ENTOMOLOGICAL AND PARASITOLOGICAL INDICES OF MALARIA TRANSMISSION AMONG RESIDENTS OF GOLD MINING AND SUGARCANE FARMING AREAS IN WESTERN KENYA

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A Thesis Submitted in Partial Fulfilment of the Requirements for the Award of the Degree of Doctor of Philosophy in Medical Parasitology of Masinde Muliro University of Science and Technology

November, 2023

DECLARATION

This thesis is my original work prepared with no other than the indicated sources and support and has not been presented elsewhere for a degree or any other award

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CERTIFICATION

The undersigned certify that we have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a Thesis entitled: "**Entomological and Parasitological Indices of Malaria Transmission among Residents of Gold Mining and Sugarcane Farming Areas in Western Kenya**".

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ACKNOWLEDGEMENT

My gratitude goes to my supervisors, Dr. Philip Ogutu and Dr. Jackson Cheruiyot for their invaluable time, ear, and words in guiding my work from inception to conclusion. I thank MMUST for accepting to admit me to pursue this program. The Department of Biological Sciences in the School of Natural Sciences for examining my work from proposal to thesis. The Directorate of post-graduate studies for ethical approval and other clearances. The lecturers who were involved in the whole process, I say thanks a lot.

I acknowledge the advice of Dr. David Mulama and Nicholas Kitungulu for their immense assistance in the identification of the area of study and technical advice on the finer details of field research. Dr. Hosea Akala, and Benjamin Opot (KEMRI/Walter Reed – Kisumu) for their advice on molecular work.

I extend my sincere gratitude to the field officers: Carolyne Njuguna, (MMUST), Eric Alusiola, Rebecca Alusiola, Redempta Mboyi (Rosterman mines), Kevin Ashuma, Derrick Alubale (Eluche), CHVs Carolyne Molenje and Gregory Juma (Rosterman), entomologists Joel Omari and Benard Isiaho (KEMRI – Kisumu). Not forgetting the officer-in-charge at the meteorological station, Kakamega.

I extend my thanks to phlebotomists/microscopists: Beatrice Malala, Caren Masulia, Mose Mulama, and Willy Aganyanya (MMUST), Brian Shitabule, Fryton Pedi, and Simon Juma for good phlebotomy and microscopy work.

My wife Joy Omutiti, sons Austin Mukabane, Bruce Mukabane, Carick Mukabane, daughter Vallary Omutiti, and nephew Uplyson Keya for understanding the meaning of my absence from family duties. My dad Wycliffe Mukabane for outstanding advice and God Almighty for the gift of life and health during the whole study period.

ABSTRACT

Despite sustained up-scaling of control programs worldwide aimed at preventing transmission of malaria and its subsequent eradication, the disease remains a major public health concern. Of the deaths due to the malaria scourge, Sub-Saharan Africa accounted for 92% with children under 5 years old accounting for 61% deaths. In Kenya, malaria accounts for 3.5 million clinical cases which resulted in 10,700 deaths annually. In as much as there was a marked decrease in malaria cases, due to up-scaling of the available control interventions, the intended goals aimed at malaria control and eventual elimination in endemic countries have not been very successful. Malaria epidemics in highlands have been associated with many factors which included climate change, drug resistance, and activities that modify land among others. The implication of malaria to land transforming activities such as artisanal gold mining, sugarcane farming, and the distance of residential houses from the mosquito breeding sites, made it possible that gold mining in Rosterman and sugarcane farming in Eluche played a significant role in the observed increased malaria incidences in Western Kenya. However, there was a lack of cogent evidence-based documentation about this scenario. Therefore, this study determined entomological and parasitological indices of malaria transmission among residents of gold mining and sugarcane growing regions in Western Kenya. This study involved both quantitative and qualitative data collection. A Survey of the mosquito breeding sites for the immature forms and collection of the adult mosquitoes by pyrethrum spray catches and light traps was done 3 consecutive days every month for 12 months starting November 2018. Blood samples by finger prick were collected on microscope slides and filter papers for parasitological studies from August 2020 to May 2021. Species identification was done for *Anopheles* and *Plasmodium spp*. *Anopheles* was identified by use of morphological keys and by molecular means, while *Plasmodium spp* were identified and quantified microscopically and molecular by use of polymerase chain reaction. Mixed method study design involving questionnaires, interviews, and focused group discussions was used to collect data from the residents. GraphPad Prism statistical package was used in data analyses. Descriptive statistics were carried out to determine relative frequencies and proportions. F-test tested the productivity of the larval habitats and the association between the month of sampling and adult *Anopheles* abundance, while the t-test tested the association between mean malaria prevalence and the site, season and age bracket at 95% confidence interval and $p \leq 0.05$ was considered statistically significant. Mosquito breeding habitats due to human activities were the most prevalent (93.22%) and had the highest productivity of the developmental stages of *Anopheles*, in which Gold Mines were the most abundant (51.29%). The average distance of the aquatic habitats from the nearest house was 251 ± 50 m. *An. gambiae s.l.* (91.96%) was the most abundant vector, while *P. falciparum* (99%) was the most prevalent malaria parasite. *Plasmodium spp* prevalence were at 8.96% by rapid diagnostic tests and 9.25% by both microscopy and polymerase chain reaction of dried blood samples. *Plasmodium spp* parasite mean densities at 95% CI was 4,840 (250 – 18,000) parasites/µl of blood. This study findings have elucidated that *An. gambiae* is the main vector of malaria and *P. falciparum* was the most prevalent malarial parasite in Rosterman mines and Eluche sugarcane farming areas in Western Kenya. It was suggested that research should be done to determine if there could be resistant markers in *Plasmodium* isolates from the people in the study sites and human blood index of the local malaria vectors to directly link them to malaria transmission.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACR	Adult child ratio
ACT	Artemisinin-based combined therapy
AGM	Artisanal gold mining
AHS	African health strategy
AL	Artmether-Lumefantrine
ANC	Ante-natal care
BLAST	Basic local alignment search tool
CHPs	Community health practitioners
CI	Confidence interval/credibility interval
CO1	Cytochrome oxidase gene 1
COVID-19	Corona virus disease – 2019
CS	Circumsporozoite
DBS	Dried blood spots
EDTA	Ethylenediaminetetra-acetic acid
EIC	Education, information and communication
FGDs	Focused group discussions
GoK	Government of Kenya
GPPS	GraphPad Prism Statistical Package
GPS	Global positioning system
IERC	Institutional ethical review committee
IIs	In-depth interviews
IRS	Indoor residual spraying

ITN	Insecticide-treated net
IVM	Integrated vector management
KAP	Knowledge, attitudes and practices
KMIS	Kenya malaria indicator survey
KNTG	Kenya national treatment guidelines
LLINs	Long-lasting insecticidal nets
LSM	Larval source management
MDA	Mass drug administration
MDGs	Millennium development goals
MoH	Kenya Ministry of health
NACOSTI	National council on science, technology, and innovation
NGO	Non-governmental organization
OR	Odds ratio
PCR	Polymerase chain reaction
PHC	Primary health care
PI	Principal Investigator
PLU	*Plasmodium* Deoxyribonucleic acid
PMI	Presidential malaria initiative
PSC	Pyrethroid spray collection
RDT	Rapid diagnostic test for malaria

rpm	Revolutions per minute
rRNA	ribosomal-Ribonucleic acid
SDGs	Sustainable development goals
SNPs	Single nucleotide polymorphisms
SSA	Sub-Saharan Africa
UHC	Universal health care
USAMRU	Unites States of America Army Research Unit – Africa/Kenya
WBCs	White blood cells

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Malaria remains a major public health concern in many countries around the world. Malaria is caused by protozoan parasite *Plasmodium spp* which are transmitted by female mosquitoes of the genus *Anopheles*. In Sub-Saharan Africa (SSA) besides endemic areas, highland areas have recorded a resurgence of malaria incidences (World Health Organization, 2020). Up to 2020, 3.4 billion people in 92 countries were at risk of malaria transmission, with 219 million cases and 435,000 deaths recorded. Of the deaths due to the malaria scourge, 92% occur in SSA and children under 5 years old account for 61% of the total deaths every year World Health Organization, 2020). The SSA region has had a reduction of 44% in malaria deaths from 680,000 to 386,000 from the period 2000 to 2019.

In Kenya, 25 million are at risk of malaria infection and the disease accounts for 3.5 million clinical cases. This accounts for up to 50% of outpatient attendance and results in 10,700 deaths annually. Malaria causes 20% of all deaths in children under 5 years in Kenya (Economic Survey, 2018). Inhabitants of the expansive western Kenya region around Lake Victoria where malaria prevalence is at 27 – 40 % and the coastal strip along the Indian ocean are at a higher risk of malaria infection because they boarder large water bodies L. Victoria and the Indian ocean respectively (World Health Organization, 2020). These malaria-endemic regions have a tropical climate with two rainy seasons, a long rainy season (March – July) and a short rainy season (September – November), and a temperature range of 19 - 27°C beside other climatic conditions (Alsop, 2007).

Regular but unpredictable malaria epidemics have been reported in Western Kenya Highlands (altitude 1200 – 1700 m above sea level) in recent years (Pascual *et al*., 2006; Stevenson *et al*., 2015). These epidemics cause devastating fatalities among the vulnerable groups of children under 5 years, pregnant women, and immunologically naïve individuals (World Health Organization, 2020). Whenever they strike, the malaria epidemics are associated with many factors which including changes in rainfall patterns, immunity (Hay *et al*., 2002; Afrane *et al*., 2006; Nawa *et al*., 2019), drug resistance by the malaria parasite, land use types, land cover types, house structure, vector control practices, socio-economic status of household and activities that modify land (Bashar *et al*., 2012; Pinchoff *et al*., 2016; Sewe *et al*., 2016; Paul *et al*., 2018; Aïkpon *et al*., 2020; Nicholas *et al*., 2021). However, for malaria to be established and spread, there must be availability of water and breeding grounds with the required ecological conditions throughout the year to provide a regular source of the mosquito vector (Pascual *et al*., 2006; Omukunda *et al*., 2013; Wang *et al*., 2019).

World-wide up-scaling of malaria control interventions has yielded positive results that have consequently resulted in a marked decline in malaria cases between the years 2000 to 2019. These control strategies include interventions that target the vector such as larval source management, environmental management and indoor residual spraying (IRS) have highly been reported to break transmission.

Stopping mosquito-human interaction by using long-lasting insecticidal nets (LLINs) is the most widespread malaria prevention method. The provision and use of the bed net have been up-scaled in SSA to upwards of 72% in the last two decades to prevent human-vector interactions, particularly at night during sleep (World Health Organization, 2020). The LLINs impregnated with synthetic pyrethroids enhance personal protection against the mosquito bite during the most vulnerable times (Kawada *et al*., 2014; Killeen, 2014). The recommendation for widespread distribution of LLINs by the Roll Back Malaria (RBM) initiative resulted in reduction of malaria from 450 million cases from 2000 to 2015 and child mortality by up to 30% (Wisniewski *et al*., 2020; World Health Organization, 2020).

The efficacy of IRS has been tremendous with the introduction of novel insecticides besides pyrethroids (Sherrard-Smith *et al*., 2018). Indoor residual spraying has resulted in the tremendous decline of population of the main malaria vectors. Therefore, it has to be practiced routinely by households to suppress indoor malaria vector numbers (Lwetoijera *et al*., 2013; Makungu *et al*., 2017). However, as these malaria control methods are in use, their full implementation is strewn with numerous challenges around the world (Karunamoorthi, 2011; Guyant *et al*., 2015).

Integrated vector management (IVM) has incorporated modern and emerging technologies such as the release of *Wolbachia*-infested mosquitoes to control mosquitoes (Walker & Moreira, 2011) in which there was reduced *Plasmodium* in *An. gambiae* infested with transient *Wolbachia*. Larviciding with either chemical or microbial insecticides has a profound effect on the prevalence of the mosquito vector and hence malaria transmission Choi *et al*., 2019; Derua *et al*., 2019; Mutero *et al*., 2020). Proper environmental management through the removal of breeding sites by draining stagnant water, removal of water-holding artificial containers, clearing bushes in the home compound, and general cleaning of the residential houses (Ng'ang'a *et al*., 2008; De Silva & Marshall, 2012; Adebayo *et al*., 2015) are critical methods employed in malaria control. Finally, medication of any sick person by receiving prompt treatment with ACT within 24 hours by ensuring functional health services, medical equipment, supplies, drugs, and competent medical personnel would drastically reduce the malaria burden (Riley, *et al*., 2016; Musuva *et al*., 2017).

In as much as there is advocacy through education, information, and communication (EIC) about malaria control through appropriate environmental management, socio-economic activities that have extensively caused land modification could be countering the malaria prevention efforts. These land-modifying activities include brick making, sugarcane farming and artisanal gold mining (Ng'ang'a *et al*., 2019; Demissew *et al*., 2020; Nicholas *et al*., 2021). Artisanal gold mining (AGM) and sugarcane farming are low-technology and labor-intensive socio-economic activities that are widespread in developing countries with a massive impact on the environment. Both artisanal gold mining in Rosterman mines and sugarcane farming in Eluche in Western Kenya are responsible for greatly modifying land. This is based on a spatial study of aquatic habitats by

(Nicholas *et al*., 2021), in Rosterman mines that established that the area has diverse mosquito breeding sites. However, their spatiotemporal abundance and productivity are not yet determined.

Both AGM and sugarcane farming have been associated with extensive use of chemicals such as mercury in gold extraction and purification and fertilizers, herbicides, and pesticides in sugarcane farming. These chemicals in the long run affect reproductive success and survivorship of mosquitoes – the carrier vector for several parasites responsible for diverse diseases. Agrochemicals have been reported to impact on the physico-chemical characteristics of the breeding aquatic habitats of the *Anopheles* vectors and other mosquito species by a study in Tanzania (Matowo, *et al*., 2010; Urio *et al*., 2022). Therefore, it is imperative to understand the influence of agrochemicals on the life cycle of the *Anopheles* vector and its potential to transmit the malarial parasite, *Plasmodium spp* which causes malaria. This will also determine the successful implementation of environmental management tools such as larval source management (LSM) aimed at the reduction of the *Anopheles* population (Imbahale *et al*., 2010; Mbuya & Kateyo 2014; Abong'o *et al*., 2020). Reducing the *Anopheles'* abundance, distribution, and composition limits their chances of gaining entry into residential houses to bite and transmit malaria and this is a major target of environmental management programs aimed at malaria control, while the contrary becomes a public health issue.

The SSA countries Kenya included through sustainable development goal (SDG) number 3, African Union (AU) agenda 2063 aspiration 1, and Government of Kenya (GoK) policy on health 2014 – 2030, are optimistic in attaining the target of eliminating malaria and other mosquito-borne diseases by 2030 through sustained implementation of control policies. This is the cornerstone of the socioeconomic development of SSA countries (Economic Survey, 2018; Africa, 2019; World Health Organization, 2020). However, the road to this is strewn with uncertainties related to several factors which include poor surveillance and response systems, lack of sufficient disease control programs, insufficient or non-existent medical infrastructure, collapsed healthcare due to insecurity and poor coordination among humanitarian agencies (Gayer *et al*., 2007 and Africa, 2019). Additionally, socio-demographic proxies such as gender, age, level of education, households incomes, household size, and area of residence (urban versus rural) are great drivers of malaria transmission (Imbahale *et al*., 2010; Pinchoff *et al*., 2016; Dawit Hawaria, 2019; Ngatu *et al*., 2019; Ugwu & Zewotir, 2020). A malaria prevalence of between 20 – 40% in the Lake Victoria region hampers Kenya's transition towards the pre-elimination phase and general socio-economic development. It is crucial to assess malaria risks at all levels to identify potential risk factors and hotspots to institute proper control strategies.

Due to the prevailing high malaria prevalence in Western Kenya highlands, it was imperative to undertake this study in the Rosterman mines and Eluche sugarcane farming rural areas to determine if the socio-economic activities being undertaken there that have modified land could be drivers for malaria transmission. The study sought to avail evidence-based scenario of entomological and parasitological malaria indices in Rosterman gold mining and Eluche sugarcane farming in Western Kenya.

1.2 Statement of the Problem

The high prevalence of malaria of between 33 – 40% in the Western Kenya is a public health concern that makes the government of Kenya not to meet its health policy 2014 – 2030. This health policy is a major plank for the socioeconomic development of the country in line with the constitution promulgated in 2010. This is despite concerted efforts by AU's Agenda 2063 and SDGs aimed at the control of malaria and other communicable diseases. Although numerous studies have described malaria risk factors in SSA, very few have attempted to study the association between socio-economic activities due to AGM and sugarcane farming to the disease dynamics. The activities result in land modification that may be responsible for the significant increase in disease vectors including mosquitoes, which transmit the malarial parasite, *Plasmodium spp*. Additionally, AGM and sugarcane farming offer employment and have been associated with the luring of poor populations who could be immunologically naïve to work in unsafe and unhealthy conditions with concomitant potential for transmission of malaria and other communicable diseases. Effective control of malaria transmission is a precursor to its elimination and the most vulnerable segments of the community must have requisite EIC. They should be knowledgeable enough about the risk factors associated with malaria to enable them to go about their socioeconomic activities without predisposing themselves to malaria infections. With this knowledge of malaria as a major health problem, the study sought to ascertain whether AGM in Rosterman mines and sugarcane farming in Eluche potentiate the resurgence of malaria transmission cases in the Western Kenya highlands

1.3 Justification of the Study

Despite heightened control measures through the provision of mosquito bed nets and making access to treatment easier as envisaged in aspiration 1 of AU's Agenda 2063, SDG 3 on universal health care (UHC) for Kenyan citizens, western Kenya continues to record an upsurge in malaria incidences. Availability of mosquito breeding habitats due to modification of the environment, lack of sufficient EIC on malaria and available control methods by the people, and a pool of asymptomatic people in the population could be contributory factors to the observed rise in malaria cases in Kakamega County, Western Kenya. This study was conducted in two areas in western Kenya that have undergone immense land modification, increasing the available mosquito breeding sites.

Availability of the larval habitats has been linked in other studies with an increase in availability, abundance, and diversity of mosquitoes, which enhance transmission of mosquito-borne diseases, among them malaria. However, there is no available information on the association between environmental modification caused by AGM and sugarcane farming on the reported malaria cases in Kakamega County, Western Kenya.

It is imperative to determine the role played by the modification of the environment due to AGM and sugarcane farming in the recorded resurgence of malaria cases. This was premised on the fact that such modifications influence entomological and parasitological malaria transmission parameters such as availability and productivity of aquatic habitats, abundance and diversity of mosquito adults and prevalence of *Plasmodium spp*.

It is also important to determine the people's EIC about malaria, the risk factors that exist, and the malaria control strategies in use. The information that will be obtained through the survey would help determine the changes in the disease burden and the efficacy of the available control interventions in the face of stiff socio-economic proxies that compel human populations to modify the environment in search of livelihoods. Additionally, the results that would be obtained would elucidate the possibility of residual transmission in the population. This will enable policy framers to offer strategies through EIC that would mitigate or synergize the existing methods or policies.

1.4 Research Questions

The research questions of the study were:

1. What is the malaria vector abundance and diversity in Rosterman gold mining and Eluche sugarcane farming areas?
2. What is the prevalence and intensity of *Plasmodium spp*?
3. What risk factors associated with malaria transmission exist in the two study sites?
4. What malaria control measures do residents of the two study sites use?

1.5 Objectives of the Study

1.5.1 General Objective

The general objective of the study was to determine malaria entomological and parasitological indices among residents of Rosterman gold mining and Eluche sugarcane farming areas in Western Kenya.

1.5.2 Specific Objectives

The specific objectives of the study were:

1. To determine malaria vector abundance and diversity.
2. To determine the prevalence and intensity of *Plasmodium spp*.
3. To identify the risk factors associated with malaria transmission.
4. To determine malaria control measures used by the residents.

1.5.3 Research Hypotheses

Ho_1: *Anopheles gambiae* is not the most abundant malaria vector in Rosterman gold mining and Eluche sugarcane farming areas.

Ho_2: Human *Plasmodium* species are not prevalent in Rosterman gold mining and Eluche sugarcane farming areas.

Ho_3: Human malaria transmission among residents of Rosterman gold mining and Eluche sugarcane farming areas is not associated with any risk factors.

Ho_4: Residents of Rosterman gold mining and Eluche sugarcane farming areas do not have good understanding of and practice various malaria interventions strategies.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Malaria Situation

Malaria prevalence globally declined from 238 million cases in 2000 to 229 million in 2019 in 87 countries where malaria is endemic. Similarly, malaria incidences reduced from 80/1000 people to 57/1000 people in the same period (World Health Organization, 2020). However, the continued population increase in the African region led to a significant increase in the risk of infection to 225/1000 people in the same period from 2000 – 2019. This region recorded a decline in deaths due to malaria from 680,000 to 386,000 representing a drop of 44% and a mortality rate reduction of 67% (World Health Organization, 2020).

To achieve its socioeconomic development, AU, through goal number 3 aspiration 1, revised its Africa Health Strategy (AHS) by creating a more comprehensive, coherent, and actionable platform for its member states and multilateral agencies. This was focused on aligning resources to improve synergy in health for its citizens. Through its Roadmap for Shared Responsibility and Solidarity in eliminating malaria and other diseases, it was viewed that there had to be the implementation of health sector framework reforms (Africa, 2019). In addition, sustainable development goal (SDG) number 3 seeks to eliminate epidemics caused by communicable and neglected tropical diseases such as tuberculosis and malaria among others.

In as much as there was a marked decrease in malaria cases, the intended goals aimed at malaria control and eventual elimination in endemic countries have not been very successful. Progress in reducing and eliminating malaria has been hindered by several factors including reduced funding and change of priority areas by governments and transnational agencies, the emergence of insecticide resistance, changes in global climate, and land use, and socioeconomic status of the communities and households to implement the recommended control strategies. Therefore, to fast-track the implementation of malaria control strategies in malaria-endemic countries, there was the need to assess risk factors at local levels to identify vulnerable groups to implement context-based prevention methods that are tailored to reverse the disparities observed in disease prevalence (Alsop, 2007; Matowo, et al., 2010; Ajayi, *et al*., 2013).

In Kenya, malaria accounts for 3.5 million clinical cases which result in 10,700 deaths annually as indicated in Figure 1. Inhabitants of western Kenya and the coastal strip were at a higher risk of malaria infection (World Health Organization, 2020). Poor utilization of malaria prevention interventions is cited as a major driver that causes these numbers to surge (Kabaghe *et al*., 2018). Elimination of the vector through LSM, indoor residual spraying (IRS), and using biological means has not yielded 100% results (Russell *et al*., 2013). The provision of bed nets and their proper use to limit vector-human interaction has yielded a significant reduction in the number of casualties but has not eliminated transmission (Steinhardt *et al*., 2017; Ashley *et al*., 2018).

