpelelezi Mkuu na Mpelelezi wa Kenya.

Uhuru wa kujiondoa

Ushiriki wako katika utafiti huu ni wa hiari na unaweza kusitisha ushiriki wako wakati wowote bila chuki na bila kuathiri huduma za afya za siku zijazo.

Fomu ya idhini

Fomu itaelezewa kwa kila mshiriki na kusainiwa na Mpelelezi au mwanasayansi mkuu anayefanya utafiti.

Matokeo mapya

Utaambiwa juu ya matokeo yoyote mapya muhimu yaliyotengenezwa wakati wa utafiti.

Je, una maswali?

Unaweza kuuliza maswali wakati wowote. Unaweza kuuliza sasa. Unaweza kuuliza baadaye. Unaweza kuzungumza nami au na mtu mwingine wakati wowote wakati wa funzo.

Harrysone Atieli	Maseno	0721347437
Guiyun Yan	UC Irvine	1-9498240175

Kwa maswali yoyote yanayohusu haki kama mshiriki wa utafiti, mtu wa kuwasiliana naye ni: Katibu, Kamati ya Mapitio ya Maadili ya Chuo Kikuu cha Maseno, Mfuko wa Kibinafsi, Maseno; Nambari za simu: 057-51622, 0722203411, 0721543976, 0733230878; Barua pepe : muerc-secretariate@maseno.ac.ke; muerc-secretariate@gmail.com.

NIMESOMA NA KUELEWA FOMU HII YA RIDHAA, NA NIKO TAYARI KUSHIRIKI KATIKA UTAFITI.

_____	_____	_____
Jina la mshiriki	Sahihi	Tarehe
_____	_____	_____
Jina la shahidi	Sahihi	Tarehe
_____	_____	_____
Jina la mpelelezi	Sahihi	Tarehe

(Kila mshiriki atapewa nakala ya fomu hii ya idhini)

Appendix J: Assent form (Kiswahili version - Kisumu and Homa Bay sites)

Kichwa cha utafiti: Marekebisho ya Mazingira katika Afrika Kusini mwa Jangwa la Sahara: Kubadilisha Epidemiolojia, Usambazaji na pathogenesis ya *Plasmodium falciparum* na *P. vivax* Malaria

Fomu hii ya idhini itaelezwa na kusainiwa na kila mshiriki wa utafiti

Jina …… Umri (miaka) ……..

Sisi ni akina nani?

Majina yetu ni Dk. Harrysone Atieli na Dk. Guiyun Yan. Dk. Atieli ni mhadhiri na mwanasayansi wa utafiti katika Chuo Kikuu cha Maseno. Dk. Yan ni Profesa Mshiriki katika Mpango wa Afya ya Umma katika Chuo Kikuu cha Carlifonia huko Irvine Amerika.

Kwa nini tunakutana nawe?

Tunataka kukuambia kuhusu utafiti unaohusisha watoto kama wewe. Tunataka kuona kama ungependa kuwa katika utafiti huu.

Kwa nini tunafanya utafiti huu?

Tunajaribu kubaini vekta ya malaria, maambukizi na mienendo ya vimelea katika Afrika Mashariki.

Je, nini kitatokea kwako ikiwa uko kwenye utafiti?

Kiasi kidogo cha damu kitachukuliwa kutoka kwa kidole chako kwa kuchomwa ili kujua kama umeambukizwa na vimelea vya malaria.

Vigezo vya kutengwa

Wakazi wanaonuia kuhama wakati wa kipindi cha utafiti na ambao hawako tayari kushiriki katika utafiti hawatajumuishwa.

Usumbufu na hatari

Njia ya kukusanya damu ya kidole-chomo husababisha usumbufu kidogo. Lanceti za damu zisizo na tasa zikifuatiwa na ethanoli tasa zitatumika kwa kila mtu, taratibu zitaleta hatari ndogo sana ya kuambukizwa na vimelea vingine vya magonjwa.

Faida kwa washiriki

Hutapokea manufaa ya kifedha kutokana na ushiriki wako, hata hivyo, ikiwa una homa au mgonjwa, utatumwa kwa kliniki ya karibu kwa ajili ya huduma.

Malipo ya matibabu

Mradi huu utakuwa na jukumu la utambuzi wa malaria na rufaa kwa kliniki za mitaa kwa tathmini. Mfanyikazi stadi wa Wizara ya Afya ya Kenya atafanya tathmini na kutoa matibabu yanayofaa. Mradi huo utagharamia tu matibabu ya kawaida ya kawaida ya ugonjwa wa malaria usio ngumu ulioidhinishwa na Wizara ya Afya ya Kenya. Utafiti hautashughulikia magonjwa mengine yasiyohusiana na malaria. Ikiwa magonjwa au magonjwa mengine yatatambuliwa wakati wa uchunguzi wa malaria, tutatoa rufaa kwa mamlaka zinazofaa za afya za mitaa. Katika hali ya dharura, usafiri, wakati wowote iwezekanavyo, utatolewa kwa kituo cha matibabu cha karibu cha serikali.

Usiri

Taarifa zinazohusiana na wewe zitashughulikiwa kwa imani kali kwa kiwango kinachotolewa na sheria. Utambulisho wako utawekewa msimbo na hautahusishwa na matokeo yoyote yaliyochapishwa. Nambari yako ya msimbo na utambulisho vitawekwa katika faili iliyofungwa ya Mpelelezi Mkuu na Mpelelezi wa Kenya.

Uhuru wa kujiondoa

Ushiriki wako katika utafiti huu ni wa hiari na unaweza kusitisha ushiriki wako wakati wowote bila chuki na bila kuathiri huduma za afya za siku zijazo.

Fomu ya idhini

Fomu itaelezewa kwa kila mshiriki na kusainiwa na Mpelelezi au mwanasayansi mkuu anayefanya utafiti.

Matokeo mapya

Utaambiwa juu ya matokeo yoyote mapya muhimu yaliyotengenezwa wakati wa utafiti.

Je, una maswali?

Unaweza kuuliza maswali wakati wowote. Unaweza kuuliza sasa. Unaweza kuuliza baadaye. Unaweza kuzungumza nami au na mtu mwingine wakati wowote wakati wa utafiti.

Harrysone Atieli	Maseno	0721347437
Guiyun Yan	UC Irvine	1-9498240175

Kwa maswali yoyote yanayohusu haki kama mshiriki wa utafiti, mtu wa kuwasiliana naye ni: **Katibu, Kamati ya Mapitio ya Maadili ya Chuo Kikuu cha Maseno, Mfuko wa Kibinafsi, Maseno; Nambari za simu: 057-51622, 0722203411, 0721543976, 0733230878; Barua pepe**: muerc-secretariate@maseno.ac.ke; muerc-secretariate@gmail.com.

UKITAKA KUWA KWENYE UTAFITI, SAINI JINA LAKO KWENYE MSTARI HAPA CHINI

Sahihi ya mtoto _____

Jina _____ Tarehe _____

Sahihi la mzazi/mlezi _____

Jina _____ Tarehe _____

Sahihi ya mplelezi _____

Jina _____ Tarehe _____

(Kila mshiriki atapewa nakala ya fomu hii ya idhini)

Appendix K: Research permit (Busia site)

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) 2722541, 2713349, 0722-205901,0733-400003, Fax: (254) (020) 2720030
Email: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 **March 2, 2021**

TO: **DR. ERIC OCHOMO**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DEPUTY DIRECTOR, CGHR,**
KISUMU

FORWARDED
for DIRECTOR - CGHR

Dear Sir,

RE: **KEMRI/SERU/CGHR/319/3931 *(REQUEST FOR ANNUAL RENEWAL & DEVIATION)*: THE IMPACT OF NOVEL VECTOR CONTROL TOOL ON ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTION PREVALENCE.**

Thank you for the continuing review report for the period **March 9, 2020 to February 4, 2021**.

The expedited review team of the KEMRI Scientific and Ethics Review Unit (SERU) noted that a protocol deviation report has been submitted as the request for annual renewal was done after the required date of submission. Measures taken to address the deviation were deemed adequate.

This is to inform you that the expedited review team of the KEMRI SERU conducted the annual review of the above referenced application and was of the informed opinion that the progress made during the reported period is satisfactory. The study has therefore been granted **approval**.