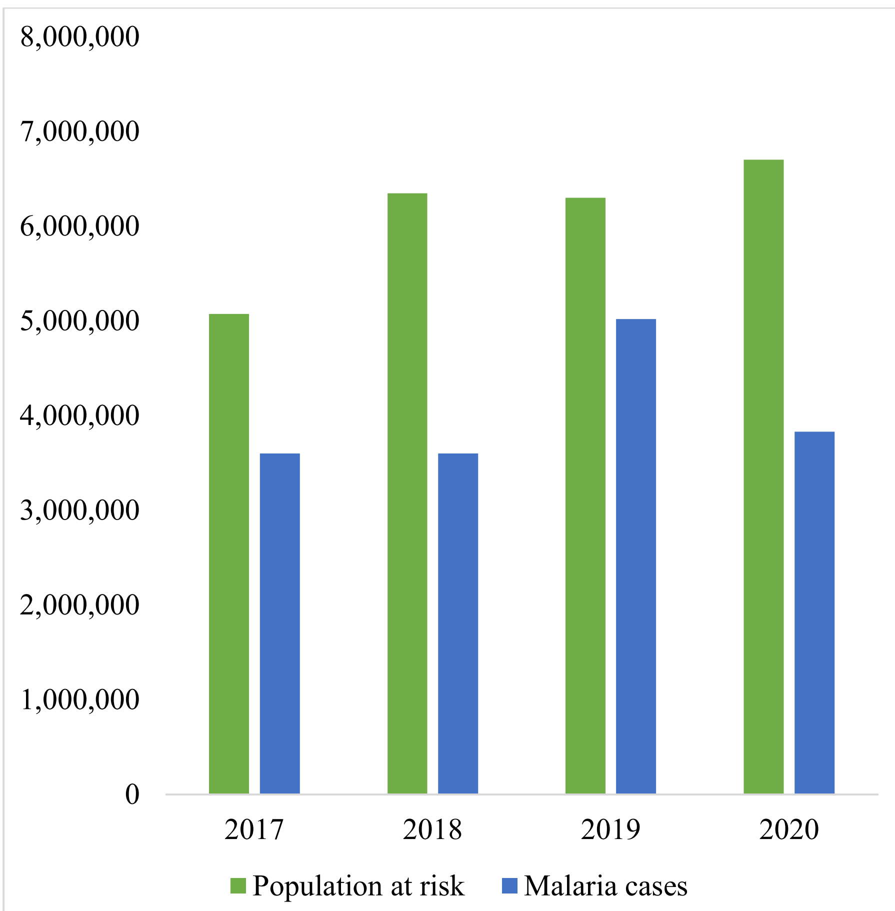

Figure 1: Malaria in Kenya (Courtesy: Kenya malaria indicator survey, 2020)

Despite GoK's commitment to make access to health care a reality for millions of its citizens at affordable or no cost, malaria epidemics still have been reported in western highlands in recent years. The last few years have seen malaria bounce back to pre-intervention times (Pascual *et al*., 2006; Mukonka *et al*., 2014; Ototo *et al*., 2015). Malaria epidemics have been associated with many factors which included changes in rain patterns, immunity, drug resistance, land use types, land cover types, house structure, vector control practices, socio-economic status of household and activities that modify land (Hay *et al*., 2002; Afrane *et al*., 2006; Bashar *et al*., 2012; Pinchoff *et al*., 2016; Sewe *et al*., 2016; Paul *et al*., 2018; Nawa *et al*., 2019; Aïkpon et al., 2020; Nicholas *et al*., 2021).

The implication of malaria to land transforming activities such as brick making and irrigated and non-irrigated sugarcane farming and the distance of residential houses from the mosquito breeding/resting sites (Kondepati, 2013; Ng'ang'a *et al*., 2019; Demissew *et al*., 2020) makes it possible that AGM and sugarcane farming could be playing a role in the observed resurgence of malaria in western Kenya highlands. Therefore, there was an urgent need to investigate other risk factors that contributed to the increased malaria cases in different regions. The results from this study provided insight into the recorded resurgence of malaria in SSA in general and Western Kenya in particular. This will help channel the malaria control interventions to the most vulnerable population.

2.2 Life Cycle of Malaria Infection

All malaria control efforts are geared at averting transmission of the parasite to the human host in which its resultant sickness causes massive morbidity and mortality. Therefore, understanding the malaria infection cycle is of paramount significance. The malaria parasite must infect successively two types of hosts, humans and female *Anopheles* mosquitoes as illustrated in Figure 2. The infected female *Anopheles* mosquito during a blood meal injects sporozoites into the bloodstream which reach the liver via systemic circulation. Here they transform into schizonts that multiply asexually in hepatocytes to release merozoites which attack erythrocytes asexually multiply rapidly until the cell bursts and release merozoites into the bloodstream to infect more erythrocytes (erythrocytic schizogony). After several generations some merozoites differentiate into sexual forms of the parasite to form gametocytes (microgametes and macrogametes), which circulate in the bloodstream. And when a female *Anopheles* picks the gametocytes during a bloodmeal, they develop into gametes in the mosquito gut. In the mosquito midgut, the gametes fuse to form zygotes which develop into ookinetes that burrow through the mosquito's midgut wall to form oocysts. In the oocysts, sporozoites develop and the oocysts eventually burst to release sporozoites into the body cavity of the mosquitoes. The sporozoites reach the salivary glands of the mosquito and are eventually released into a healthy human being during bloodmeal by the mosquitoes to start the infection again (Adopted from Centers for Disease Control and Prevention website, www.cdc.gov >malaria>biology)

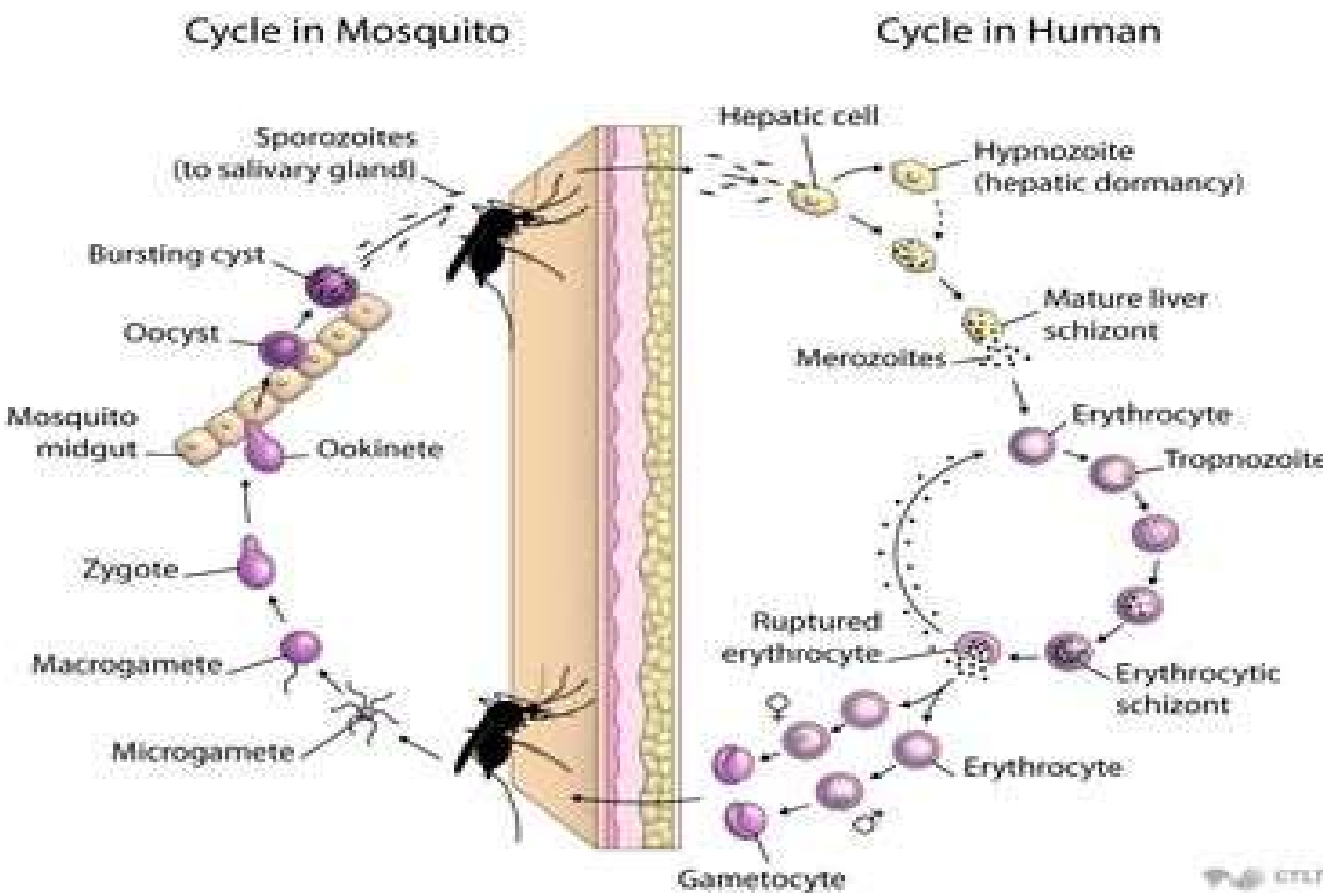

Figure 2: Life cycle of *Plasmodium* [Source: http://ocw.jhsph.edu/].

2.3 Abundance and Diversity of Malaria Vectors in Sub-Saharan Africa

Transmission of malaria has greatly been attributed to the occurrence, abundance, and distribution of the anopheline mosquitoes, environmental conditions, rainfall, altitude, and other ecological factors (WHO, 1982; Minakawa *et al*., 2005; Protopopoff *et al*., 2009; Sinka *et al*., 2010; Ndenga *et al*., 2011).

Over 140 species of anopheline mosquito, the most important of which is *Anopheles gambiae* complex, *Anopheles funestus*, and *Anopheles coluzzii* among others have been implicated in the transmission of malaria parasites Coetzee *et al*., 2013; Coetzee, 2020). The geographical distribution of the dominant malaria vectors had been hampered by inadequate data for the individual sibling species and therefore, rely heavily on mapping complexes. Within the *An. gambiae* complex there are two efficient malaria vectors namely; *An. gambiae* and *An. arabiensis*. On the other hand, *An funestus* is a main human *Plasmodium* transmitter in SSA (Coluzzi, 1984; Hunt *et al*., 2010; Sinka et al., 2010).

The anopheline vectors breed in a wide range of habitats but have a proclivity for fresh brackish water with right temperature and other optimum physicochemical properties (Mereta *et al*., 2013). However, *An. quadriannulatus*, *An. melas*, *An. merus*, and *An. bwambae* have been found to breed in salt water and mineral springs (Hunt *et al*., 2010) and have also been implicated in malaria transmission but have low vectorial capacity. Implementation and sustenance of effective malaria control measures require regular monitoring of malaria vector occurrences and distribution (Nicholas *et al*., 2021).

The *Anopheles* vectors are well distributed in Kenya with *An. arabiensis* found in the Kano plains, along the Coast, Central and Turkana areas (Bayoh *et al*., 2010). *An. funestus* and *An. gambiae* s.s., *An, arabiensis*, and *An. merus* at the coast (Mbogo *et al*., 2003), while *An. pharaoensis* has also been reported at the Coast, Central and Lake Victoria basin (Beier *et al*., 1999).

In western Kenya, *An. gambiae* s.s, *An. arabiensis* and *An. funestus* are the notable vectors of malaria). In the western Kenya highlands *An. coustani*, *An. squamosus* and *An. implexus* have been added to malaria transmitters as secondary malaria vectors (Mustapha *et al*., 2021; Nicholas et al., 2021). High spatial variability of the abundance of the main vectors has been reported and as a consequence variation in transmission intensity (Ndenga *et al*., 2006). The observed changes in vector abundance have been attributed to rainfall and proximity to permanent breeding grounds). A clear understanding of the spatial and temporal malaria vector population dynamics is crucial to enable defining regions where the largest malaria risks occur to re-evaluate the existing control strategies (Eba *et al*., 2021).

For mosquitoes to breed, there must be availability and sustainability of the breeding grounds. These sites must ensure the immature forms metamorphose and emerge as adults. The existence of suitable breeding sites for the *Anopheles* mosquito vectors throughout the year in a particular area sustains the vectors in that area and hence their capacity to transmit malaria (Al-Sheik, 2011). Evaluation of breeding habitats in areas where malaria is endemic is critical in the provision of information required for malaria surveillance and control. Western Kenya has been reported to have abundant and diverse natural and human-made, permanent and temporary breeding habitats for the anophelines. As the human population density grows in the region, socioeconomic costs of malaria are proportionally likely to grow (Carlson *et al*., 2004). Land cover changes are critical drivers for temperature rise in the micro-habitats for the mosquito larval stages which subsequently increases the abundance of the malaria vectors (Kweka *et al*., 2016).

Human-made breeding sites that have been described included fishponds preferred by *An. gambiae* s.l., burrow pits and goldmines for *An. funestus* and *An. implexus*, drainage ditches for *An. coustani* s.l., tire track, and footprints were abundant with *An. squamosus*, and cultivated swamps for *An. gambiae* and *An. funestus*). Artificial containers such as water storage tanks have also been shown to be important vector breeding sites contributing to malaria transmission (Al-Sheik, 2011). On the other hand, naturally occurring aquatic larval habitats include rockpools, swamps, slow-moving rivers, and puddles.

Understanding the ecology of these larval habitats is crucial in the implementation of malaria mitigation strategies, particularly environmental and larval source reduction. Control measures should target all aquatic larval habitats regardless of their productivity rates to result in sufficient reduction in the malaria vector densities and as an additional malaria control strategy (Ndenga *et al*., 2011; Nicholas *et a*l., 2021).

2.4 Prevalence and Diversity of Malaria Parasites in Western Kenya

Malaria epidemics in western Kenya have been reported to be unpredictable and vary in transmission intensity from one region to another. Of the four malaria parasites, *P. falciparum* has been consistently associated with malaria on the Kenyan coast and the western highland region (Ernst *et al.*, 2006; Kalayjian *et al*., 2013; Aidoo *et al*., 2018). While *P. vivax* having been reported in Kenya in 2023 and *P. ovale* are fairly same in Kenya co-infection with *P. malariae* and *P. falciparum* comprise about 40% but does not constitute an epidemic at all. However, malaria transmission is not static and therefore, the other *Plasmodium* species are present in different proportions. *P. falciparum* infections account for over 90% of malaria infections in western Kenya while *P. ovale*, *P. vivax,* and *P. malariae* account for less than 10% prevalence (Wanyonyi *et al*., 2018; Aidoo *et al*., 2018). However, *P. ovale*, and *P. malariae* have been observed as mixed infections in microscopic smears during surveys and hospital visits by those sick with malaria (Omondi, 2017, Antony *et al*., 2019; Waiswa *et al*., 2022).

Failure to comprehend the impact of non-*Plasmodium falciparum* malaria infections is bound to impact the control strategies instituted (Lo *et al*., 2017). Malaria still ranks as one of the neglected tropical diseases in rural villages of SSA because of the continued use of microscopy as the gold-standard diagnostic tool available among other factors. Microscopy is less sensitive and may not detect the other plasmodial species if parasitemia is low (Niño *et al*., 2016) or the patient is asymptomatic. However, *P. ovale*, *P. vivax,* and *P. malariae* have been observed as mixed infections in microscopic smears during surveys (Omondi, 2017). This implied that unless the microscopist was keen enough, the mixed infections may be missed.

Epidemiologic information about the prevalence and distribution of the other *Plasmodium* species in modified environments considered potential hotspots for malaria transmission is still scanty or not available. The information gained would add to the pool of knowledge about the prevalence and the diversity of the plasmodial species circulating in the epidemic-prone areas of western Kenya. And the outcome of this would be beneficial in the pursuit of a healthy population according to AU's Agenda 2063 and SDG inspiration 3.

The development of resistance of the malaria parasites to multiple anti-malarial drugs has been cited as a likely obstacle to malaria control in many parts of SSA. Increased provision of bed nets has resulted in falling numbers of malaria cases. On the contrary, the emergence of resistance by *Plasmodium* to conventional antimalarials such as artemisinins, calls for precise diagnostics and surveillance of malaria transmission to enable the attainment of the AU Agenda 2063 on the elimination of communicable diseases. However, sufficient data is lacking to highlight the extent of development of resistance in the *Plasmodium* as the focus has been on endemic regions (Zhong *et al*., 2008; Dondorp *et al*., 2009; Daniels *et al*., 2012; Cheruiyot *et al*., 2014).

Drug resistance in *Plasmodium* parasites is due to genetic mutation in targeted genes. To ascertain the development of mutations, molecular methods can be used to monitor major point mutations (Ogouyèmi-Hounto *et al*., 2013). Single nucleotide polymorphisms (SNPs) in *P. falciparum* multi-drug resistance gene 1 (*pfmdr1*) at N86Y, Y183F, S1034c, N1042D, and D1246Y modulate resistance to chloroquine, artemisinin derivatives, and arylaminoalcohol drug lumefantrine and (Hayton & Su, 2004; Sisowath *et al*., 2007; Cheruiyot *et al.*, 2014). Besides *pfmdr1* mutations in *P. falciparum* confer chloroquine resistance transporter (pfcrt), *P. falciparum* dihydrofolate reductase (pfdhfr) and *P. falciparum* dihydropteroate synthetase (pfdhps) genes confer resistance to chloroquine, lumefantrine, antifolates, and pyrimethamine and quinine (McCollum *et al*., 2006).

The prevalence of resistance markers in *P. falciparum* to commonly used antimalarials in Kakamega County of Western Kenya is conspicuously missing. In highland regions of Western Kenya, the role played by drug resistance as a driver for the resurgence of malaria epidemics has not been established (Ogouyèmi-Hounto *et al*., 2013; Wakoli *et al*., 2022). Studies involving malaria drug resistance depend largely on samples collected from medical facilities from patients presenting malaria signs. However, none has delved in drug resistance indices in asymptomatic population in environmentally modified areas of western Kenya.

2.5 Risk Factors for Malaria Transmission

Malaria transmission is a function of a number of risk factors including socio-demographic factors, proximity of residential houses to aquatic habitats, house structure characteristics, bed net use and status, and environmental factors. Knowledge of these factors would enable the agencies concerned to tailor and probably improve malaria prevention strategies (Protopopoff *et al*., 2009;).

Transmission of malaria is aggravated by different human activities. Knowledge of these activities would give a scientific insight into what is needed to be done to reduce malaria episodes within populations. Socioeconomic activities such as evening roadside trade/business, visitations, bathing, fishing, and guarding crops/livestock against wild animals among others, have been identified as some of the potential risk factors of malaria within households (Moshi *et al*., 2018; Bamou *et al*., 2021).

Age has been identified as risk factor for persistent malaria occurrence within various setups. Studies have identified children under 5 years and adolescents below 17 years of age, as the most vulnerable groups (Pinchoff *et al*., 2016). Pregnant women form another group of the most vulnerable segment to malaria transmission (Waiswa *et al*., 2022).

The level of education and occupation have been reported as key socio-demographic characteristics that determined the risk for malaria transmission (Antony *et al*., 2019; Maseko &

Nunu, 2020; Bamou *et al*., 2021). In these studies, unemployed people with little or no formal education were at a higher risk of malaria infection. As the level of education increased as well as the income levels, the risk of contracting malaria inversely reduced. Higher education, employment, and increased household incomes improved house structures and environmental management. As a result occurrence of malaria within the families drastically reduced (Protopopoff *et al*., 2009; Wanyonyi *et al*., 2018;). The lack of this vital information in AGM and sugarcane farming regions of Western Kenya acted as a hindrance to the attainment of millennium development goals (MDGs).

The life cycle of a malaria vector involves two critical stages, the aquatic stage, and the adult stage. Female *Anopheles* mosquitoes must oviposit in a suitable breeding habitats that were heterogeneously dispersed to maintain spatial heterogeneous transmission within the human host population. Further, studies have shown that when densities of adult mosquitoes peak and the breeding sites were closer to human settlements, biting rates were often higher (Smith *et al*., 2004; Midega *et al*., 2012; Nixon *et al*., 2014).

In the mining regions, control mitigation methods could be lacking, more so if the activity is illegal or on a small scale. Most of the malaria vector biology studies have dwelt on climatic effects on vector abundance and distribution. Rarely have human-modified environments been accorded attention to warrant targeting as potential hotspots for malaria surveillance and control (Naranjo-Díaz *et al*., 2014).

In Rosterman mines and Eluche sugarcane growing regions, alteration of the land for socioeconomic reasons creates larval habitats in close proximity to residential houses (Nicholas *et al*., 2021). Studies have shown that when adult mosquitoes emerge, they fly to seek blood meal in the nearest possible residential house and that wind has a significant contribution to blood meal search by the adult *Anopheles* (Midega *et al.*, 2012). This places people who reside in upwind houses at much more risk of contracting malaria.

A clear understanding of how the distance of the nearest residential house from the larval breeding sites affects the epidemiology of malaria will avail evidence-based information on the incorporation of appropriate malaria control strategies in addition to LSM (Munyekenye *et al*., 2005). Additionally, it would point at where the availability of breeding habitats that cause most malaria cases were to guide the location and nature of the control measures to be instituted.

House structure characteristics play a role in transmission by limiting the ability of the vector to gain access into the houses. Better housing significantly reduced malaria prevalence in the studied households and residing in well-constructed houses reduced mosquito numbers within the houses. Poorly and haphazardly constructed residential houses with eaves, cracks in the walls and roofs, and the absence of screens in doors and windows sustained malaria transmission by enabling the mosquito to enter. (Lwetoijera *et al*., 2013; Ng'ang'a *et al*., 2019; Bamou *et al*., 2021). The number of rooms, number of occupants, and sharing the house with domestic animals increased the likelihood of mosquitoes biting the occupants and hence high incidence of malaria.
The availability of separate bedrooms for adults and children enabled the hanging of the bed net) as a result protecting the occupants from infective mosquito bite. House structure characteristics were a major means of removing the risk of malaria among households. Houses in many rural communities had a cultural dimension to them. The cultural factors influenced the wide adoption of malaria prevention strategies (Liu *et al*., 2014; Ngadjeu *et al*., 2020 Musiime *et al*., 2022).

Human behavior guides the adoption of various malaria prevention practices. Houses with good paintwork/ceiling/more rooms and without cracked openings in walls/roofs/eaves/vents reduced significantly the likelihood of the occupants to contract malaria (Lwetoijera *et al*., 2013; Liu *et al*., 2014; Guerra *et al*., 2018).

In rural setups, house designs may not be as uniform as in urban estates. This could be a contributory risk factor in the house structure that could be increasing the malaria cases in the larger Kakamega County. Investigation of the risk factors related to the structure of the house would provide a clearer picture of the successes of the methods used in malaria control.

The bed net is the gold standard method of malaria prevention used in large parts of SSA. As the net continues to be used, its quality deteriorates and this opens the likelihood of the *Anopheles* mosquito gaining access to bite the sleeping occupants. This is because the continued use of the bed net in the house causes a shift in the feeding habits of *An. gambiae*, the principal vector of malaria in Kenya. Continued use of the bed net coupled with whether the family users slept in a bed or not, presence of a bed net in a household, results in a significant reduction in indoor mosquito prevalence (Mutuku *et al*., 2011; Mukabane *et al*., 2022).

Failure to properly tuck the net in the bed or mattress/mat correctly offered avenues for entry for the mosquito to bite the people sleeping under it (Lindsay *et al*., 2021). In the recent past, there has been heightened provision of the ITNs to meet the target of more than 80% of the at-risk population sleeping under nets. However, damage to the bed net created holes through which mosquitoes passed to reach the occupants.

Continued washing diminishes the ITNs' insecticide integrity and consequently puts the users at risk of being bitten by infective mosquitoes. Damaged and regularly washed nets were a feature of low-income earning households who rely on donations by governments and other multi-national agencies. Donations by governments come after a lapse of not less than three years. The long duration between distributions of the ITNs means the integrity of the net was lost before subsequent distributions (Mutuku *et al*., 2011; Kawada *et al*., 2014; Minta *et al*., 2017; Lindsay *et al*., 2021). The unavailability of this vital information on the impact of the bed net status on the increase of malaria cases within the larger Kakamega County in Western Kenya inhibited the evaluation of the efficacy of the ITNs as primary malaria control tools.

Malaria prevention is not an indoor activity only, but through IVM, proper management of the immediate surroundings of the house was also critical. Improper utilization of the environment creates ample habitats for the mosquito vector to breed and rest. Mosquitoes breed within available aquatic habitats regardless of the site. The *Anopheles* vector has a proclivity for fresh brackish water with the right temperature to oviposit their eggs. These types of breeding sites are temporary and are preferred by the vector. The inability of households and the community to synergize efforts in the maintenance of free mosquito breeding grounds would put them at risk of malaria transmission. The lack of information about the state of the environment and the risk it contained in malaria transmission in AGM and sugarcane growing areas, created a lacuna that this study sought to fill.

2.6 Integrated Malaria Vector Management

Malaria control is a core strategy in forestalling malaria transmission. Several strategies that target the vector such as larval source and environmental management and IRS have highly been proposed for breaking transmission. The efficacy of IRS has been tremendous with the introduction of novel insecticides besides pyrethroids (Sherrard-Smith *et al*., 2018; Choi *et al*.; 2019). However, stopping mosquito-human interaction by using the LLINs was the most widespread malaria prevention method in use in endemic regions.

Integrated vector management (IVM) has incorporated modern and emerging technologies such as the release of Wolbachia-infested mosquitoes to control mosquitoes (Walker & Moreira, 2011) in which there was reduced *Plasmodium spp* in *An. gambiae* infected with transient Wolbachia. Larviciding has been advocated by a large swathe of proponents of mosquito control. Larviciding with either chemical or microbial insecticides had a profound effect on the abundance of the mosquito vector and hence malaria transmission Choi *et al*., 2019; Derua *et al*., 2019; Mutero *et al*., 2020). However, this requires strong collaboration and coordination between health and environmental regulators to reach a common ground.

The provision and use of the bed net has been up-scaled in SSA to upwards of 72% in the last two decades to prevent human-vector interactions, particularly at night during sleep (World Health Organization, 2020). The LLINs impregnated with synthetic pyrethroids enhanced personal protection against the mosquito bite during the most vulnerable times (Kawada *et al*., 2014 and Killeen, 2014).

With the observed development of resistance of *An. gambiae* to pyrethroid-based insecticides, it was critical to continue to assess the efficacy of LLINs in malaria control programs. This is because many mosquito vector species maintain robust transmission of malaria in endemic regions (Killeen, 2014). The indoor-feeding mosquito tends also to rest indoors for a period of up to two days to enable the blood meal to be fully digested. This rest period ensured that the eggs developed before the female mosquito ventured out to search for sites to oviposit (Lengeler, 2004). It was imperative to keep LLINs hanged in the houses for them to deter engorged mosquitoes from resting indoors.

The recommendation for widespread distribution of LLINs by the Roll Back Malaria initiative has widely borne fruit. This recommendation averted more than 450 million cases from 2000 to 2015 and reduced child mortality by up to 30% (World Health Organization, 2018; Wisniewski *et al*., 2020).

A lot of financial, technical, and operational input has been incorporated increasing the LLIN coverage in SSA to three LLINs per household (Masaninga *et al*., 2018). Kenya was among the first adopters of the requirement for free mass distribution and continuous distribution of the LLINs.

The up-scaled distribution of nets through mass distribution and antenatal caregiving centers (ANC) to pregnant women had seen the operational LLIN coverage and ownership rise to over 80% in western Kenya counties (Zhou *et al*., 2014). However, the distribution and use of the bed net were not without challenges. The hindrances include inaccessibility, loss of integrity and deterioration, loss of the net, longer periods between distributions (Zhou *et al*., 2014; Tassew *et al*., 2017; Ng'ang'a *et al*., 2021), population growth, migration into a new area from a non- bed net coverage region and use of the net for non-intended purposes (Sangare et al., 2012; Scates *et al*., 2020).

Despite high LLIN coverage in western Kenya counties, malaria episodes were still experienced by households in the region (Ng'ang'a *et al*., 2021). This called for the need to study the role of LLINs in the resurgence of malaria transmission in western Kenya. This would help the policymakers to base the distribution of the bed net on epidemiological considerations.

To suppress indoor malaria vector numbers IRS must be practiced routinely by households. Declining malaria transmission over the decades has been attributed to the implementation of insecticide-based vector control methods. IRS has resulted in the population of the main malaria vectors to tremendously decline. Therefore, it has to be practiced routinely by households to suppress indoor malaria vector numbers (Lwetoijera *et al*., 2013 and Makungu *et al*., 2017). However, little data was available about the large-scale deployment of IRS and the impact on the observed development of malaria vector resistance to WHO-recommended insecticides (Tangena *et al*., 2020).

Various factors hinder the successful utilization of the IRS in endemic areas as recommended by WHO. These include choice of the compounds for spray manufacture, accessibility and cost, social acceptance and incentivization to use the bed net, policy and practice and technical flaws in the application of the process (Chanda *et al*., 2015; Sherrard-Smith *et al*., 2018; Shahandeh & Basseri, 2019; Abong'o *et al*., 2020; Tangena *et al*., 2020). Utilization of IRS by regions, countries, communities, and households would be driven by a desire for malaria elimination as envisaged by WHO.

In Kenya use of IRS has had a significant impact on anopheline population changes in the location and time of seeking blood meal by the mosquito (Gimnig *et al*., 2003; Ototo *et al*., 2015), variations in the composition of the vector species and the shift in the host selection (Bayoh *et al*., 2010; Ndenga *et al*., 2016). The use of IRS has been shown to affect the anopheline feeding behaviors by shifting to seeking blood meals early in the morning or earlier in the evening. These are the times when human hosts are out or not protected by insecticides (Cooke *et al*., 2015) and are exposed to the anthropophagic mosquito bite. However, the lack of regular surveillance of human and vector behavior patterns defeats the goals of the compilation of data and mapping of potential malaria transmission hotspots. The adaptability of the female *Anopheles* mosquitoes to selective pressures in response to the heightened use of IRS was a major concern that could not be overlooked (Githinji *et al*., 2020). This provided a basis for further studies to be carried out on the impact of the adoption of IRS on the reduction of malaria transmission in Kenya.

Environmental factors play a crucial roles in the prevalence, distribution, and abundance of anopheline mosquitoes (Minakawa *et al*., 2006; Ndenga *et al*., 2006; Kondepati, 2013). There have been recorded environmental modification practices such as deforestation, reclamation of wetlands, and a shift from cash crop farming to short seasonal crop farming due to population growth. Land use change is a great driver for the increase in the abundance of malaria vectors. Therefore, proper management of the environment must be incorporated into the malaria control strategies to reduce the breeding and resting sites of the mosquito vector (Kweka *et al*., 2016; Nicholas *et al*., 2021).

Several studies have associated man-made and permanent habitats to be the main contributory factors for increased anopheline larval distribution. As such authorities and communities should target more anthropogenic activities that result in the availability of breeding places. Therefore, proper environmental management would drastically reduce mosquito breeding sites (Mattah *et al*., 2017; Nicholas *et al*., 2021). This strategy was achievable through understanding the vector behavior such as preferred breeding sites, resting places, and feeding characteristics.

Removal of breeding sites through draining stagnant water, removal of water-holding artificial containers, clearing bushes in the home compound, and general cleaning of the residential houses was quite critical in malaria control (Ng'ang'a *et al*., 2008; De Silva & Marshall, 2012; Adebayo *et al*., 2015).

People's knowledge, attitudes, and practices also determined the successful implementation of environmental management tools such as larval source management aimed at the reduction of the *Anopheles* population (Imbahale *et al*., 2010). Reducing the *Anophele's* abundance, distribution, and composition limits their chances of gaining entry into residential houses to bite and transmit malaria.

Sub-Saharan Africa suffers the greatest malaria burden majorly in women and children because of low immunity to malaria parasites and low incomes occasioned by a poor socio-economic environment. Differential socio-economic proxies play a key role in the perception that malaria is a disease of the poor. Poverty is not limited to the people, but also the inability of the government to provide affordable primary health care, train its personnel, and do proper promotional campaigns (Ricci 2012; Yadav *et al*., 2014).

Additionally, the health facilities should have requisite disease testing kits and adequate drugs). How prompt the sick members of a household seek medication was dependent on the knowledge of the household head about malaria and its primary signs and symptoms. When they seek treatment, all steps of diagnosis, prescription and follow-up through observed direct drug intake in the treatment pathway as envisaged by the Roll Back Malaria (RBM) initiative were adhered to (Chuma *et al*., 2009; Smith *et al*., 2010). However, delayed medical care seeking was still observed in vulnerable groups probably due to distance to caregiving centers, stock-outs of ACTs, lack of personnel at existing primary health centers, and if they were available, failure to follow medical guidelines.

In Kenya, MoH through the Kenya national malaria treatment guidelines recommend that good malaria case management practices have to comprise universal availability of affordable health services to all citizens. Any sick individual have to receive prompt treatment within 24 hours through the provision of functional health services, medical equipment, supplies, drugs, and competent medical personnel (Riley *et al*., 2016; Musuva *et al*., 2017).

The Kenya national and county governments have collaborated in ensuring the millennium development goal of UHC is achieved. This highlighted the Bill of Rights as underlined in the Constitution of Kenya 2010 in which the citizens are entitled to the fundamental right to the highest attainable standards of health (Africa, 2019). Strong government leadership, the building of functional partnerships, and the incorporation of community health practitioners (CHPs) in the provision of Primary Health care (PHC) (Hussein *et al*., 2021).

In 2004 the Kenya government initiated a policy change in the anti-malarial drug that is used for uncomplicated *P. falciparum* malaria to AL and ACT as a first-line drug for the general public while pregnant women still continued to use Fansidar®. However, the adoption of drug policies around the world and in Kenya face policy, financial and legislative challenges that consequently affect the availability of the drug to the masses that need them. To counter this, Mass Drug Administration (MDA) has been done in malaria-endemic areas of Lake Victoria islands and the coastal strip with significant outcomes (Kangwana *et al*., 2009; Gitaka *et al*., 2017; Hussein *et al*., 2021).

Proper treatment for malaria and drug compliance were critical components of malaria eradication in endemic countries. Education, information, and communication (EIC) form the core of the activities that are needed to pass drug and non-drug issues to the recipients). There must not be any ambiguity in drug messages regarding branding, costing, availability, dosage, and side effects. Failure to adhere to the AL regimen would result in the malaria parasite developing resistance and consequently jeopardize the purpose of malaria prevention). Factors that contributed to non-adherence to AL regimens included forgetting, losing, storage for future use and adverse effects on the users. Sharing drugs with members of the family or neighbors and lack of food to take the medication have also been documented as other reasons for failure to complete a dosage (Gerstl *et al*., 2010; Pereira *et al*., 2011; Sambili *et al*., 2016).

The use of AL has reduced morbidity and mortality due to malaria in many areas as envisaged by AU's Agenda 2063, but some areas still recorded contrasting anti-malarial use outcomes (Kapesa *et al*., 2018). A resurgence of *P. falciparum* malaria in western Kenya defeats the intended purpose of AL and MDA. This has demonstrated the need for stronger community engagement in assessing factors that guide promptness to accessing medical care whenever a member of a household had a malaria episode. This would provide evidence-based information to policy makers on where more malaria eradication strategies through the provision of medicines need to be channeled more.

The information that will be obtained from this study would aid in determining the changes in the malaria disease burden, the risk factors posed by land modification due to gold mining and sugarcane farming, and the efficacy of the available control interventions in the face of stiff socio-economic proxies that compel human populations to change the environment in search of livelihoods. Additionally, the results will elucidate the possibility of residual transmission and the probability of the development of resistance to available antimalarial drugs. This would enable policy makers to offer strategies through EIC that would mitigate or synergize the existing methods or policies.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Sites

The study was carried out in two purposively chosen sites in Kakamega County, Western Kenya (Figure 3) which had been reported through a spatial study to have significant aquatic habitats for *Anopheles spp* mosquitoes, the principal vectors for malaria in the region, and abundant *Anopheles gambiae* s.l in the laboratory-reared mosquitoes (Nicholas *et al*., 2021). This made them potential areas for malaria transmission. The survey was part of a wider pilot study in Western Kenya regions that have undergone massive environmental modifications to determine their role in the observed increased malaria cases. Therefore, the two study sites were chosen as part of a wider pilot study in Western Kenya.

Kakamega County (0° 17' 0" North and 34° 45' 0" East) is at an average elevation of 1535 meters above sea level. Kakamega town the county headquarters is 31 kilometers north of the equator and receives high rainfall of between 1280 and 2214 millimeters annually which is evenly distributed. The long rains peak from March to July with a monthly average of 205 millimeters, while short rains are between September and November. The temperature ranges between 18 °C to 29 °C with November to February being the hottest months with an average monthly rainfall of 79 millimeters (Kakamega Meteorological Station).

Over the past few decades, the Western Kenya areas have undergone significant land-use disturbances such as deforestation for settlement, brick making, quarrying, and wetland reclamation which have induced micro-climatic changes that enhance the reproduction and survivorship of *Anopheles* mosquitoes (Carlson *et al*., 2004; Afrane, *et al.*, 2006; Minakawa *et al*., 2006; Ndenga *et al*., 2011; Imbahale et al., 2012).

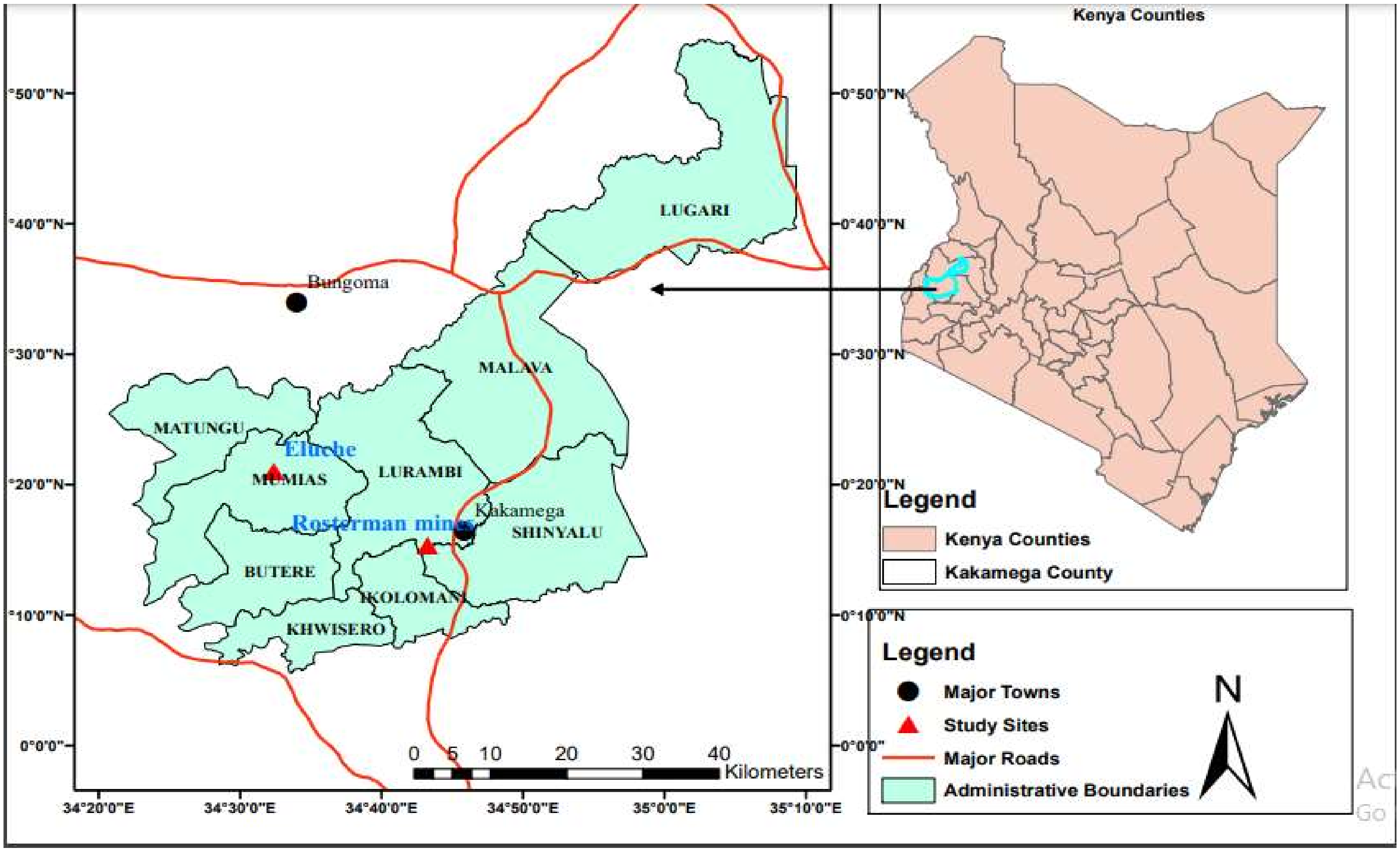

Figure 3: Map showing the two study sites (created from Google maps and Rosterman and Eluche inserted by the author)

3.1.1 Rosterman Gold Mines Study Site

Rosterman mines in Lurambi sub-county (Latitude 0.28°N, Longitude 34.75°E) at an altitude of 1450 (±34) meters above sea level and 5 km from Kakamega town. The area is 5 km by 5 km with 608 homesteads and a population of about 5,000 people (Courtesy of County Government of Kakamega). The village is dominated by the Isukha sub-tribe of the Luhya tribe. Rosterman is an old gold mine that was vacated by the British Company, Rosterman Incorporated in 1952. Despite the cessation of large scale mining of gold, artisanal miners still scavenge for gold dust to date (Kyalo *et al.*, 2015).

Derelict pits filled with water are scattered all over the village. Shallow water holding pans are a common feature due to the continuing "jua kali" mining activities. Besides artisanal gold mining, the residents engage in agricultural activities in which they grow a variety of food crops and rear animals on a small scale. Crop growing has been extended up to the river/stream valleys where reclamation of land has been done. Other economic activities include quarrying, sand harvesting, small-scale trade, and bicycle riding (bodaboda). Rosterman Mines village does not have any public or private health centers. And so the residents seek treatment from neighboring health facilities at Elwesero, the administration police line, and GoK Prisons in Kakamega town. St Paul Imusonga is the only nearby private health facility. To access any of these caregiving centers, the residents walk, use bodabodas that they consider cheaper, hire three-wheelers (tuk-tuks), and private vehicles, or call for ambulance services from the County referral hospital in Kakamega town. Ease with which a population accesses government health facilities enables achievement of GoK's aspiration on universal health care (UHC). However, on the positive side, the Rosterman mines area has a dedicated team of community health volunteers (CHVs) who provide medical assistance to the residents.

3.1.2 Eluche Study Site

Eluche in Mumias East sub-county (Latitude 0.33°N, Longitude 34.48°E) is at an altitude of 1340 (± 35) meters above sea level and lies in the sugarcane belt of Mumias. Eluche has an area of about 6 km by 6 km with 488 homesteads and a population of about 4500 people (Courtesy of County Government of Kakamega). The village is dominated by the Wanga sub-tribe of the larger Luhya tribe. The collapse of the giant Mumias Sugar factory impacted the lives of the residents. This resulted in them converting their sugarcane farmlands to growing food crops and rearing livestock. Just like Rosterman mines, river/stream basins have been reclaimed for growing a wide variety of food crops including maize, beans, groundnuts, vegetables, and yams. Other socio-economic activities the residents engage in are small-scale trade and bodaboda. Residents of Eluche are served mainly by the Eluche health center. However, they can also get services from Lusheya and Shianda health centers and private health service providers such as Shadarpha medical center - Mwitoti, Testimony, Bethel Medical Center, and Shianda Clinic.

3.1.3 Feasibility Study

A feasibility study of the two study sites was done from October to November 2017. The purposes of the reconnaissance was to determine their suitability for the study, map out areas to be covered in the study (approximately 2.5 km radius from Rosterman and Eluche Secondary schools respectively). This was intended to familiarize the principal researcher with the area administrators and community health practitioners (CHPs). After which to discuss with the locals about their opinions on malaria transmission.

3.2 Determining of Malaria Vector Abundance and Diversity

All the aquatic habitats where mosquitoes were likely to breed, were examined for the aquatic forms, while selected residential houses were assessed and used for adult mosquitoes catches in them.

3.2.1 Sampling Aquatic Habitats in Both Sites

This was purposed to determine the abundance and productivity of the mosquito breeding habitats. A total of 32.224 potential aquatic habitats were first inspected for the presence of mosquito larvae. Then using a handheld GPS device (*GPS 12 XL, 15 meters accuracy, Garmin Ltd. 2003, Olathe, Kansas, USA*), the coordinates (longitude, latitude, and elevation) of the exact locations of the larval sites were recorded.