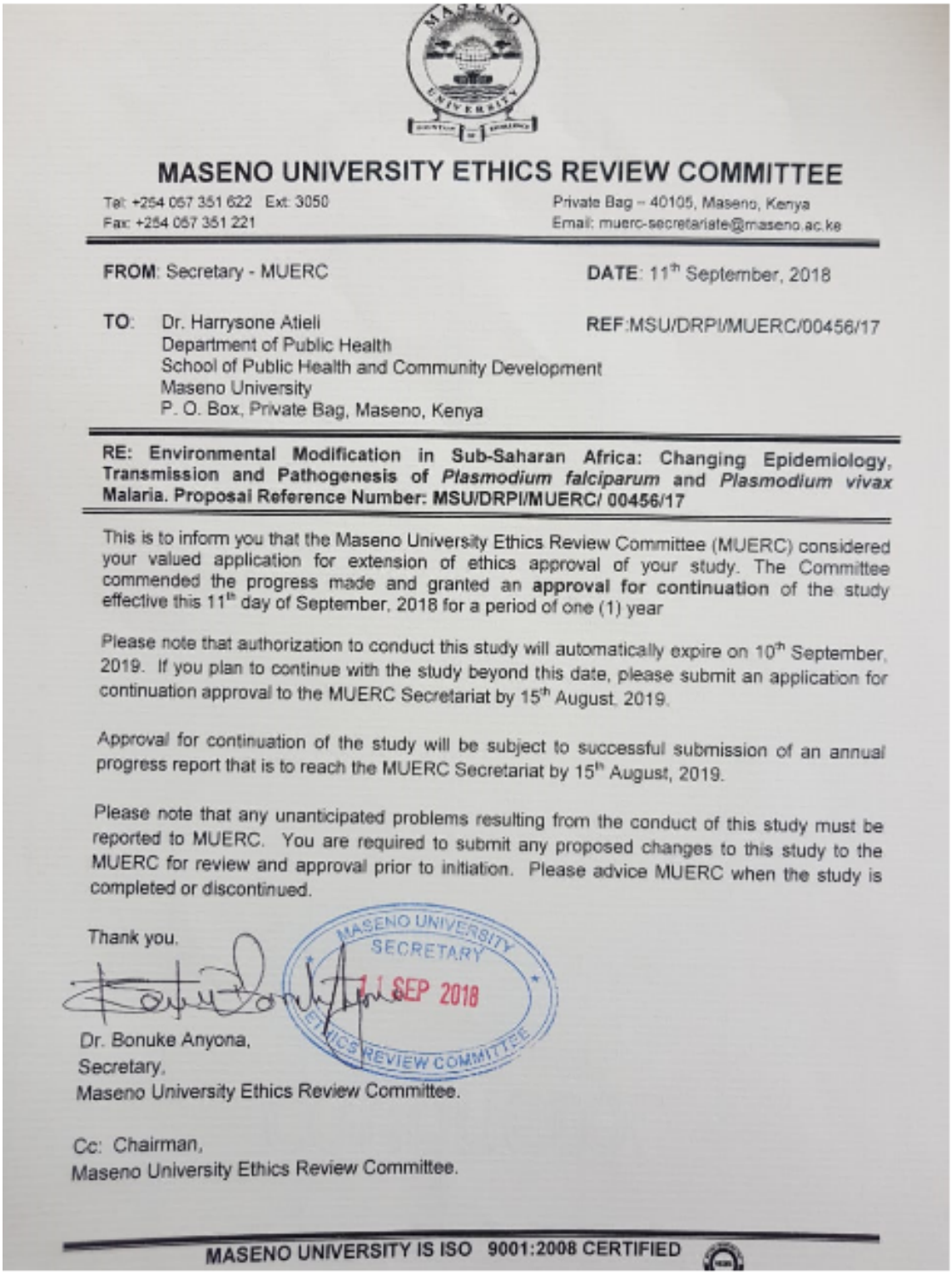
This approval is valid from **March 9, 2021** through to **March 9, 2022**. Please note that authorization to conduct this study will automatically expire on **March 9, 2022**. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the SERU by **January 26, 2022.**

You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to the SERU for review prior to initiation. You may continue with the study.