Two to fifteen dips into the mosquito breeding sites were taken using a standard 350 ml mosquito dipper (*Bioquip, Gardena, CA, USA*) with a wooden handle and poured onto white plastic trays as described by Service, (1993). The dips were taken where developmental stages are expected (edges of the habitat/shallows/around vegetation). The larvae were morphologically identified as either culicines or anophelines in which culicines rest at an angle to the water surface while anophelines rest parallel to the water surface. Anophelines were identified to species level using keys described by Gillies and Coetzee (Coetzee, 2020), counted using suction of teat droppers, and recorded in datasheets. Larval density was calculated using a formula adopted from Bashar *et al*., (2016):

$$\text{Mosquito larval density} = \frac{\text{The total number of larvae counted}}{\text{Total number of dips}}$$

The stages of development of the sampled aquatic forms were also recorded as either larva, first - second (early), third - fourth (late) instars, or pupa. The habitats were categorized as drainage ditches, foot/hoof prints, gold mines, burrow pits, cultivated swamps, tire tracks, rock pools, fish ponds, puddles, and artificial containers as described by Nicholas *et al*., (2021) and in *Appendix 6*. Rivers and streams were not considered for sampling of mosquito larval stages because *Anopheles* mosquitoes oviposit in stagnant water.

Other aquatic habitats characteristics that were considered included the length and width of the water surface, vegetation cover, and distance to the nearest house. A tape measure (*IndiaMart, India*) was used to measure the length and width of the water surface, distance from the larval habitat to the nearest residential house, while distance of more than 60 meters to the nearest residential house was approximated. This distances were used to find area covered by vegetation in the aquatic habitat and proximity of the habitats to residential houses. The vegetation cover for elongate habitats was estimated and expressed as a percentage using the given formula while circular/ovoid habitats area was estimated by diameter squared as described by Omukunda *et al*.,, (2013)

$$\text{Vegetation cover} = \frac{\text{Area covered by vegetation } (l \times w)}{\text{Area of the aquatic habitat } (L \times W)} \times 100\ \%$$

Where: L = length of aquatic habitat,

l = length of the part covered by vegetation,

W = breadth of aquatic habitat, and

w = breadth of the part covered by vegetation.

Additional information for the characterization of larval habitats included the date, month of sampling, and the name of the study site.

3.2.2 Collection of Adult Mosquitoes

This was aimed at collecting indoor feeding and resting adult mosquitoes by using both pyrethroid spray collection (PSC) and CDC light traps. The data obtained would be used to determine the abundance and diversity of the *Anopheles* vector. To determine the number of households to be sampled the following formula was adopted from Mugenda & Mugenda (1999) at 95% confidence interval (CI). The sample size was obtained by putting the prevalence rate of malaria at 40% in Kakamega County KMIS, (2020).

$$n = \frac{Z^2P(1-P)}{d^2} + 10\%$$

Where: d = margin of error (0.05),

n = minimum expected sample size from each study site (from an estimated population size of 500 to 600 households with 6 occupants = 3,000 to 3,600 people),

P = estimated prevalence from the previous study (40%), and

Z = standard normal deviation that corresponds to 95% CI (1.96).

This gave 368 respondents per study site that was obtained as below:

$$n = \frac{1.96^2 \times 0.4 \times (1-0.4)}{0.05^2} + 10\% = 404$$

The adult mosquitoes were collected from 10 – 15 randomly selected houses for 3 consecutive days every month for twelve months from November 2018 to October 2019. Only indoor mosquitoes were trapped using PSC (WHO, 1995) and CDC light traps. PSC was done between 0600 and 0900 hours, during which mosquitoes were still resting indoors and there was minimal disturbances in the houses. The locations of the houses sprayed were recorded using differential GPS. Before spraying, foodstuffs and water containers were all covered well to minimize contamination with the spray. White sheets were spread on the floor and the furniture onto which the knocked-off mosquitoes landed. The doors and windows were closed to prevent mosquitoes from escaping. The spray (0.025%) was prepared by mixing emulsifiable pyrethrum concentrate with 0.1% piperonyl butoxide in 5 liters of kerosene. The house was sprayed well starting from outside through the eaves (if present) and then the inside. After 15 minutes had elapsed, the knocked-down mosquitoes on the sheets were collected, sorted, and recorded, after which, the occupants were asked to leave the door(s) and window(s) open for 15 – 30 minutes before entering the sprayed houses.

Six light traps were set between 1900 and 0600 hours for 3 consecutive nights inside six randomly selected houses at least 50m apart (Killeen et al., 2006). In each house, the light trap was placed at a height of 1.5 m at the leg end of the bed or a place in the bedroom shown by the house owner. PSC and the use of light traps to collect the adult mosquitoes were done on separate days in different houses. Some of the houses were repeated during the twelve-month study period to act as sentinels for the study.

In the field, all the mosquitoes were sorted and recorded (*Appendix 7*) and non-anopheline species discarded. The anophelines collected were placed in Petri dishes with moistened cotton wool and carried in a cool box to the Biotechnology laboratory at MMUST for further morphological identification. During the collection of adult mosquitoes, the characteristics of the houses were observed and recorded using questionnaires. These characteristics of the houses recorded included walls (mud, iron sheet, concrete), roof (grass/thatch, iron sheet), presence/absence of eaves, windows (number, with/without screen), new/old, number of rooms, number of sleepers as was described by Minakawa *et al*., (2005). The presence or absence of the bed net and its state (as measured by the number of holes due to wear/tear) was also recorded as indicated in *Appendix 7*.

3.2.3 Adult *Anopheles* Species Identification

Morphological keys were used to identify the adult *Anopheles* mosquitoes that were brought into the lab from the field up to species level using a dissecting microscope. In case a particular aspect was not got right in the field or the field officers had different opinions. Further, molecular work was done on the mosquito vectors as a confirmatory test. At MMUST Biotechnology lab further sorting and classification according to Gillies & De Meillon, (1968) was done for quality assurance. During scoring at the lab, emphasis was placed on abdominal (fed/unfed) and gonotrophic (gravid and half-gravid) status. The mosquitoes were kept in labeled (date, species, abdominal/gonotrophic status, and site of collection) 1.5 ml Eppendorf tubes containing 70% isopropanol and stored at -20°C.

To extract DNA, the legs and wings of each adult mosquito were placed in 1.5 ml Eppendorf tubes, then 20 µl of TE buffer, tris – EDTA added. This was incubated at 95°C for 15 minutes in a heating block. The tubes were then vortexed for 2 minutes and then the DNA containing supernatant was separated by centrifugation at 12,000 revolutions per minute (rpm) at room temperature (24 - 27°C) for 2 minutes.

Amplification and sequencing of the cytochrome oxidase subunit 1 (CO1) (Folmer *et al*., 1994) region was performed. Mwea strain (established in 2004) from ICIPE, Nairobi, Kenya acted as standard reference positive and negative controls for *An. gambiae* s.l. sibling species identification.

Polymerase Chain Reaction (PCR) was done at International Center for Insect Physiology and Ecology (ICIPE) – Mbita for sibling species identification using protocols of Scott *et al*. (1993) and Koekemoer *et al*. (2002). For instance, 10 µl of PCR reaction were prepared with 0.5 µM final concentration for each primer, 2 µl of 5× Hot Firepol Evagreen HRM mix (*Solis BioDyne, Tartu, Estonia*), and 1 µL of DNA template. Thermal cycling conditions for CO1 were as follows: initial denaturation at 95° C for 15 min, followed by 40 cycles of denaturation at 95° C for 30 s, annealing at 50° C for 30 s, and extension at 72° C for 1 min 30 s, and a final extension at 72° C for 7 min. PCR reactions for CO1 were conducted on a Veriti thermocycler (*Applied Biosystems*).

Following PCR, HRM analysis of amplicons was conducted by gradually increasing the temperature by 0.1° C after every 2 s from 75 to 92° C, resulting in a plot of the change in fluorescence with time (dF/dT). PCR-HRM protocols were validated for accuracy and sensitivity using standard reference controls. ExoSAP-IT (*USB Corporation, Cleveland, OH, USA*) was used to remove unincorporated dNTPs and PCR primers before sequencing. Sequences were edited in Geneious 7.0.5 (http://www.geneious.com) and used to query GenBank (Kearse *et al*., 2012).

3.3 Determination of the Prevalence and Intensity of the *Plasmodium* species among the Residents

The observation of *Plasmodium* microscopically, immunologically, and molecularly was an indicator of sickness with malaria and its presence in the population. Participants of the age range 6 months (youngest) to 80 years (oldest) of both sexes were enrolled to participate in the study. Both written and verbal consent was sought for adults to participate in the study while assent for children under 18 years was sought from the head of the family, parent, or guardian (Appendix 1 & 2). Each participant was allocated a random number (for identification and reference). The survey was done on 3 consecutive days (Wednesday, Thursday, and Friday) in August 2020, November 2020, February 2021, and May 2021 to represent the 4 seasons i.e. cool dry, short rainy, hot dry, and long rainy seasons respectively. Ethical clearance was obtained vide MMUST/IERC/090/19 (*Appendix 4*).

3.3.1 Inclusion and Exclusion Criteria

The objective of the study was explained to participants before being enrolled to participate. Compulsion or coercion was not used during the study and there were no direct financial benefits for the participants. To be included in the study, the participant had to:

1. Willingly and be present during the blood sample taking exercise.
2. Be a resident of Rosterman and Eluche for at least 30 days before the blood samples are taken.
3. Willingly let the researcher into their compound or house.
4. Not be on malarial treatment

For a resident to be excluded from the study:

1. Unwilling to participate in the study.
2. Being absent during blood sample taking exercises.
3. Unwilling to have their blood sample taken.
4. Not being a resident of the two study sites for at least 30 days before the day of the exercises.

5. Being on malarial treatment.

3.3.2 Blood Sample Collection and Processing

Blood samples were obtained by qualified phlebotomists from the Kakamega County Referral and Teaching Hospital by the standard finger prick method. The fingertip was wiped clean with an alcohol pad and the prick was made. The first blood drop was wiped off. A sterile loop applicator was used to collect a drop of blood which was placed on a rapid diagnostic test (m-RDT) device (*OptMal®, DiaMed, Cressier, Switzerland*), Buffer was added to the device cassette to enable the sample of blood to flow by capillarity through reaction area. Results were recorded after 15 minutes by observing appearance of red lines near points marked 'C' and 'T' on the m-RDT for positive and no line near 'C' for negative slides respectively. The result were recorded in data sheets (*Appendix 8*), as positive or negative. Those found to have malaria were referred to the nearest health center. Two drops of blood were placed on a labeled clean grease-free frosted slide (*Fusion Biotech, Shilpent, India*), and four drops were placed on Whatman filter papers, 125 mm (*IndiaMart, India*) for subsequent microscopic and molecular analysis respectively.

3.3.3 Prevalence and Intensity of *Plasmodium spp* by Microscopy

Two drops of respondents' blood were used to prepare thick and thin blood films and the slides labeled with random numbers (date, age, site, and time of collection). The slides were air-dried in a horizontal position for 45 minutes then placed in a vertical position in a slide box and transported to the Biotechnology lab in MMUST for further processing. The thick blood smears were not fixed while the thin blood smears were fixed in absolute alcohol for 30 minutes and then air-dried. Both slides were stained with 5% Giemsa (*Sigma-Aldrich, Darmstadt, Germany*) for 45 minutes as described by Iqbal *et al*., (2003). The asexual forms of the malaria parasites (trophozoites and schizonts) were identified using an oil immersion objective ×100 and recorded. Malaria parasites density per microlitre (µl) of blood was calculated using:

Thick blood smear

$$\frac{\text{Malaria parasites counted}}{\text{200 white blood cells}} \times 8{,}000 = \text{number of parasites/µl}$$

as modified from Payne, (1988). The parasite densities were classified as 1) light infection (40 – 5,000), 2) moderate infection (5,001 – 10,000), and 3) heavy infection (> 10,000) parasites/µl of blood. A third microscopist examined the slides for quality control and in case there were discordant results between the two first microscopists with regards to parasite intensity and species.

3.3.4 Molecular Identification of *Plasmodium* Species

The filter paper with the four blood drops (DBS) of each participant was well labeled with the random numbers of the participant and malaria status (either negative or positive). The DBS packed in zipped folders were carried in a cool box to the lab at MMUST for storage at -20°C before processing.

One hundred and thirty-nine positive DBSs were carried to the United States Army Medical Research Unit – Africa/Kenya (USAMRU) laboratories in Kisian, Kisumu for molecular confirmation of species of the *Plasmodium* by PCR The protocol for DNA purification from DBS by QIAamp DNA Mini Kit was used to extract and purify the *Plasmodium* DNA from the DBSs.

Three circles were punched out from each DBS using a 3-millimeter (mm) puncher (*Harris Uni-Core™, Qiagen, Hilden, Germany*) and placed in a 1.5 ml microcentrifuge tube. The puncher was cleaned each time between successive DBSs as described by Strøm *et al*. (2014). To the 1.5 ml microcentrifuge tubes containing the three circles, 180 microlitres (µl) of Buffer ATL was added, after which it was incubated at 85°C for 10 minutes. The microcentrifuge tubes were then briefly centrifuged to remove drops from inside the lids.

Twenty µl of proteinase K stock solution was added, and the mixture vortexed and incubated at 56°C for one hour. After which the tubes were centrifuged and 200 µl of Buffer AL was added. The mixture was vortexed and incubated at 70°C for 10 minutes and then centrifuged. To each tube, 200 µl of ethanol (96 – 100%) was added and the mixture vortexed thoroughly and centrifuged. The mixture from each tube was carefully applied to the QIAamp Mini spin column (2 ml collection tube) without wetting the rim.

The caps were closed and the mixture centrifuged at 8,000 revolutions per minute (rpm) for one minute. The QIAamp Mini spin columns were then placed in clean 2 ml collection tubes and the tubes containing the filtrate were discarded. The QIAamp Mini spin columns were then carefully opened and 500 µl Buffer AW1 was added without wetting the rim. The caps were closed and centrifuged at 8,000 rpm for 1 minute. The QIAamp Mini spin columns were placed in clean 2 ml collection tubes while the tubes containing the filtrate were discarded, then 500 µl Buffer AW2 was added without wetting the rim, the cap was closed and centrifuged at 14,000 rpm (full speed) for three minutes.

The QIAamp Mini spin columns were then placed in new 2 ml collection tubes and the tubes containing the filtrate were discarded, then centrifuged at 14,000 rpm for one minute. The QIAamp Mini spin columns were then placed in clean 1.5 ml microcentrifuge tubes and the tubes containing the filtrate were discarded. To each QIAamp Mini spin column, 150 µl Buffer AE was added, incubated at room temperature for one minute, and centrifuged at 8,000 rpm for one minute. The eluates were stored at 4 - 8°C to await preparation for PCR.

For detection of plasmodial DNA, Multiplex real-time PCR (RT-PCR) was done on the eluates targeting *Plasmodium* DNA (PLU) and a housekeeping control gene RNase for quality control purposes (to determine the efficiency of the extraction process and determine the source of the samples – either human or nonhuman blood). Four µl of master mix, 1 µl primers, 1 µl probes (*QuantiFast® Probe PCR Kit 2000, Qiagen, Hilden, Germany*), and 3 µl distilled water (in the ratio 11 master mix: 1 primer: 1 probe: 3 distilled water) were put in each well on the 96-well PCR plate. Two µl of the sample was added to each well with two wells containing positive control (*P. falciparum* reference strain 3D7) and negative control (*P. falciparum* reference strain W2). The plate was sealed tightly, centrifuged at 2,500 rpm for one minute, and loaded into the RT-PCR machine (*QuantStudio™6 Pro Real-Time PCR System, 96-well, 0.2 mL, Applied Biosystems, US*).

3.4 Determination of the Risk Factors Associated with Malaria Transmission among the Residents

Data was collected in the households and the environment that were acting as risk enhancers to the transmission of malaria within the population of the two study sites. A semi-structured questionnaire (*Appendix 3*), IIs, FGDs, and observation (*Appendix 7*) of the confounding factors were carried out to determine the socio-demographic characteristics of participants and the household heads' knowledge of malaria. The questionnaire was adopted from a knowledge, attitudes, and practices (KAP) one used by the CDC in an area of urban malaria transmission (Siri *et al*., 2008).

After modification, it was translated into the KiIsukha dialect and was verbally translated to Kiswahili by the interviewers to those who had difficulties understanding English or KiIsukha dialect. The questionnaire was pretested by interviewers (community health practioners) for validity and consistency on 15 residents of Sichirai village (Kakamega Cental sub-county) and village administrators in Rosterman and Eluche. Appropriate corrections were then made and the interviewers were further trained on community entry techniques, seeking consent, asking questions, and recording responses.

Filling questionnaires was systematic from household to household. A household was taken to be a house where people slept. The head of the household was the person interviewed or given authority to answer the questions. Other members of the household were allowed to listen to the interview and offer minimal participation. In the case of a polygamous household, wives were preferred as household heads because they were in direct control of their households.

3.4.1 Socio-demographic Characteristics of the Participants

The socio-demographic factors considered in the questionnaires were gender, age, marital status, the highest level of education main occupation, and average monthly income. These socio-demographic factors have been reported to play a significant role in malaria transmission. In assessing the household heads' knowledge about malaria, the questions asked were about the participant having heard of malaria, the source of that information, malaria transmission, the cause of malaria, where mosquitoes breed, and symptoms of malaria.

3.4.2 House Structure Characteristics

House characteristics are key drivers of malaria transmission (Bashar *et al*., 2012). Data on house characteristics was collected by direct observation on two occasions, during the sampling of aquatic habitats for larval stages of the mosquito and PSC. The characteristics in the house structure that were studied included: house (new/old), wall (mud/concrete), roof (grass thatched/iron sheets), eaves (present/absent), doors/windows (screened/unscreened), number of rooms, and number of occupants. The GPS of the surveyed houses was also taken and recorded.

3.4.3 Bed net Status

Data on the status of the mosquito net was collected through direct observation while the occurrence of malaria episodes in the household was by interviewing the head of the household. The GPS of the surveyed houses in which data on the state of the ITNs were sought was also taken and recorded.

3.4.4 Environmental Management Status in the Study Sites

Environmental management by individual households and communities played a critical role in reducing the mosquito vector burden and thus malaria transmission. The questionnaire sought to find out how the residents of the two study sites dealt with their environment in the face of malaria disease. In addition, questions asked in the questionnaires, IIs, and FGDs were on mosquitoes breeding and resting sites, and care of the environment by the residents, to reduce the risk of being bitten by the *Plasmodium*-carrying mosquitoes. This information obtained from the study of the risk factors would go a long way to re-think the success of the available malaria control strategies.

3.5 Determination of Malaria Control Measures Used by Residents and Communities in the Two Study Sites

The institution of malaria control strategies is significant in reducing the malaria burden. However, this should be done with cognizance of the challenges individual households and communities face in the implementation of any documented intervention. Semi-structured questionnaires (*Appendix 3 & 4*), IIs, and FGDs were carried out to determine mosquito control methods they employed and medication behaviors. FGDs were prominent in this section concerning the successes/challenges of the control programs available. FGDs were purposely selected to include 3 – 7 recruits from community leaders, administrators, and (CHPs) and a guide. The recruits had prior knowledge of malaria and had primary school certificate. A total of 63 (37 female and 26 male) participants were involved aged 18 years and more. FGDs met for five sessions during the survey period and each session lasted for up to one hour. The IIs were conducted during the filling of the questionnaire and in FGDs in which standing out issues on the malaria cycle were discussed.

3.5.1 Indoor Malaria Prevention Methods Used by Households

Both quantitative and qualitative data were collected on the methods employed by the households to prevent members from malaria. In these questions, five aspects were critical: the bed net (availability, ownership, use, and maintenance), insecticide sprays, preventing mosquito breeding and resting places, mosquito coils/repellents, indoor residual spray (IRS), and treatment. Data on the methods used by households particularly indoors to prevent mosquitoes from gaining access into their houses and from their bites were collected using questions as indicated in the questionnaire in *Appendix 3 & 4*, the strategies used to maintain their bed nets (re-treatment) to ensure continuous efficacy and if they practiced IRS.

3.5.2 Outdoor Malaria Control Methods Used the Residents and Communities in the Two Study Sites

Data was also collected on the environmental management methods the households employed to prevent mosquitoes from breeding and resting using methods described in 3.4.5. Through the FGDs, qualitative data was sought to find out the extent of communal collaboration in preventing the proliferation of *Anopheles* and hence malaria episodes.

A couple of questions in this section were open-ended. Information obtained from this section would go a long way in giving evidence-backed need for improved malaria control interventions in the two study sites and other rural settings with similar socio-demographics.

3.6 Participants' Treatment-seeking and Drug Use Behaviour

Questionnaires, IIs, and FGDs were used to find out the treatment-seeking and drug-use behaviors of the participants in the two study sites. The data collected would give a sneak preview into the much-communicated information on seeking prompt treatment for malaria and completion of the prescribed anti-malarial drugs recommended by MoH. The questions asked involved, what action household heads took when members contracted malaria, what drugs were prescribed/bought, if there were any side effects and challenges in completing anti-malarial regimens, and what reasons made them not complete given doses.

3.7 Data Management and Analysis

All data that was collected was entered into Microsoft Excel Spreadsheets, cleaned, and coded. It was statistically analyzed using different tests to address particular aspects. Descriptive statistics were carried out to determine relative frequencies and proportions. Tables and Graphs (by MS Excel) were generated to show the distribution of relative frequencies of various variables. The data were exported to GPPS version 5.0 (*GraphPad Software Inc, San Diego, CA*) for analysis of the variables: 1) Specific objective 1: the F-test was used to test the productivity of aquatic habitats, the association between the month of collection and the adult mosquito densities; 2) Specific objective 2: the t-test was used to test the association between mean malaria prevalence and the site of the study, the season of the study, and age brackets in which the disease was recorded, and 3) Specific objectives 3 & 4: the Pearson's Chi-square test and Fisher's exact test to test the association between socio-demographic variables and the knowledge of malaria, malaria prevention, and control, malaria episodes, treatment-seeking, and drug use behavior at 95% CI (not including zero) and appropriate odds ratios (OR) drawn at $p \leq 0.05$ statistical significances.

3.8 Ethical Considerations

The protocols for this research were approved by Masinde Muliro University of Science and Technology Institutional Ethical Review Committee (MMUST-IERC) vide approval number MMUST/IERC/090/19 (*Appendix 4 & 5*). While the research license was obtained from the National Council for Science, Technology and Innovation (NACOSTI) vide license number: NACOSTI/P/20/3215 (*Appendix 6*). National and County administrative authorities permitted the study to proceed. The risks and benefits of participation in the study were explained to the respondents. Participation in the study was voluntary, with no monetary compensation provided to participants for their time. Informed written and verbal consent and assent were provided by eligible respondents before recruitment in the FGDs, IIs, or administration of the questionnaire (*Appendix 1 & 2*).

Before the collection of blood samples for malaria testing, informed consent was requested from the parents or guardians (or nominated household heads). All data and other information collected were confidential and were entered in data sheets as indicated in appendix 8. Respondents' names and identification numbers were removed from the final data before analyses were conducted. Blood samples were stored with unique identification numbers to protect respondents' identities. The absence and unwillingness of a resident to participate excluded them from the process. For quality assurance, meetings between the PI and field officers were conducted before and after the exercise regularly.

3.9 Limitations of the Study

The inability to collect both indoor and outdoor adult mosquitoes due to logistical and financial constraints, may not be quite representative of the malaria vector prevalence and diversity in the

study sites. Additionally, the inability to use all the adult mosquito collection methods besides PSC may have underpowered the total number of anophelines collected during the study period.
The principal investigator had no control over the participants selected by the community contact person and therefore there may have been a selection bias of the participants.

The COVID-19 pandemic had a partial effect on the willingness or lack of it thereof of the participants to take part in the study. This made a certain segment of the population not participate in malaria testing and blood collection.

During molecular work on DBSs, less DNA was extracted making troubleshooting constantly done at each level of PCR, and down streaming was not possible.

CHAPTER FOUR

RESULTS

4.1 Abundance and Diversity Malaria Vectors

Both the developmental stages of the mosquito vector and the adults were analysed for their relative abundance and species diversity to correlate with the confounding factors that potentiated for the observed recorded rise in malaria in the Western Kenya region.

4.1.1 Aquatic Habitats and their Productivity

The availability of breeding habitats, their relative abundance, and productivity posed risk factors for transmission of malaria. A summary of the potential breeding habitats, relative abundance and productivity surveyed from November 2018 to October 2019 in the two sites is shown in Table 1. Eighty-nine thousand seven hundred and ninety-one (89,791) immature mosquitoes were recorded. Of this, 64.11% (57,567) were culicines while anophelines accounted for 35.89% (32,224).

Of the anophelines, 84.43% were first to second instar, 14.14% third to fourth instar, and 1.43% pupae. Mosquito breeding sites due to human activities were the most prevalent (93.22%), with gold mines being the most productive aquatic habitats accounting for 51.29% of the anopheline larval density. This was followed by drainage ditches (13.39%), fish ponds (12.65%), human/animal footprints (6.85%), cultivated swamps (6.55%), burrow pits (4.82%), rock pools (2.45%), puddles (1.15%), tire tracks (0.68%), and artificial containers (tins/cans/tanks/bottles, unused/old household items) (0.20%).

Aquatic breeding sites sampled had a mean length of 6.14 meters and width of 2.49 meters while the vegetation cover median was 22.82%. The majority (52.15%) of the larval habitats sampled had vegetation growing in them. The occurrence of larvae in the habitats differed from one habitat to the next, with those with less vegetation cover more likely to have early instar stages.

Table 1: Distribution of aquatic breeding sites and their productivity in Western Kenya (Rosterman mines and Eluche) from November 2018 to October 2019

Habitat Type	Study site		Habitat Type N (%)	Culicines	Anopheline Developmental stages			Total anophelines N (%)
	Rosterman	Eluche			L1-L2	L3-L4	Pupa	
Drainage ditches	624	528	1,152 (16.45)	6,376	3,732	568	4	**4,304 (13.36)**
Footprints	516	660	1,176 (16.79)	4,180	1,860	348	0	**2,208 (6.85)**
Gold mine	1,764	000	1,764 (25.19)	22,288	13,037	3,168	324	**16,529 (51.29)**
Burrow pits	240	204	444 (6.34)	1,603	1,339	144	70	**1,553 (4.82)**
Cultivated swamp	408	36	444 (6.34)	6,212	2,020	80	12	**2,112 (6.55)**
Tire tracks	48	48	96 (1.37)	3,607	200	16	3	**219 (0.68)**
Rock pools	180	144	324 (4.63)	1,242	720	70	0	**790 (2.45)**
Fish ponds	240	636	876 (12.51)	5,604	3,884	144	48	**4,076 (12.65)**
Puddles	324	252	576 (8.23)	1,239	361	9	0	**370 (1.15)**
Container	78	73	151 (2.16)	5,216	55	8	0	**63 (0.20)**
Total	**4,422**	**2,581**	**100.01**	**57,567**	**27,208**	**4,555**	**461**	**32,224 (100.00)**

During the survey period, 97% of the sampled habitats had mosquito larvae at any one time of sampling. The mean larval densities per every aquatic habitat type were as shown in Figure 4. The gold mines (9.37), besides being the most productive regarding overall larval mosquito abundance, also had the highest mean densities. This was followed by rock pools (5.49), while artificial containers (tins/cans/tanks/bottles, unused/old household items) had the least mean densities of the breeding sites sampled.

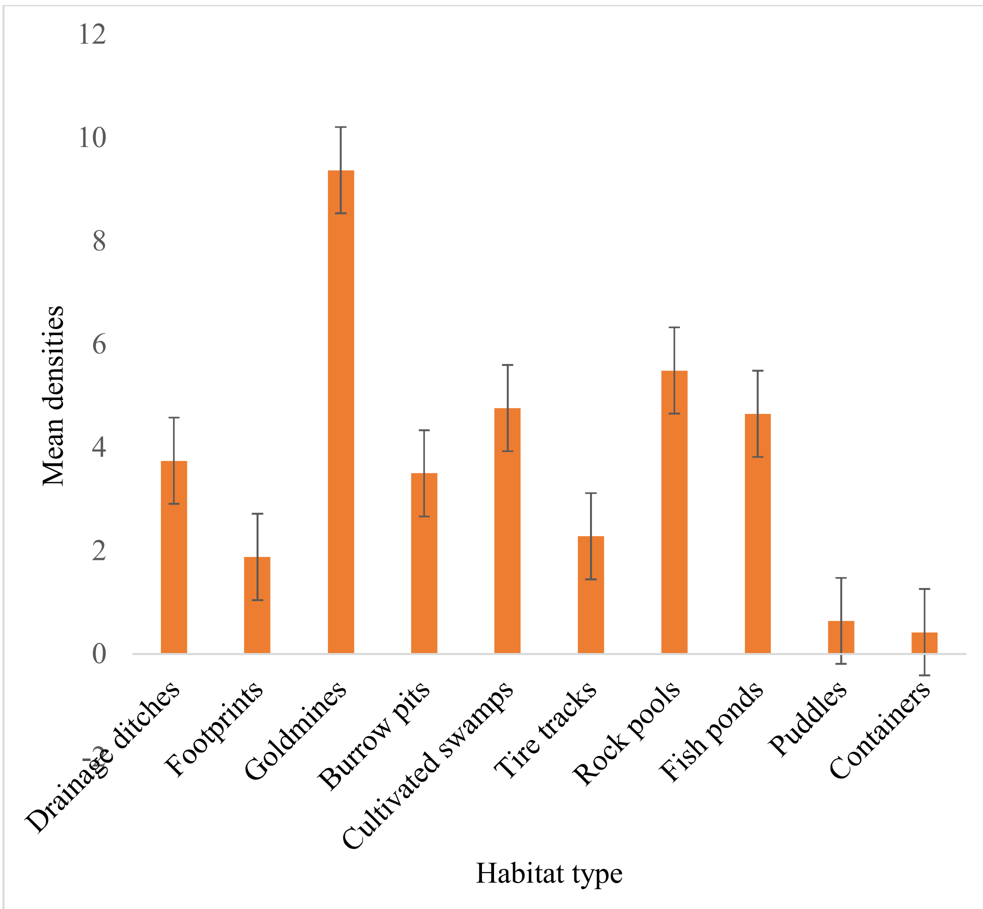

Figure 4: Mean densities of mosquito developmental stages in the different habitats in Western Kenya (Rosterman mines and Eluche) from November 2018 to October 2019

Larvae of the anophelines mosquitoes showed seasonal differences in densities as shown in Figure 5. It was deduced that larvae of the malaria vector were available in the study sites all year round. The drier months of January to March still recorded a greater abundance of larvae. This indicated that water habitats were still available for breeding by the mosquito. On the other hand, wetter months showed a reduced abundance of mosquito larvae despite the availability of water. A comparison of the month of the year when sampling was done and anopheline larval density in the potential breeding habitats showed no significant correlation (r^2 = 0.2427, 95% CI -0.8888 – 0.3249, p = 0.2148).

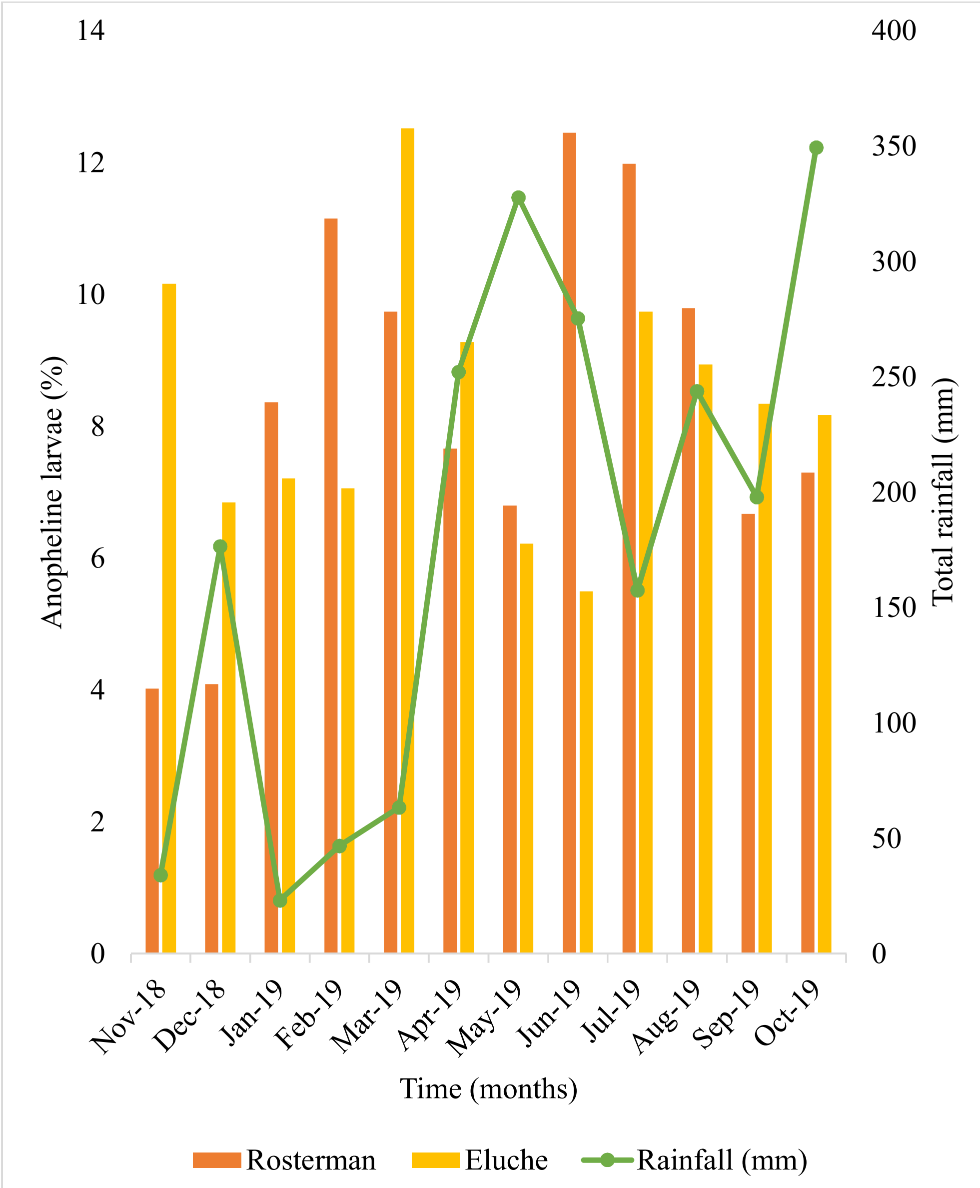

Figure 5: Monthly anopheline larvae dynamics in aquatic habitats in Western Kenya (Rosterman mines and Eluche) from November 2018 to October 2019

4.1.2 Abundance and Diversity of Anopheline Larval Stages

Morphological analysis of the larval stages showed that the majority of the larvae were *An. gambiae* at 91.96% followed by *An. funestus* (5.53%), *An. coustani* (1.68%), *An. implexus* (0.52%), and *An. squamosus* (0.31%). *An. gambiae* and *An. funestus* the principal vectors of malaria were the most dominant anopheline larval stages. The secondary vectors, *An. coustani,* *An. implexus* and *An. squamosus* did not play a significant role in the malaria transmissionin the study areas.

4.1.3 Abundance and Diversity of Adult Anopheline Vectors

A total of 11,937 adult mosquitoes were collected during the study period (November 2018 – October 2019). Morphologically the genus *Culex* accounted for 93.93% (n = 11,212) and the genus *Anopheles* was 6.08% (n = 726). No *Aedes* mosquitoes were sampled. Of the anophelines, females represented 78.37% (n = 569) and males 21.63% (n = 157). In Rosterman female *Anopheles* collected were 42.36% (n = 241) while in Eluche 57.64% (n = 328). The seasonal abundance of the female *Anopheles* was as represented in Figure 6. In Rosterman, the frequency peaked in July, while in Eluche the frequency showed little variation across the months. However, in Eluche the number of mosquitoes showed an increase from October to January. On comparison of the months of the year when sampling was done in Rosterman and Eluche and the adult mosquito densities, showed no significant correlation (r^2 = 0.007069, 95% CI -0.6598 – 0.7447, p = 0.8431).

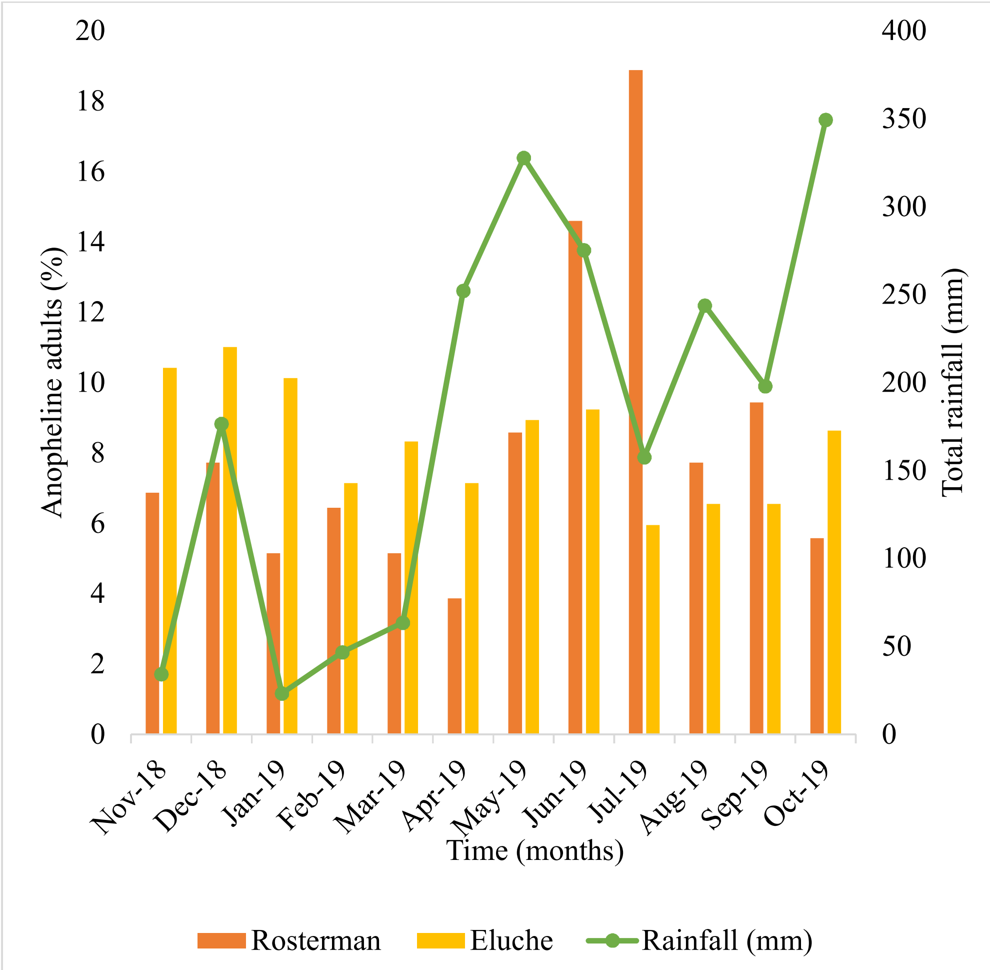

Figure 6: Monthly female anopheline adults collected by PSC and CDC light traps in Western Kenya (Rosterman mines and Eluche) from November 2018 to October 2019

Of the anophelines collected *An. gambiae* s.l White was the predominant species at 63.27% (n = 360), followed by *An. funestus* Giles at 21.09% (n = 120), *An. squamosus* Theobald 7.56% (n = 43), and *An. implexus* 5.97% (n = 34), and *An. coustani* Laveran 2.11% (n = 12). The greatest frequency of *An. gambiae* s.l White in Rosterman was in July and the least frequency was in April, whereas in Eluche the frequency was greatest in December and the least frequency was in July. Moreover, a total of 480 specimens of *An. gambiae* s.l White and *An. funestus* Giles were identified to species level by PCR, of which all were successfully amplified. They were identified as *An. funestus* 48.33% being the majority, *An. arabiensis* 30% and *An. gambiae* s.s 21.67%.

From all the adult female *Anopheles* species collected, species was *An. gambiae* s.s was the most abundant followed by *An. arabiensis* White ($F_{8, 99} = 24.593$, $p \leq 0.0001$) as shown in Table 2.

Table 2: Mean *Anopheles* mosquito densities by species in Rosterman mines and Eluche from November 2018 to October 2019 (by morphological diversity)

Female *Anopheles* species	**Mean ± SE**	***p*-value**
An. gambiae	30.000 ± 2.2500	0.0025
An. funestus	10.000 ± 1.9920	0.0025
An. squamosus	3.583 ± 0.6211	0.0024
An implexus	2.833 ± 0.4051	0.0036
An coustani	1.00 ± 0.2752	0.0119
Sites		
Rosterman	23.670 ± 2.3430	0.0024
Eluche	24.500 ± 2.4700	0.0025

4.1.4 Correlation of the Developmental Stages and Adult Vectors

When correlation was performed on anopheline developmental stages and the adults, there was no significant correlation ($r^2 = 0.002205$, 95% CI -0.5725 – 0.5755, $p = 0.9946$), for the twelve-month study period. This showed that the availability of the anopheline larvae in the habitats was not a guarantee that adult vectors would be in the two study areas and the reverse was also true, that the occurrence of adult vectors did not indicate that larval stages were be available.

4.2 Prevalence and Diversity of Malaria Parasite among Residents of Rosterman and Eluche

The availability of the malarial parasite in the population causes malaria infections and is readily picked by the vector and transmitted to a healthy individual, thus sustaining transmission.

4.2.1 Prevalence of Malaria

Prevalence of malaria within the study population by various tests was 8.96% (214/2389) by rapid diagnostic tests, 9.25% (221/2389) by microscopy and 9.25% (221/2389) from DBS respectively. Polymerase chain reaction of the DBS results indicated one of the samples had a mixed infection with *P. falciparum* and *P. malariae* from a sample collected in Eluche sugarcane growing area during the hot dry season. Based on the four seasons and age, the results were as shown in Table 3.

Malaria was most prevalent during the rainy season (31.8%) and least prevalent during the cool dry season (15.4%). Comparatively, by sex, mean malaria prevalence was significantly different (t=107, df = 1, 95% CI 94.29 – 119.7, p = 0.0059), with males being more likely to suffer from malaria than females. There was a significant difference in mean malaria prevalence across the four seasons (long rainy, cool dry, short rainy and hot dry) (t=7.144, df = 3, 95% CI 29.67 – 77.33, p = 0.0056), with a resident likely to report a bout of malaria during the rainy seasons than the drier seasons.

Table 3: Frequencies of *Plasmodium* prevalence in the population by sex and season in the two study sites (by microscopy)

Sex	Rosterman N (%)	Eluche N (%)	Total N (%)	*p*-value
Female	49 (50)	59 (50.9)	108 (50.5)	0.0059

Male	49 (50)	57 (49.1)	106 (49.5)	
Total	**98 (100)**	**116 (100)**	**214 (100)**	
Season				
Cool dry	7 (2.5)	26 (8.7)	33 (15.4)	0.0056
Short rainy	36 (9.1)	24 (11.2)	60 (28.0)	
Hot dry	23 (7.5)	30 (10.3)	53 (24.8)	
Long rainy	32 (10.6)	36 (12.2)	68 (31.8)	
Total	**98 (45.8)**	**116 (54.2)**	**214 (100)**	

When the adult-to-child malaria ratios (ACRs) were computed for the two study sites the results were as shown in Figure 7. ACR was highest in the short rainy season and least in the cool dry season.

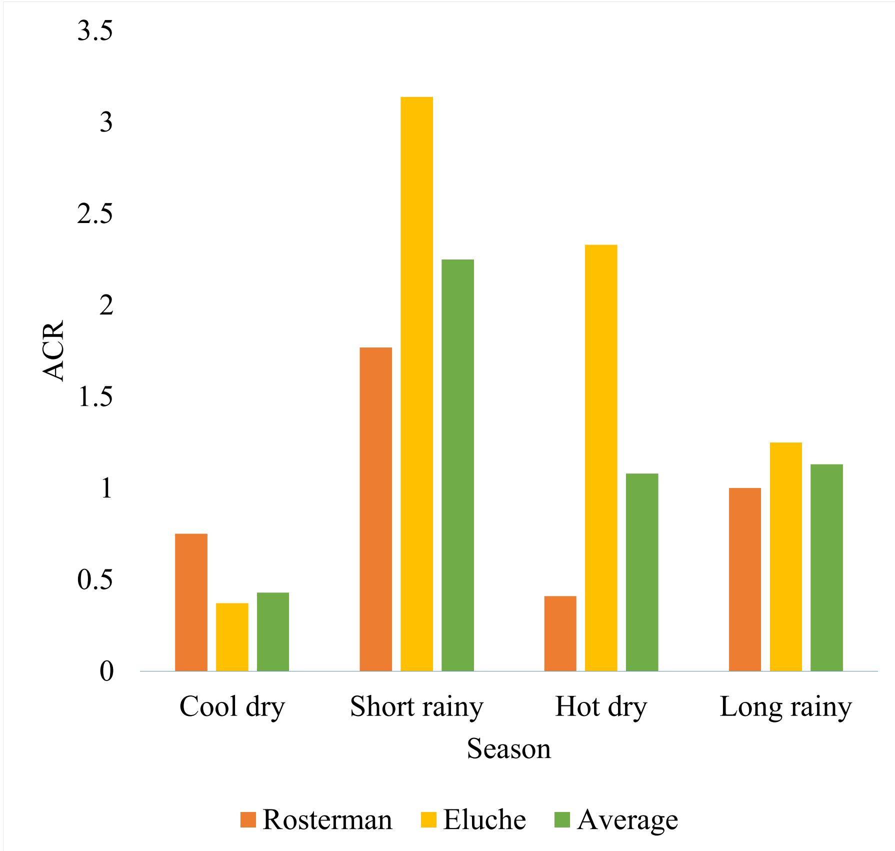

Figure 7: Adult and child vulnerability to malaria

4.2.2 Prevalence of *Plasmodium spp* by Seasons

Results from the m-RDT and microscopy showed the mean *Plasmodium* infection prevalence in the surveyed population was at 8.96% as shown in Figure 8. The long rainy season had the highest malaria prevalence at a mean of 11.4%, followed by the short rainy season (10.2%), hot dry season (8.9%) and cool dry season had the lowest mean of 5.6%. There was a significant difference in mean malaria prevalence during the four seasons (t=7.135, df = 2, 95% CI 8.67 – 17.12, p = 0.0072). This indicated that rainfall had a strong correlation with *Plasmodium spp* infection.