Yours faithfully,

ENOCK KEBENEI,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT.

Appendix L: Research permit (Homa Bay and Kisumu sites)



MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221

Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 11th September, 2018

TO: Dr. Harrysone Atieli
Department of Public Health
School of Public Health and Community Development
Maseno University
P. O. Box, Private Bag, Maseno, Kenya

REF:MSU/DRPI/MUERC/00456/17

RE: Environmental Modification in Sub-Saharan Africa: Changing Epidemiology, Transmission and Pathogenesis of *Plasmodium falciparum* and *Plasmodium vivax* Malaria. Proposal Reference Number: MSU/DRPI/MUERC/ 00456/17

This is to inform you that the Maseno University Ethics Review Committee (MUERC) considered your valued application for extension of ethics approval of your study. The Committee commended the progress made and granted an **approval for continuation** of the study effective this 11th day of September, 2018 for a period of one (1) year

Please note that authorization to conduct this study will automatically expire on 10th September, 2019. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 15th August, 2019.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 15th August, 2019.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to the MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

Thank you.

MASENO UNIVERSITY SECRETARY 11 SEP 2018 ETHICS REVIEW COMMITTEE

Dr. Bonuke Anyona,
Secretary,
Maseno University Ethics Review Committee.

Cc: Chairman,
Maseno University Ethics Review Committee.

MASENO UNIVERSITY IS ISO 9001:2008 CERTIFIED

Appendix M: NACOSTI research license

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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RESEARCH LICENSE

This is to Certify that Mr.. Colins oduma of Egerton University, has been licensed to conduct research in Homabay, Kisumu on the topic: IMPACT OF SEASONALITY AND MULTIPLICITY 1 OF Plasmodium falciparum INFECTION ON PATTERNS OF GAMETOCYTE AMONG MALARIA ASYMPTOMATIC INDIVIDUALS FROM SELECTED SITES IN WESTERN KENYA for the period ending : 26/November/2022.

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Appendix N: Participants, households, and cluster level characteristics

Numerical variable					
	variable	min	max	median	mean
Household level	Altitude (in meters)	1077	1284	1149	1162
	Distance to the nearest river (in meters)	0.4	1933.6	510.5	581.8
	Household population	1	16	5	5.5
	Habitat number within 250m of household	0	17	2	2.9
	Distance to the nearest habitat within cluster	0.5	881.3	127.1	160.9
Cluster level	Bed net coverage %	73.9%	100%	93.5%	92.9%
	Habitat number per cluster	3	34	15	15.5

Categorical variable			
	variable	category	percentage
Household level	Education of household head	Below secondary	76.5%
		Completed secondary	23.5%
	Kitchen location	Indoor	19.6%
		Outdoor	80.4%
	Roof material	corrugated iron	84.4%
		non corrugated iron	15.6%
	Screens window	No	97.2%
		Yes	2.8%
	Eave type	Closed	32.7%
		Open	67.3%
Individual level	Age category (years)	<5	16.8%
		5-14	25.9%
		15-39	37.7%
		40-59	12.4%
		>59	7.1%
	Sex	female	59.5%
		male	40.5%

PLOS GLOBAL PUBLIC HEALTH

RESEARCH ARTICLE

Altitude, not potential larval habitat availability, explains pronounced variation in *Plasmodium falciparum* infection prevalence in the western Kenya highlands

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Abstract

Progress in malaria control has stalled over the recent years. Knowledge on main drivers of transmission explaining small-scale variation in prevalence can inform targeted control measures. We collected finger-prick blood samples from 3061 individuals irrespective of clinical symptoms in 20 clusters in Busia in western Kenya and screened for *Plasmodium falciparum* parasites using qPCR and microscopy. Clusters spanned an altitude range of 207 meters (1077–1284 m). We mapped potential mosquito larval habitats and determined their number within 250 m of a household and distances to households using ArcMap. Across all clusters, *P. falciparum* parasites were detected in 49.8% (1524/3061) of individuals by qPCR and 19.5% (596/3061) by microscopy. Across the clusters, prevalence ranged from 26% to 70% by qPCR. Three to 34 larval habitats per cluster and 0–17 habitats within a 250m radius around households were observed. Using a generalized linear mixed effect model (GLMM), a 5% decrease in the odds of getting infected per each 10m increase in altitude was observed, while the number of larval habitats and their proximity to households were not statistically significant predictors for prevalence. Kitchen located indoors, open eaves, a lower level of education of the household head, older age, and being male were significantly associated with higher prevalence. Pronounced variation in prevalence at small scales was observed and needs to be taken into account for malaria surveillance and control. Potential larval habitat frequency had no direct impact on prevalence.