Comparing *Plasmodium spp* infection prevalence based on the study site, Eluche had a higher mean (10.6%) than Rosterman mines (7.4%) as shown in Figure 8. Therefore, sugarcane farming in Eluche could be associated more with increased Plasmodium spp prevalence than gold mining in Rosterman.

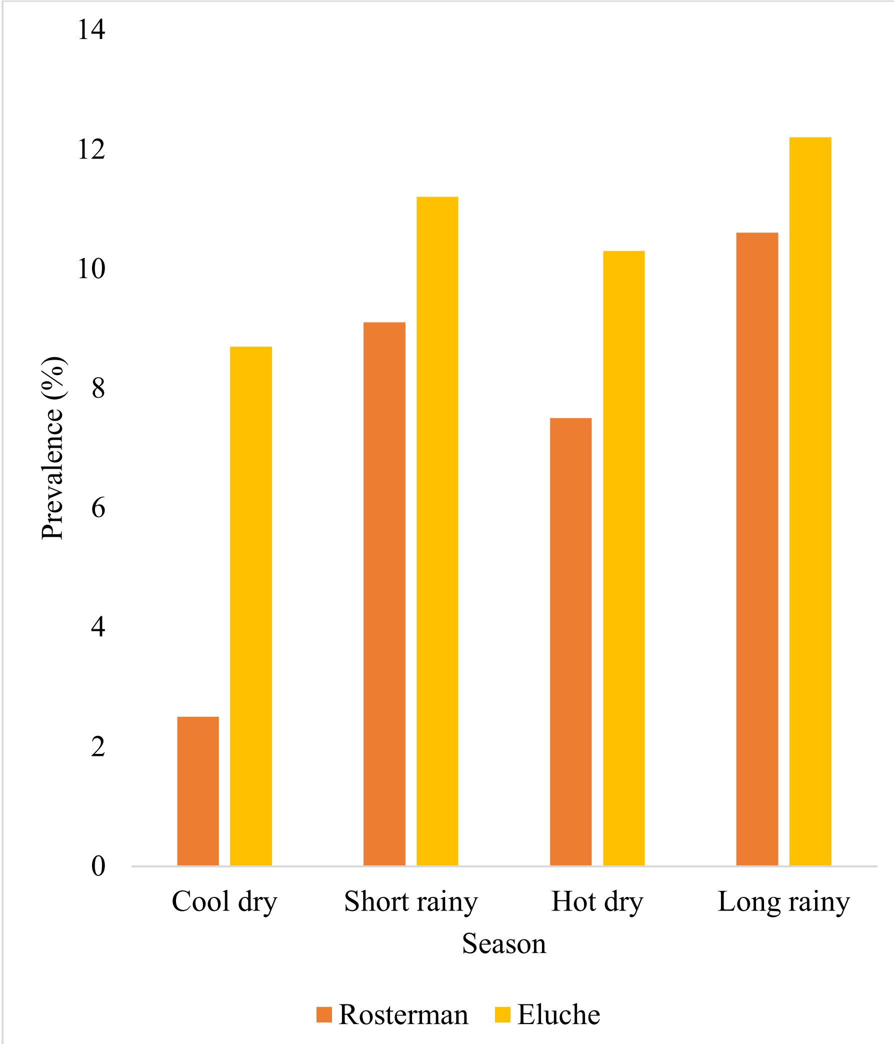

Figure 8: Seasonal *Plasmodium spp* prevalence in Rosterman and Eluche

4.2.3 Prevalence of *Plasmodium spp* by Age

The prevalence of *Plasmodium spp* parasites based on age of the participants was as indicated in Figure 9. Malaria parasite was more prevalent in people aged 19 years or more (44.9%), followed by the 6 – 19 years bracket at a prevalence of 39.3% and least prevalent in children aged 5 years or below (15.9%). When tested for association, *Plasmodium spp* prevalence was not age-dependent (t=3, df = 2, 95% CI -10.36 – 153, p = 0.0641) and this suggested that members of households would contract malaria regardless of the age.

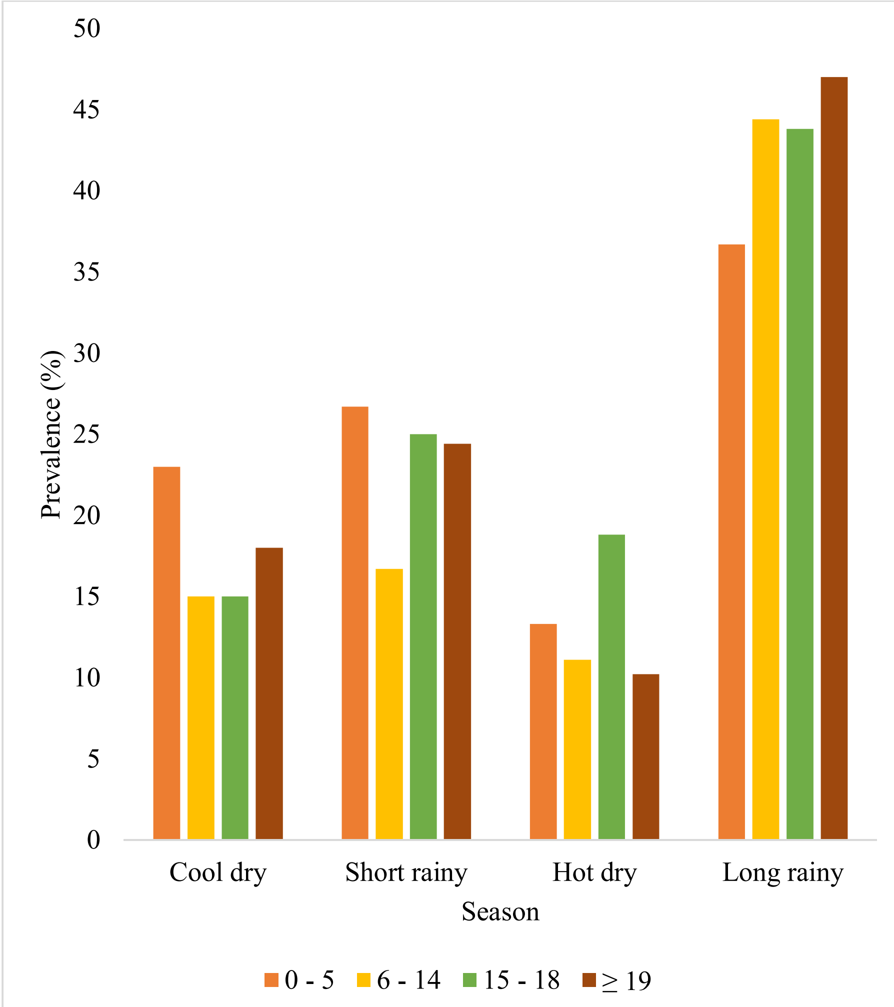

Figure 9: *Plasmodium spp* prevalence by age in Rosterman and Eluche

4.2.4 Intensity of *Plasmodium spp* Parasites in the Study Participants

Based on the seasons, the intensity of *Plasmodium* density was as shown in Figure 10. The cool dry season had the highest mean density of 5,635 parasites/µl of blood, followed by long rainy (5,040), short rainy (4,660), and hot dry (4,030) parasites/µl of blood respectively. The cool dry season (August) had the peak *Plasmodium* density. This indicated a 1-month lag period after the rains.

The *Plasmodium spp* parasite mean densities at 95% CI was 4,840 (250 – 18,000) parasites/µl of blood. Males had a higher mean density of 6,160 (300 – 18,000) parasites/µl of blood than women who had a mean density of 4,125 (250 – 15,000) parasites/µl of blood. Rosterman mines had a

Plasmodium mean density of 4,190 parasites/ µl of blood, lower than Eluche which had a mean of 4,975 parasites/ µl of blood. Of the 214 *Plasmodium* infections recorded, 59.81% (128/214) had a light infection, 32.71% (70/214) had a moderate infection and 7.48% (16/214) had a heavy infection.

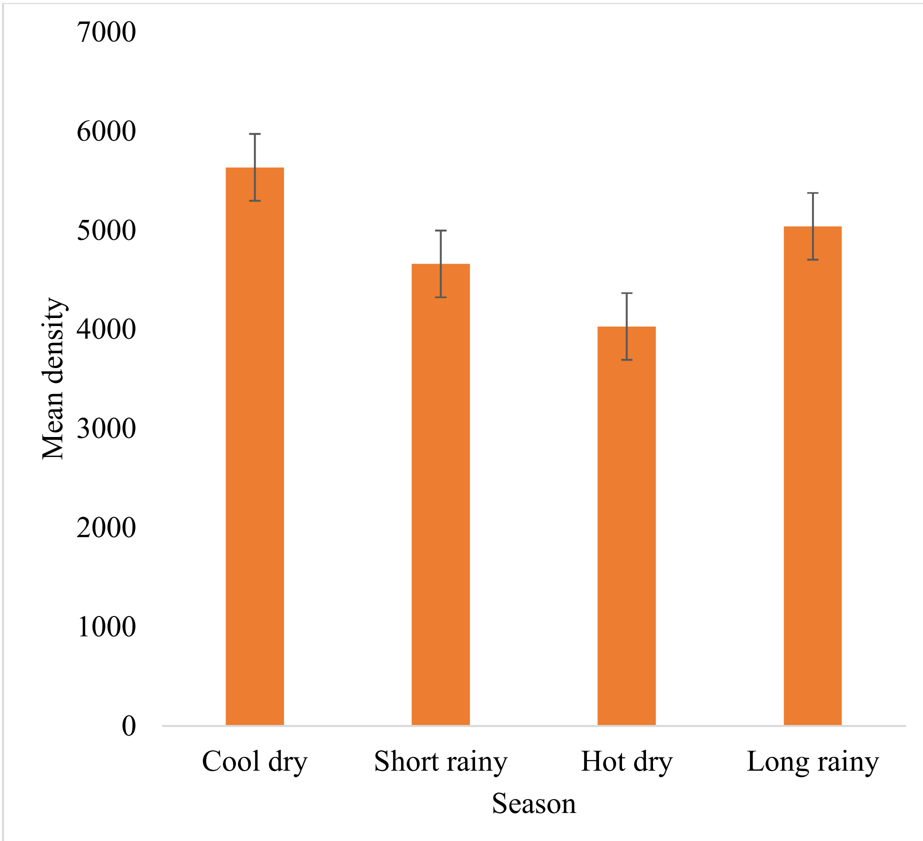

Figure 10: Seasonal *Plasmodium spp* mean densities for Rosterman and Eluche

4.2.5 Malaria Parasite Diversity

By microscopy, results indicated that infection by *P. falciparum* was 99% of the 214 positive samples, while one slide collected in August (cool dry season) in Eluche showed mixed infection of *P falciparum* and *P. malariae*. Of the 139 DBSs from asymptomatic participants who tested negative by m-RDT and microscopy were analysed for species diversity by PCR, in which 53.96% (n = 75) tested positive for *Plasmodium* parasites. The result of PCR, indicated a higher sensitivity that enable it to detect submicroscopic *Plasmodium* infection. It is therefore, crucial to say that PCR was far much more sensitive compared to microscopy and m-RDT.

4.3 Risk Factors Associated with Malaria Transmission in the Two Study Sites

In the two rural study sites, a number of risk factors exist that put the general population at risk of malaria transmission.

4.3.1 Socio-demographic Characteristics of Participants

The socio-demographic variables were distributed among participants as shown in Table 4. Of the participants in both FGDs, interviews and filled the questionnaires, 56.5% of the cumulative total were female, 47.0% were aged between 20 – 39 years, 70.2% were married, 39.8% attained post-primary education, 32.5% were in formal and informal employment, and 15.3% earned KShs 5,000 or more per month.

Table 4: Distribution of socio-demographic characteristics of the heads of the households surveyed

Variable	category	Site				Cumulative total	
		Rosterman		Eluche		N	%
		N	%	N	%		
Gender	Male	191	50.3	129	36.2	320	43.5
	Female	189	49.7	227	63.8	416	56.5
Age (years)	≤ 19	46	12.1	35	9.8	81	11.0

	20 – 39	180	47.4	166	46.6	346	47.0
	40 – 59	97	25.5	95	26.7	192	26.1
	≥ 60	57	15.0	60	16.9	117	15.9
Marital status	Married	256	67.4	261	73.3	517	70.2
	Unmarried	74	19.5	49	13.8	123	16.7
	Widowed	43	11.3	44	12.4	87	11.8
	Divorced	7	1.8	2	0.6	9	1.2
Level of education	No education	48	12.6	62	17.4	110	14.9
	Primary	160	42.1	173	48.6	333	45.2
	Secondary	129	33.9	101	28.4	230	31.2
	Tertiary	43	11.3	20	5.6	63	8.6
Main occupation	Self-employed in agriculture	72	8.9	91	25.6	163	22.1
	Self-employed in business	85	22.4	57	16.0	142	19.3
	Employed	82	21.6	37	10.4	119	16.2
	Housewife	101	26.6	138	38.8	239	32.5
	Unemployed	40	10.5	33	9.3	73	9.9
Average monthly income (Ksh)	≤ 2,000	204	53.7	171	48.0	375	51.0
	2,000 – 5,000	111	29.2	138	38.8	249	33.8
	5,000 – 10,000	23	6.1	18	5.1	41	5.6
	> 10,000	41	10.8	17	4.8	58	7.9
	N/A	1	0.3	12	3.4	13	1.8

4.3.2 Knowledge of the Head of the Households about Malaria

To determine the knowledge, attitudes and practices (KAP) of the head of the household on malaria, questions on if they had heard of malaria, source of information about malaria, cause of malaria, mode of transmission, methods of prevention, mosquito breeding sites, signs of malaria, and use of mosquito net were asked. Participants who gave at least three correct responses to the first five key questions were considered knowledgeable enough. Ninety-seven percent (n = 719) of the total respondents admitted to having heard, read, or seen information about malaria as shown in Table 5. The radio/television (58.8%) was the most reported source of the information, while other sources (3.9%) of information mentioned were schools, chief's announcements, churches/mosques, and reading materials. *Plasmodium* organisms (52.9%) were the most cited cause of malaria followed by germs (17.7%), bites by the mosquito (17.0%), and dirty stagnant water (4.8%) while 7.7% of respondents said they did not know the cause of malaria.

On the mode of malaria transmission (Table 5), 90.6% cited bites by mosquitoes, in which 31.8% correctly reported bites by a mosquito that had bitten a malaria patient. Bushes (55.0%) were the most cited mosquito breeding sites followed by others (water-holding containers, tanks) at 25.2%, tall grass at 12.6%, and stagnant water at 7.2%.

Over 95% of the respondents were able to identify at least three symptoms of malaria. They correctly mentioned shivering, headache, sweating, vomiting (n = 5, cited vomiting yellow phlegm), loss of appetite, joint pains, high fever, and feeling cold

Table 5: Respondents' knowledge of the cause and transmission of malaria and breeding sites of the vector

Variable	Response	N	%
Heard of malaria	Yes	719	97.7
	No	17	2.3
Source of information	Home	98	13.3
	Radio/Television	433	58.8
	Health center	51	6.9
	Health workers	27	3.7
	Suffered from malaria	98	13.3
	Others	29	3.9
Cause of malaria	Germs	130	17.7
	Dirty stagnant water	35	4.8
	Mosquito bites	125	17.0

	Plasmodium organisms	389	52.9
	Do not know	57	7.7
Malaria transmission	By bites of any mosquito	403	54.8
	Bites of mosquito which has bitten a malaria patient	234	31.8
	Others	46	6.3
	Do not know	53	7.2
Mosquito breeding sites	Stagnant water	53	7.2
	Tall grass	93	12.6
	Bushes	405	55.0
	Others	185	25.2

In the FGDs, it was reported that lack of proper information, education and communication (IEC) had made some segments of the people to believe that malaria is a disease of pregnant women and children.

4.3.3 Association Between Selected Socio-demographic Variables and Knowledge of Malaria, Bed net use, and Environmental Management in the Two Study Sites

When the socio-demographic characteristics of the participants were associated with the household heads' knowledge of malaria, the bed net, and environmental management. The results were as shown in Table 6. The socio-demographic variables had no significant association with the knowledge of malaria by the heads of the households.

Table 6: Association between selected socio-demographic factors and knowledge of malaria

Variables	N	Odds Ratio	95% CI	*p-value*
Factors associated with knowledge of malaria				
Gender				
Male	320	0.782	0.518- 9.212	0.287
Female	416	-0.782	0.109-1.929	0.287

Education				
No education	110	17.205	0.000	0.848
Primary	333	16.467	0.000	0.996
Secondary	230	0.000	0.000	0.997
Tertiary qualifications	63	1.000	0.000	0.837

4.3.4 Mosquito Breeding Sites

Availability and proximity of the breeding sites for the mosquito to residential houses was a risk that was assessed. The study found that the distance of the houses from anopheline larval habitats had a median distance of 251 ± 50 m. Thirty-seven percent (37.88%) of the houses were less than 50 m from the nearest anopheline-positive larval habitats. The distance of the residential house from the nearest larval habitat was significantly associated with the risk of malaria transmission (OR = 0.1177, 95% CI = 0.1170 – 0.1864, p = 0.0001).

4.3.5 House Structure Characteristics as Risk factors

Characteristics in the house design contribute to the ability of the mosquito vectors to gain access into the house and bite the occupants and subsequently transmit the malaria parasite during a blood meal.

The results as represented in Table 7 showed that 78.13% of the houses were old. Mud-walled houses accounted for 92.76% of all the houses with grass thatch at 0.90%. A majority (59.33%) of the houses had eaves and other openings such as cracks. Thirty-seven percent (37.19%) had doors and windows without screens. Eight nine percent (89.55%) had more than one room with 56.41% of them with more than four sleepers at any one given time.

When the house structure characteristics were tested for association with the risk of malaria transmission, all of them had a significant association with the risk of malaria transmission. An individual in a grass-thatched house had a higher risk of malaria transmission than those in a tinroofed house (OR = 1.73, $p < 0.0001$). On the other hand, an individual in a single-room house was four times more likely to suffer from malaria than one in a house with more than one room (OR = 4.14, $p < 0.0001$). The number of sleepers was significantly associated with a history of malaria episodes (OR = 0.63, $p < 0.0001$).

Table 7: House structure as risk factor for malaria transmission (univariate analysis)

Risk factor	N	%	Odds Ratio (OR)	95% CI	*p* - value
House					
New	157	21.87	Ref		
Old	561	78.13	1.7280	1.3640 – 2.1870	0.0001
Wall					
Mud	666	92.76	Ref		
Concrete	52	7.24	0.0378	0.0273 – 0.0521	0.0001
Roof					
Grass	5	0.70	Ref		
Iron sheets	713	99.30	68.94	28.210 – 168.50	0.0001
Eaves					
Absent	292	40.67	Ref		
Present	426	59.33	0.7053	0.5685 – 0.8752	0.0001
Doors/windows					
With screen	451	62.81	Ref		
Without screen	267	37.19	0.2862	0.2303 – 0.3560	0.0001
Number of rooms					
1	75	10.45	Ref		
> 1	643	89.55	4.1450	3.1150 – 5.5150	0.0001
Number of occupants					
≤ 4	313	43.59	Ref		
> 4	405	56.41	0.6256	0.5047 – 0.7755	0.0001

4.3.6 Bed net Status

The bed net is the primary line of defense against malaria for most households. This subsection sought to find out the status and occurrence of malaria in the sampled households. Ninety-two percent (92%) of the households had a mosquito bed net. Of these nets, 33.84% had five or more holes and 12.64% were only hanged when it was time for sleep by the owner. The status of the bed net was significantly associated with the risk of malaria transmission (OR = 0.3254, 95% CI = 0.2598 – 0.4077, $p = 0.0001$).

4.3.7 Environmental Management

A proper environment was critical in curtailing mosquito breeding and resting sites. This section sought to find out the practices the residents used to reduce the risk of malaria transmission. The most notable environmental management practices mentioned were the removal of water-holding waste containers, draining stagnant water, clearing bushes around the houses, and cleaning the homesteads regularly.

However, participants observed that mosquitoes become more abundant when the maize crop was blooming. This was because some of them would cultivate their lands a few meters from their households. This data was important in highlighting the challenges individual households and the two communities underwent.

4.4 Malaria Control Measures Used in the Study Sites

The results from the determination of the malaria control measures practiced showed that the residents used different approaches to minimize the likelihood of transmission. This was attributable to good EIC by the village administration and CHVs.

4.4.1 Bed net Ownership and Use

The data on the ownership and use of the bed net by the residents is as presented in Figure 11. On bed net ownership in the sampled households, 97% of the families had the LLIN type. The net was bought, freely given by health campaigners in early 2018, or given at health centers during neonatal and postnatal care.

When probed if they used the bed net the previous night, 93.17% reported having used it while the rest did not. When asked why they did not use the net, they reported that they were out on duty. This information was corroborated in the FGDs where it was reported some community members worked at night.

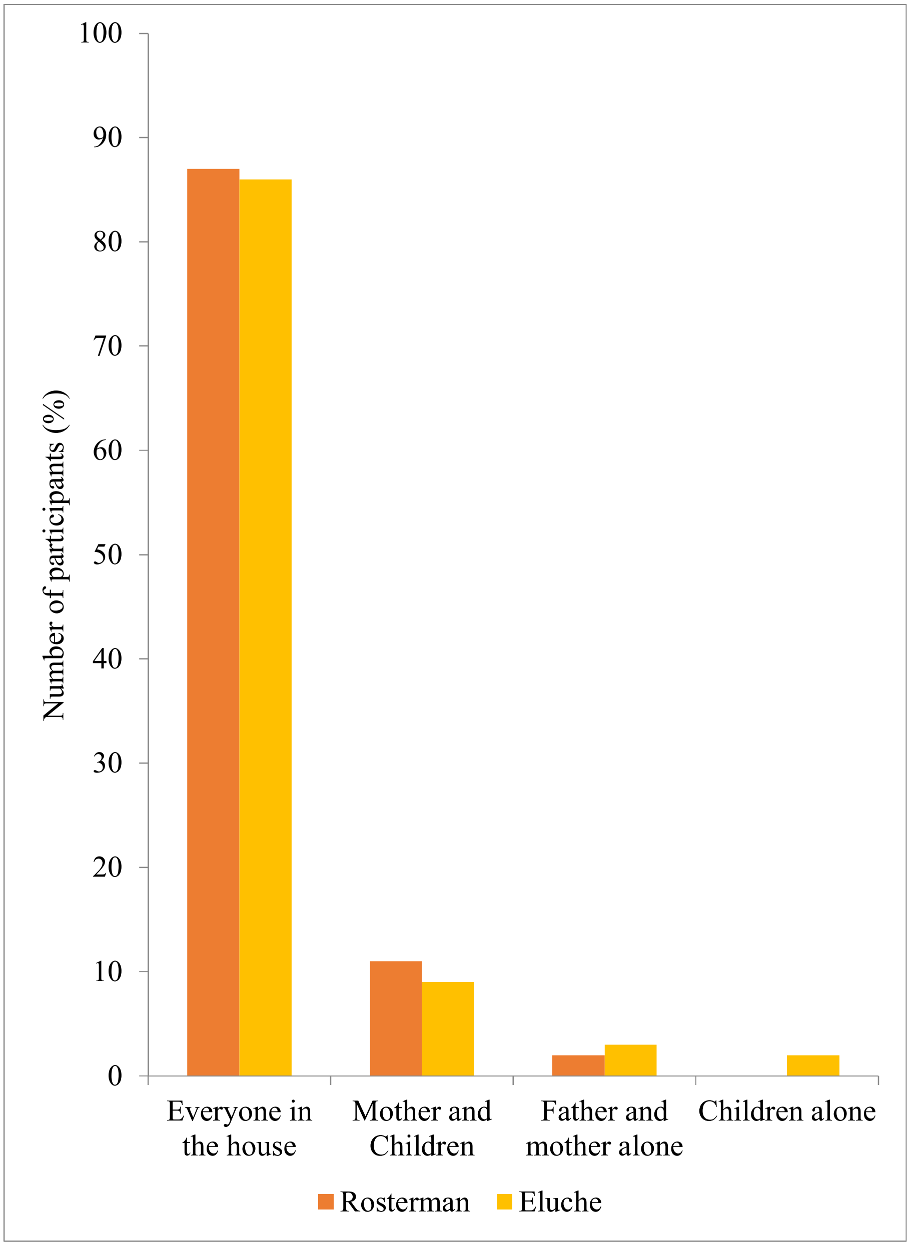

Figure 11: Respondents' bed net ownership

When selected socio-demographic characteristics of the respondents were associated with net ownership and use, the following results were obtained as shown in Table 8. The results showed that ownership and use of the bed net were not significantly associated with the households' heads' socio-demographic characteristics. Bed net ownership and use were not in any way determined by the age or education of the respondent.

Table 8: Association between selected socio-demographic factors and knowledge of the bed net

Variables **Factors associated with knowledge of bed nets**	N	Odds Ratio (OR)	95% CI	*p*-value
Age				
≤19	77	0.114	0.012-1.062	0.056
20-39	340	0.275	0.026-2.924	0.284
40-59	205	0.485	0.485-4.4745	0.534
≥60	114	1.000	0.031- 4.123	0.271
Education				
No education	110	1.311	0.127-13.498	0.820
Primary	333	1.166	0.138-9.824	0.888
Secondary	230	1.238	0.140-10.959	0.848
Tertiary qualifications	63	1.000	0.171- 9.628	0.812

4.4.2 Malaria Prevention Methods Preferred by the participants in the two Study Sites

The results obtained are as represented in Figure 12. The question on malaria prevention had sub-sections on malaria control at both household and environmental levels. A majority (87.2%) of those interviewed had proper knowledge of malaria prevention and control. The bed net (86.0%) was the most reported method, followed by treatment at 31.0%, using insecticide sprays at 12.0%, using mosquito coil at 6.5%, removing breeding and resting places of mosquitoes at 2.0%, and other methods at 1.0%. In the FGDs, it was reported that the villagers had good IEC about malaria prevention.

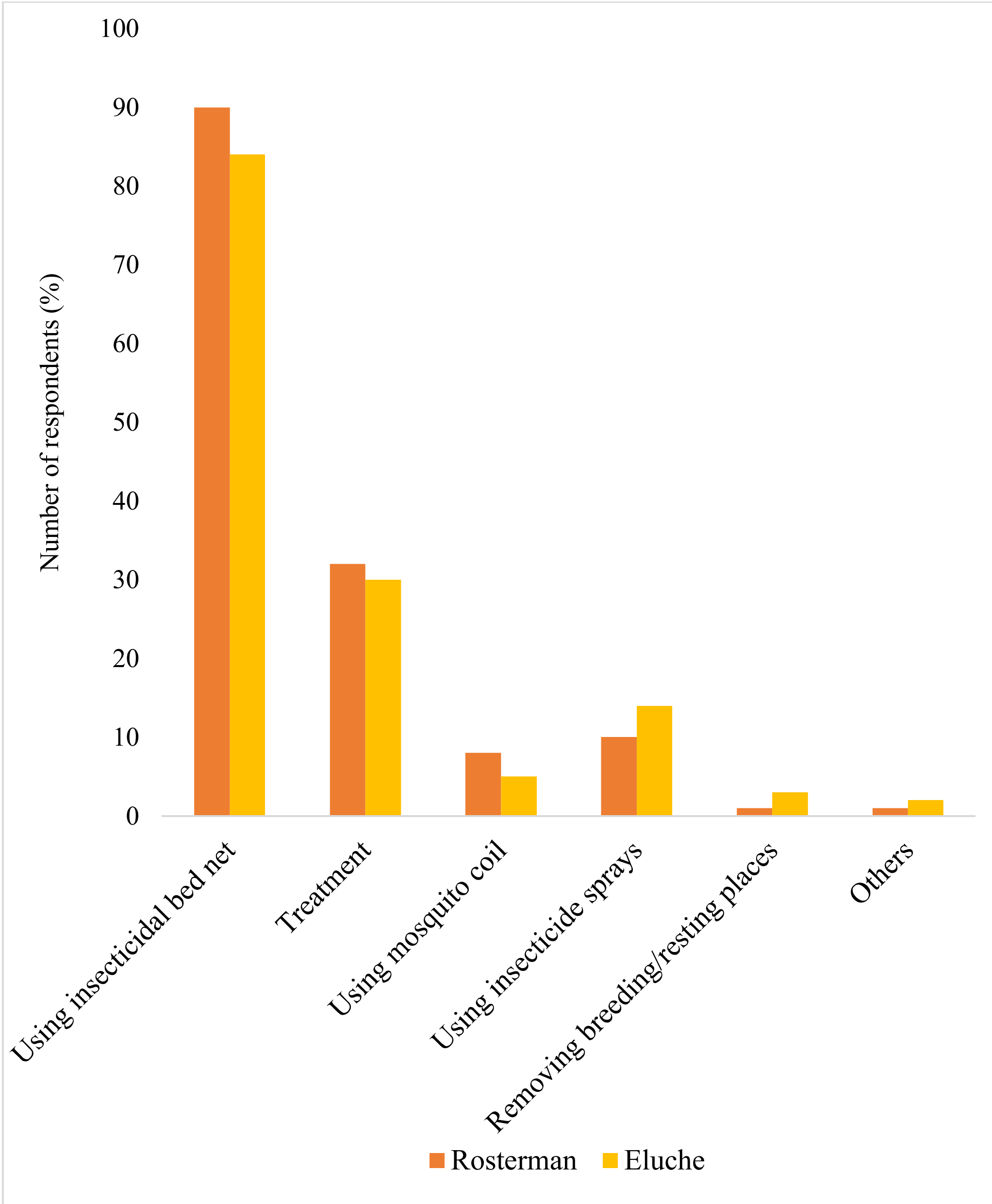

Figure 12: Respondents' communicated malaria control methods

4.4.3 Knowledge and Use of Environmental Management Methods

Proper environmental management activities greatly reduce the availability of the mosquito vector consequently decreasing the likelihood of transmission of malaria. This section reported the results of the environmental malaria prevention methods the participants knew and used to prevent them from contracting malaria through mosquito bites. These methods were used to reduce breeding and resting sites for the mosquito. The environmental practices applied are shown in Figure 13.

A cleaner environment was the most central point highlighted by the respondents. Draining stagnant water (55.4%) and removing water-holding containers remove likely breeding sites, while clearing bushes around the house (14.5%) and cleaning house surroundings (22.6%) remove the resting sites for the vector from around the houses. FGDs corroborated this information which was reinforced through EIC strategies.

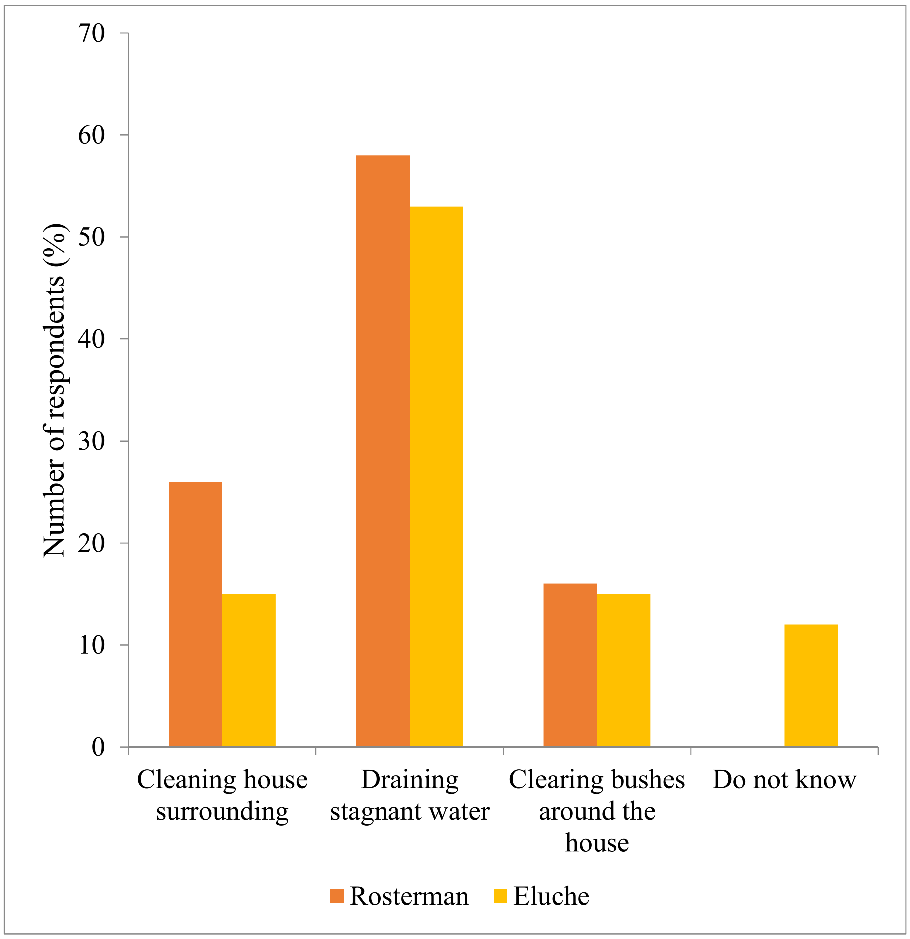

Figure 13: Known and used environmental management methods for malaria control

This section associated selected socio-demographic factors of respondents and their knowledge of environmental management. The results were as indicated in Table 9, in which neither gender nor level of education had any association with knowledge of environmental practices that households employed to prevent malaria transmission. This was a clear indicator that the participants had prior knowledge of the outdoor activities that were taken to control malaria despite their gender or level of education.

Table 9: Association between selected socio-demographic factors and knowledge of environmental management

Variable **Factors associated with environmental management**	N	Odds Ratio (OR)	95% CI	*p*-value
Gender				
Male	320	-0.015	0.555-1.750	0.959
Female	416	0.015	0.959-1.015	0.959
Education				
No education	110	1.930	0.868-54.680	0.068
Primary	333	1.699	0.730-41.013	0.098
Secondary	230	1.228	0.435-26.761	0.243
Tertiary qualifications	63	1.318	0.623-28.217	0.378

4.5 Treatment-seeking and Drug Use Behaviour by Participants

This study findings indicate that the participants sought prompt treatment whenever a household member had malaria. They use the prescribed anti-malarial drugs or would buy from the drugstores in the area. However, 22.96% of the respondents at times failed to comply with the instructions for the proper use of the drugs given/bought.

4.5.1 Action Taken when Household Member had Malaria Bout

This study found that household members were likely to contract malaria disease. The action taken by the heads of the particular households had a profound impact on treatment success. The study found that treatment sought by a member of a household was based on knowledge of malaria symptoms by the household head. The onset of any one or more of the reported signs and symptoms of shivering, headache, sweating, vomiting, loss of appetite, joint pains, high fever, and feeling cold prompted respondents to seek medical assistance or buy anti-malarial drugs. The actions taken when a member of the household fell sick were as shown in Figure 14. Going to a health facility was the most preferred action followed by self-treatment while seeking traditional healers and no treatment received negligible responses.

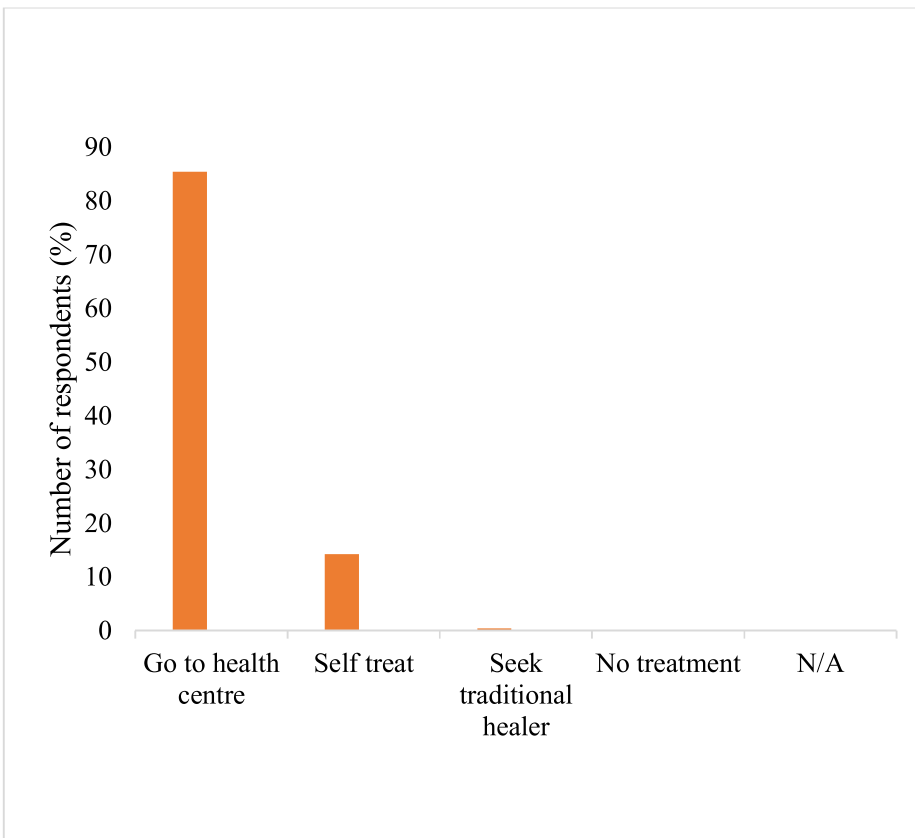

Figure 14: Action taken when members of households had malaria bout

The onset of malaria signs and symptoms prompted some to seek medication immediately, but others would wait for a day or two before going for treatment. This formed a born of contention during the FGDs in both Rosterman gold mines and Eluche sites.

FGDs reported that differences in the time of seeking medication were because of the economic situation of the household, distance to the health center, and availability of antimalarial drugs in the health facilities.

However, 86% of the participants preferred going to the health center because the diagnosis was performed on the sick first before drugs were prescribed. This raised the likelihood of successful malaria treatment and not any other ailment that had similar signs and symptoms as malaria (such as typhoid and brucellosis). The unavailability of anti-malarial drugs in government medical facilities was highly mentioned.

Figure 15 shows the drugs that were prescribed or bought from drug stores. AL was the main drug that patients were given at health facilities or bought from drug stores. AL and Panadol/Paracetamol were the most preferred combination (90.19%) followed by AL and Brufen (Ibuprofen) (6.81%). It was reported in all FGDs that AL was the most available antimalarial drug and that the most dispensed drug combination was AL and a pain killer.

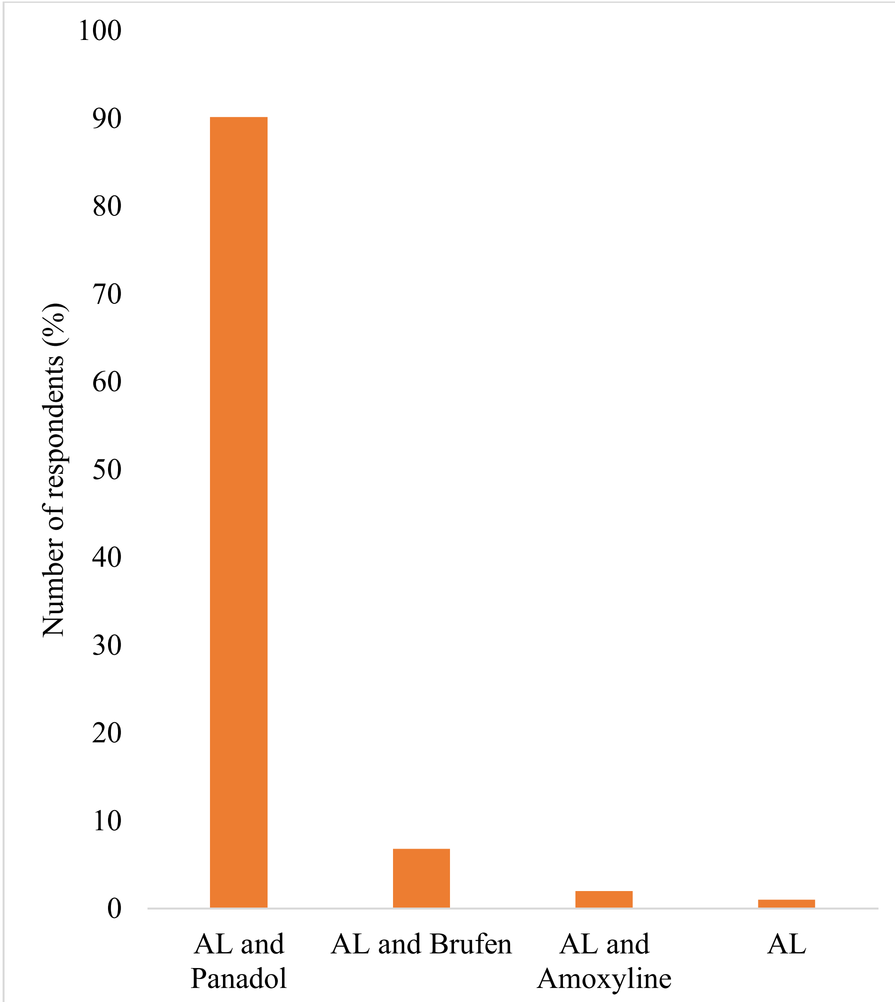

Figure 15: Drug used when one had malaria

4.5.2 Anti-malarial Drugs Compliance

A good portion (22.96%) of the respondents reported having failed to complete the prescribed AL dosage giving a range of reasons as indicated in Figure 16. Having recovered from a malaria bout (79.14%) was identified by most participants as the main reason for failure to complete the dose. While cases of development of allergy towards the AL were minimally mentioned at 0.5%.

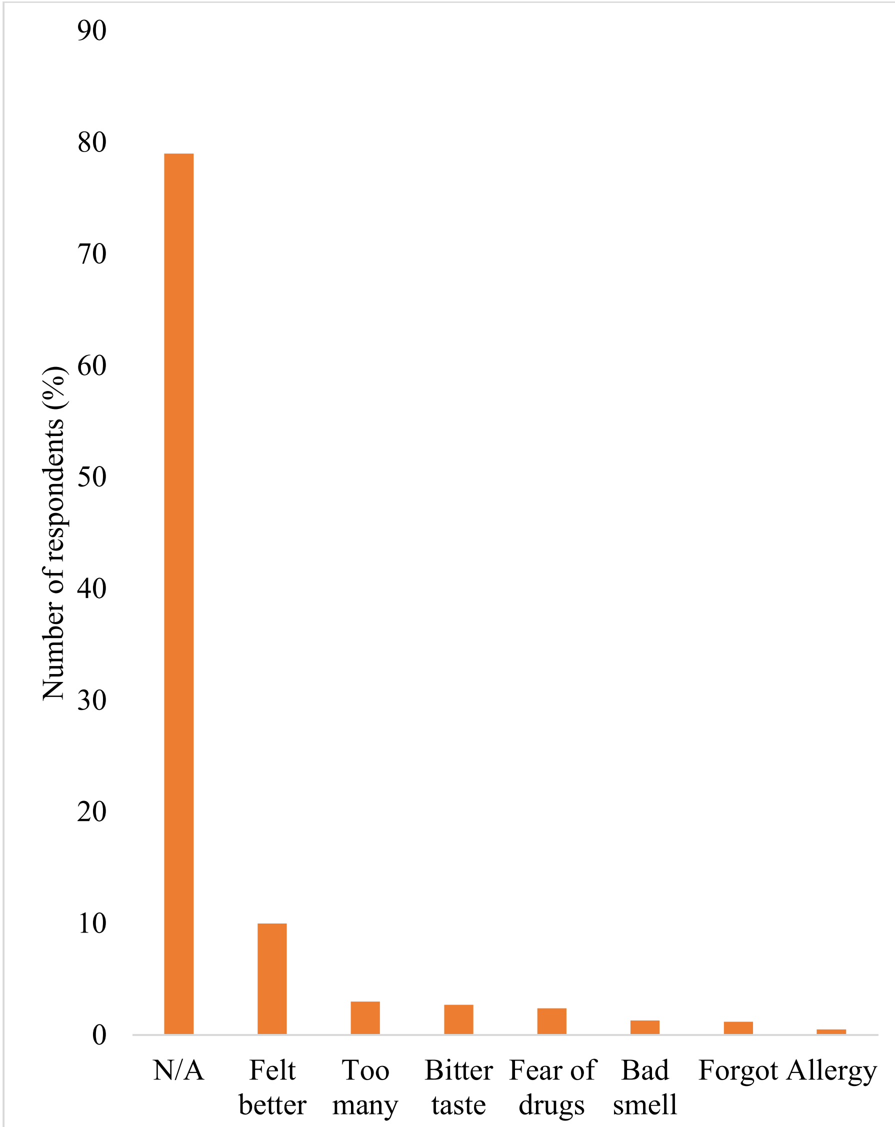

Figure 16: Reason given for not completing the antimalarial drug dose

Figure 17: shows the reported uses of the remaining part of the dose. The remainder of AL being re-used (9.80%) and being thrown away (8.84%) were the most stated reasons. The finding show that a segment of the population did not comply with antimalarial drug regimen as prescribed medically.