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BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Increased investment in gametocytes in asymptomatic *Plasmodium falciparum* infections in the wet season

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Abstract

Background: Transmission stemming from asymptomatic infections is increasingly being recognized as a threat to malaria elimination. In many regions, malaria transmission is seasonal. It is not well understood whether *Plasmodium falciparum* modulates its investment in transmission to coincide with seasonal vector abundance.

Methods: We sampled 1116 asymptomatic individuals in the wet season, when vectors are abundant, and 1743 in the dry season, in two sites in western Kenya, representing different transmission intensities (Chulaimbo, moderate transmission, and Homa Bay, low transmission). Blood samples were screened for *P. falciparum* by qPCR, and gametocytes by *pfs25* RT-qPCR.

Results: Parasite prevalence by qPCR was 27.1% (Chulaimbo, dry), 48.2% (Chulaimbo, wet), 9.4% (Homabay, dry), and 7.8% (Homabay, wet). Mean parasite densities did not differ between seasons ($P = 0.562$). *pfs25* transcripts were detected in 119/456 (26.1%) of infections. In the wet season, fewer infections harbored detectable gametocytes (22.3% vs. 33.8%, $P = 0.009$), but densities were 3-fold higher (wet: 3.46 transcripts/uL, dry: 1.05 transcripts/uL, $P < 0.001$). In the dry season, 4.0% of infections carried gametocytes at moderate-to-high densities likely infective (> 1 gametocyte per 2 uL blood), compared to 7.9% in the wet season. Children aged 5–15 years harbored 76.7% of infections with gametocytes at moderate-to-high densities.

Conclusions: Parasites increase their investment in transmission in the wet season, reflected by higher gametocyte densities. Despite increased gametocyte densities, parasite density remained similar across seasons and were often below the limit of detection of microscopy or rapid diagnostic test, thus a large proportion of infective infections would escape population screening in the wet season. Seasonal changes of gametocytemia in asymptomatic infections need to be considered when designing malaria control measures.

Keywords: *Plasmodium falciparum*, Transmission, Gametocyte, Asymptomatic, Season

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Plasmodium falciparum and *Plasmodium vivax* Adjust Investment in Transmission in Response to Change in Transmission Intensity: A Review of the Current State of Research

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Malaria parasites can adjust the proportion of parasites that develop into gametocytes, and thus the probability for human-to-vector transmission, through changes in the gametocyte conversion rate. Understanding the factors that impact the commitment of malaria parasites to transmission is required to design better control interventions. *Plasmodium* spp. persist across countries with vast differences in transmission intensities, and in sites where transmission is highly seasonal. Mounting evidence shows that *Plasmodium* spp. adjusts the investment in transmission according to seasonality of vector abundance, and transmission intensity. Various techniques to determine the investment in transmission are available, i.e., short-term culture, where the conversion rate can be measured most directly, genome and transcriptome studies, quantification of mature gametocytes, and mosquito feeding assays. In sites with seasonal transmission, the proportion of gametocytes, their densities and infectivity are higher during the wet season, when vectors are plentiful. When countries with pronounced differences in transmission intensity were compared, the investment in transmission was higher when transmission was low, thus maximizing the parasite's chances to be transmitted to mosquitoes. Increased transmissibility of residual infections after a successful reduction of malaria transmission levels need to be considered when designing intervention measures.

Keywords: ***Plasmodium falciparum*** **(Pf),** ***Plasmodium vivax*** **(pv), gametocyte carriage, investment in transmission, seasonality, transmission intensity**