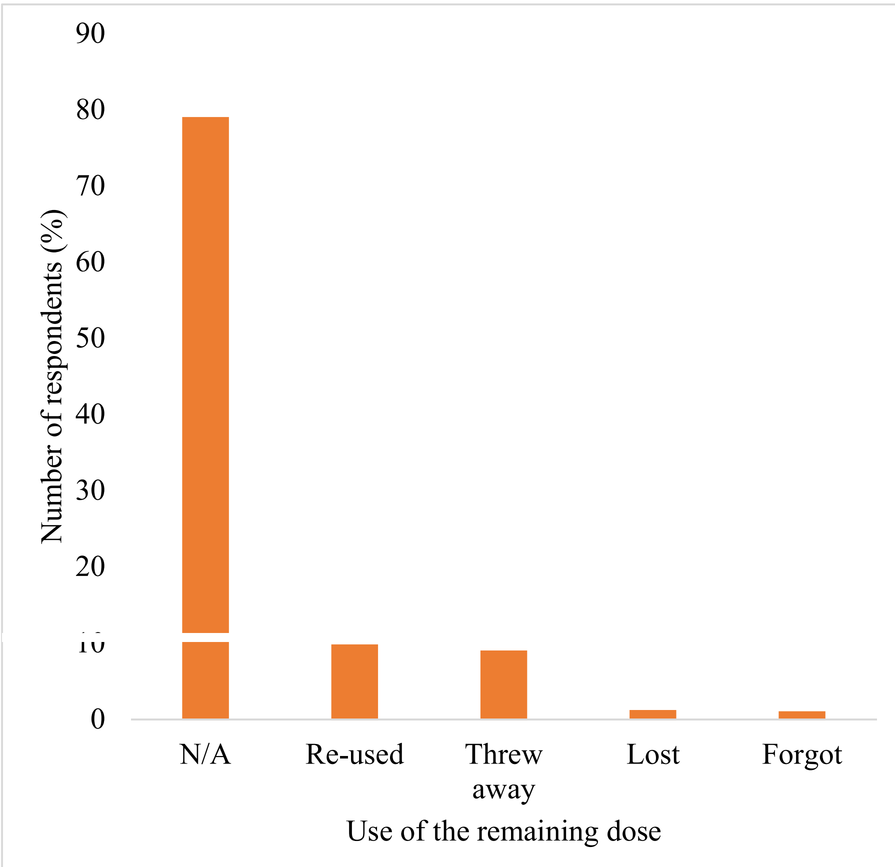

Figure 17: Use of the remaining antimalarial drug dose

The study reported that there was a trend in which some respondents to re-use some of the antimalarial drugs dosage having failed to complete the initial dose that was prescribed to them. However, there was no significant association at 95% CI, $p \leq 0.05$, between selected socio-demographic factors and knowledge of anti-malarial drug use, as shown in Table 10. Proper drug compliance was not dependent on gender, level of education nor occupation.

Table 10: Association between selected demographic variables household response to malaria and drug use

Variable	N	Odds Ratio (OR)	95 % CI		p-value
Action taken when having malaria			Lower	Upper	
Gender					
Female	416	1.195	0.791	1.805	0.397
Male	320	1.026	0.966	1.090	0.459
Level of education					
Educated	626	0.908	0.512	1.609	0.887
Not educated	110	0.987	0.913	1.067	0.741
Failure to complete the dose					
Gender					
Female	98	0.929	0.656	1.315	0.678
Male	71	0.983	0.908	1.064	0.724
Occupation					
Employed	25	0.855	0.392	1.863	0.693
Not employed	144	0.984	0.903	1.072	0.409
Use of the remaining dose					
Level of education					
Educated	65	0.530	0.237	1.186	0.112
Not educated	112	0.950	0.902	1.002	0.131
Occupation					
Employed	64	0.855	0.392	1.863	0.693
Unemployed	105	0.984	0.903	1.072	0.676

CHAPTER FIVE

DISCUSSION

The findings of this study show that the two socio-economic activities studied – artisanal gold mining and sugarcane farming modify the environment in the Rosterman gold mines and Eluche areas of Western Kenya. Thus they play a critical role in sustaining malaria transmission. The diverse mosquito breeding sites showed that the areas were conducive for *Anopheles* vector proliferation. The availability of adult female *Anopheles* within residential houses showed that there was transmission in the areas in site of the instituted malaria prevention interventions. The malaria cases that were reported and the high prevalence of *P. falciparum* in circulation indicate that despite concerted and up-scaled malaria control interventions and EIC, malaria was still a major public health concern. It was with this in mind that this study suggested that policy implementers should inform the people in the two villages that in as much as they go about their socio-economic activities such as gold mining and sugarcane farming among others, they needed to take great care of themselves and their loved ones not to contract malaria. Falling sick with malaria resulted in immense loss of man-hours that would have been used in productive activities to improve their socio-economic standing.

5.1 Abundance and Diversity of Malaria Vector

This study showed that malaria vectors were abundant and diverse in the study ecosystems all year round. This was attributed to the availability of breeding and resting places for the *Anopheles* mosquitoes. Spatio-temporal abundance, distribution, and diversity could be used to elucidate a plausible index to the sustenance of the recorded malaria transmission. This information is crucial because it identified the two sites as possible hotspots for malaria resurgence in the Western Kenya. And therefore called for a re-evaluation of the existing control intervention and/or shoring up the existing malaria prevention methods.

The higher proportion of the diverse habitats available in the study sites were categorized broadly into natural and human-made habitats. Human-made mosquito breeding habitats associated with gold mining and farming activities were the most widespread and the most productive. Their abundance and productivity could be associated with the abundant mosquito vectors that maintain malaria transmission. This study finding is consistent with other studies in four sub-counties of Kakamega county and Nyabondo (Ng'ang'a *et al*., 2019;), both endemic areas of Western Kenya, hypoendemic rural Bandarban, Bangladesh and Jazan region, Saudi Arabia (Alam *et al*., 2018), in which artificial habitats were the most diverse and most productive, the aquatic habitat characteristics notwithstanding.

However, a study conducted elsewhere in Kakamega County and Southern Ghana found that there was significant variability in the distribution of aquatic habitats across regions and seasons (Mattah, *et al*., 2017; Nicholas *et al*., 2021). This was contradictory to the finding of this current study in which there was marginal variation in the nature and distribution of the breeding sites. This was attributable to the nature of the socio-economic activities of these two study sites. Proper knowledge of mosquito breeding sites would go a long way in understanding the best environmental practices by the locals in the two villages and others in areas with similar settings that will mitigate malaria infection.

The ability of the *Anopheles* vector to locate an aquatic site and oviposit to start its life cycle ensured availability mosquito vector. The anopheline larval stages were abundantly present in the aquatic habitats sampled all year round, regardless of the availability or absence of raint. This was an indication that the two sites had a greater potential for sustaining the transmission of malaria. Elsewhere, studies have shown that the availability of aquatic habitats sustained the mosquito vector, based on environmental and climatic predictors. Additionally, agro-systems have been shown to contribute to a higher risk of malaria transmission (Wanjala & Kweka, 2016; Mattah *et al*., 2017; Eba *et al*., 2021) and in which mosquitoes clustered around riverine ecosystems. The implication of this availability of the developmental stages was that either environmental management malaria control strategies were not bearing fruit or the socio-economic activities of the people in the two study sites over-rode effects of malaria.

The land modification processes in both study sites could have played a significant role in the maintenance of the vector through numbers. Human-made breeding sites including mines, ditches, and fish ponds were the most productive. Studies in Saudi Arabia and Bangladesh found that human-made and artificial aquatic habitats were productive for *An. arabiensis*, thereby sustaining vector abundance and consequently malaria transmission (Al-Sheik, 2011; Alam *et al*., 2018). Illegal gold mining that results in the proliferation of mosquito breeding sites has been significantly associated with malaria, as was the case in Bolivar and Cordoba in Colombia and The Philippines by the creation of pools of stagnant water which favour the reproductive ecology of the mosquitoes (Castellanos *et al*., 2016; Pagel, 2022). Irrigated sugarcane farming in Ethiopia was also associated with increased malaria cases through an increased population of the vectors (Demissew *et al*., 2020).

These findings further highlight what other studies found that other human activities such as agro-systems, land use types and artificial forests, brick making and deforested margins modified the microclimate for breeding of mosquitoes (Naranjo-Díaz *et al*., 2014; Ng'ang'a *et al*., 2019; Eba *et al*., 2021; Nicholas *et al*., 2021). Therefore, changes in the microclimate of the mosquito breeding sites through land-modifying activities such as artisanal gold mining and sugarcane farming increased the abundance and diversity of the malaria vector.

The information presented here will form an important component of the malaria epidemiological study because it makes available potential drivers that lead to the presence of the principal vector in the two rural villages in Western Kenya. Additionally, this information will guide on areas where malaria control strategies need to be channeled. Vector control should target more larval source management and larviciding and the reduction of resting sites for adult mosquitoes.

The study findings showed that the adult mosquito vector was reported indoors all year round and that the adults had higher densities the following sampling period after a previous rainy month. For instance, the months of March to May had higher monthly rainfall recorded. This was followed by increased adult densities in June and July. This mosquito lag phase after rains had been reported elsewhere in other studies such as in Mali and Guangdong, China, in which perhaps battering of the immature *Anopheles* by torrential rain or being washed away by surface run-off reduced the number that emerged (Magombedze *et al*., 2018; Wang *et al*., 2019). Strong winds would blow the vulnerable adults to long distances thereby, either increased or reduced the mosquito vector numbers (Dunphy, 2019). This could be the reason the mosquitoes were abundant in the two study sites throughout the study period.

Adult female *Anopheles gambiae* s.l. vectors were sampled in peoples' residential houses in reasonably high densities. This group of vectors posed the greatest danger to humanity regarding the residual transmission of malaria. It implied that the houses permitted entry of mosquitoes or that the mosquitoes had devised mechanisms of gaining access to the houses to bite the occupants. Generally, mosquitoes have been documented to bite from dusk to dawn. The high numbers caught by both PSC and light traps indoors illustrated that the inhabitants of Rosterman gold mines and Eluche were at higher risk of being bitten while indoors. This finding supported other studies conducted in Marani and Iguhu, Western Kenya in which indoor humidity could have been the driving factor, (Ndenga *et al*., 2006; Omukunda *et al*., 2013) and another study in Antula, Guinea Bissau which found that indoor collected mosquitoes were higher during the drier transmission season than rainy season (Pålsson *et al*., 2004). Unless the occupants of the houses in which the adult female *Anopheles* were collected practiced indoor malaria intervention methods, they were at a higher risk of contracting malaria infection through bites by infected mosquitoes.

This study's findings showed that the study sites had abundant and diverse mosquito vectors that sustained malaria transmission. *An. gambiae*, *An. arabiensis* and *An. funestus* were the most abundant vector species, while *An. coustani* which was categorized as a secondary vector, was also found in the study sites. These anopheline species have been implicated in enhanced malaria transmission. Studies by Nicholas *et al*. (2021) and Killeen *et al*. (2016) found that the three species were also the most abundant malaria vector species in western Kenya. This increased abundance of *An. funestus* is attributable to its breeding habits which transcend the rainy season. So it is possible to persist beyond this period and be available throughout the year (Sinka *et al*., 2010). This supported finding by by Zhou *et al*., (2016) which observed that the introduction of ITN uses in Kenya, had led to a fall in *An. gambiae* but led to an increased abundance of *An. funestus* in Western Kenya. This was due to vector developing resistance to insectides that impregnate ITNs. While Abong'o *et al*., (2020) indicated that by use of light traps indoors, a higher proportion of *An. funestus* were collected in malaria endemic Migori, L. Victoria basin and that IRS substantially reduced *An. funestus* numbers in the same study area. The role of the other malaria vectors, *An. coustani* inclusive should not be overlooked when instituting malaria intervention practices because of their substantial proportions in the study sites. The most common malaria control practice was the use of ITNs/LLINs which target indoor resting/foraging mosquitoes. Much more has to be done if the intended malaria elimination targeted by 2030 has to be achieved in Kenya and the other 14 countries in the WHO Africa malaria region (World Health Organization, 2020).

Relating female *Anopheles* abundance to precipitation is significant in making near-future predictions about malaria occurrence and hence offers seasonal rapid response to avert fatalities. Additionally, knowledge of how *Anopheles* survived long non-rainy seasons to emerge again during the rainy season to continue transmitting malaria was crucial in knowing when to target mosquito residual breeding sites and adoption of control measures such as IRS and the use of repellents.

5.2 Prevalence and Diversity of *Plasmodium spp* among Residents of Rosterman and Eluche

Findings of this study reported that malaria was still a serious public health concern for the people of Rosterman mines and Eluche. *P. falciparum* was the most prevalent malaria parasite species in the two areas. Results showed that children under 5 years had lower malaria cases than people above 12 years of age. People sought treatment from health facilities whenever they had a malaria bout in their household, on the assumption that they were bound to get proper diagnosis and treatment. The findings call upon relevant stakeholders to review the existing malaria prevention means as exemplified below.

Results of the analysis of blood revealed that the malaria prevalence was 8.96% by m-RDT and 9.25% by both microscopy and PCR. This confirmed that malaria prevalence was high. The malaria infections were reported in all the four seasons with the rainy season showing a significant rise in malaria prevalence in both study sites. These malaria prevalence rates were lower than that of studies done in various parts of Kenya including Baringo, Iguhu, Emutete and Marani, in children between 6 – 14 months in Busia and Bungoma (Wanjala & Kweka, 2016; Omondi, 2017; Bashir *et al*., 2019), in asymptomatic pregnant women in Kakamega county and in healthy pregnant women attending ANC in Kinshasa, DR Congo (Matangila *et al*., 2014; Waiswa *et al*., 2022). The malaria prevalence rates in Rosterman mines and Eluche were higher than the Kenya national average rate of 8% and the results of another study on pregnant women attending a teaching hospital in Nigeria (Isah *et al*., 2011; KMIS, 2020). Therefore, it is worthy reporting that it would be better if the two areas were considered for enhanced malaria prevention EIC.

This study observed that males were more at the risk of infection with malaria than women in the study sites. This attributed to the kinds of socio-economic activities of artisanal gold mining and sugarcane farming that they were mainly engaged in more than their female counterparts. From the questionnaire, it was observed that more females filled out the questionnaires than males. This implied more females were found in the homesteads than males because of the call of work. This occurrence had earlier been observed in other studies in a rural setting in the equatorial rainforest of the South West region of Cameroon, Burkina Faso, Assam, India and the University of Gondar specialized Referral hospital, Ethiopia (Yadav *et al*., 2014; Guglielmo *et al*., 2021; Nyasa *et al*., 2021; Mulugeta *et al*., 2022). The reason fronted for the higher malaria prevalence in males was, that they frequently stayed out carrying out more outdoor activities, and also left their homes very early in the morning before dawn to tend to farms. However, other studies have disputed this and reported that females were more prone to malaria than males. Such are studies in Kisumu county, Kenya, public health facilities in Uganda, an urban setting in Ghana (Otieno, 2015; Quaresima *et al*., 2021; Okiring *et al*., 2022) because women were exposed to the vector bite during the most dangerous hours, at dusk. Malaria studies have labeled females as one of the vulnerable groups. In these two settings, it would be in order to consider the males as the group of concern. The finding of this study that malaria prevalence was high, called for concerted efforts that will reduce transmission of the parasite within the communities in the two study sites.

This study found that prevalence of *Plasmodium spp* parasite was not dependent on age but a random occurrence within the asymptomatic population of the two sites. This indicated a varying degree of dynamics of transmission. A study in Iguhu, Kakamega County, Kenya reported that malaria parasite prevalence reduced proportionately with age. This was attributed to the acquisition of corresponding immunity as age increased. The occurrence of *Plasmodium spp* in asymptomatic members of a study population has been reported and in school children in western Kenya (Kepha *et al.*, 2016; Wanjala & Kweka, 2016; Oduma *et al*., 2023) The inability of the observed *Plasmodium spp* presence to develop into full-fledged malaria is a concern in the desire to reduce malaria transmission through prompt and proper treatment.

This study reported varying *Plasmodium spp* infection intensities across sites, sex, and seasons. Eluche sugarcane site had a higher *Plasmodium spp* intensity than Rosterman. On the other hand, males in the study sites had higher *Plasmodium spp* intensity than the females and the cool dry season recorded the highest *Plasmodium spp* intensity compared to any other season. The reason for the higher *Plasmodium spp* intensity in Eluche could be related to unstable malaria transmission that has been reported in this area or perhaps that the population in Rosterman was faced with a stable transmission pattern that could have increased a herd immunity that limited full-blown disease.

One study showed that low *Plasmodium spp* intensities could be due to the development of immunity against the malaria parasite, consequently reducing the development of the disease within stable transmission areas and various populations in different areas were exposed differently to the malaria vector (Wanjala & Kweka, 2016). Studies on *Plasmodium spp* intensities can be used as malaria epidemic prediction measures that can inform authorities to institute relevant control measures to avert epidemics. The prevalence of asymptomatic malaria infections could act as a reservoir segment that maintains transmission within communities. Therefore, they could act as major obstacles to malaria control (Oduma *et al*., 2023). Researchers have to re-think malaria prevention methods that target the asymptomatic cases in the population to stem transmission.

This study reported that the most prevalent malaria parasite was *P. falciparum*. This corroborated other findings in the same Western Kenya but different sites that *P. falciparum* was the prominent cause of malaria in the region. Studies in pregnant women attending various ANC centers in Kakamega county, pregnant women attending Bungoma county hospital, Iguhu, Kakamega county, in school children in Baringo, (Omondi, 2017; Antony *et al*., 2019; Waiswa *et al*., 2022) showed that *P. falciparum* was the most prevalent malaria parasite species. Mixed infection by *P. falciparum* and *P. malariae* was reported in a respondent in Eluche. This supported the findings of other studies in which mixed infections were also reported. It was crucial to continue targeting *P. falciparum* in malaria control through treatment by the use of ACTs Wanyonyi *et al*., 2018; Antony *et al*., 2019; Waiswa *et al*., 2022). Additionally, the focus should be on the role of mixed infections in the population of the two areas.

5.3 Risk Factors Associated with Malaria Transmission in the Two Study Sites

This study findings indicate that the socio-demographic factors of the study participants had significant association with some risky predisposing factors to malaria transmission. Many studies have highlighted the need to continuously assess risk factors that predispose to malaria infections.

Knowledge, attitudes, and practices (KAPs) of the people in the two study sites were investigated against their socio-demographic characteristics. Females, people aged below 40 years, married, with at least a primary school level of education, employed, and earning below KShs. 5,000 monthly constituted the greater percentage of the respondents in the study sites. This was supported by other studies elsewhere, in which women are usually in direct control of households and play a significant role in determining the preventive mechanism used, reading the clinical signs in the children and acquisition of medical assistance (Bashar *et al*., 2012; , Thomas *et al*., 2018; Ramdzan *et al*., 2020). Formal education and occupation of the household head were also key determinants in having proper KAPs about malaria. This was in concurrence with studies in Kakamega county, Kenya (Kepha *et al*., 2016; Mukabane *et al*., 2022), in South Africa where individuals with low economic status had suffered from malaria in the past, the Democratic Republic of Congo (DRC) (Mutegeki *et al*., 2017; Ngatu *et al*., 2019), which showed a similar trend in which people in dire economic status and rural areas were at a higher risk of getting infected than other groups.

Findings in other studies on children in Enugu, Nigeria showed that malaria episodes reduced significantly with the improved socio-economic status of the household (Uzochukwu *et al*., 2018; Ugwu & Zewotir, 2020). On the contrary, a study in India found that there was no significant association between socio-demographic characteristics and malaria transmission (Thomas *et al*., 2018). The findings of this study in support of others averred that socio-demographic factors can be used as indices for curtailing the transmission of malaria in the probable hotspots such as Rosterman mines and Eluche sugarcane growing ecosystems. As governments of various countries in SSA strive to eliminate malaria, they should strive to better the economic status of their citizens. An economically empowered population will protect itself against malaria and other diseases.

It was evident that study participants had heard of malaria from different sources including mass media, school curriculum, CHPs and administrators. The study reported that the gender, age, and marital status of the respondents had no significant association with the knowledge of malaria, the method of prevention, and the control strategy applied by the respondent. This is a vindication that knowledge and information about malaria were available to everyone regardless of their socio-economic status. The choice of the malaria preventive method used was equally not dependent on the socio-economic standing of the respondent. A study in Zambia and other one showed that variations in demographic variables raised the risk of malaria transmission by contributing to weakness in program interventions (Yadav *et al*., 2014; Nawa *et al*., 2019; ngatu *et al*., 2019).

Prior information from diverse sources enhanced the understanding of malaria as a disease of greater public concern. The people in the study sites had proper prior EIC and therefore were not disadvantaged. However, they cited that they were not involved in the formulation of policies about malaria control, but were only recipients. It had been reported that people in rural settings were at a higher risk of getting infected than other groups (Ngatu *et al*., 2019; Abubakar *et al*., 2018). The Association of the mosquito with malaria transmission by the responders enabled them to institute personal and communal control practices that reduced the likelihood of contracting the disease. This concurs with a study in a village in South Africa, in which having past experience with mosquitoes transmitting malaria, led to the affected implementing an appropriate control strategy (Mutegeki *et al*., 2017). Proper EIC about malaria is vital in understanding the preventive method to use in different settings.

The proportion of houses within a range of 50 m to the nearest mosquito larval habitats was high. This close proximity of residential houses to mosquito breeding site may have enabled the newly emerged vector to get to human blood meal source easily. This provided a significant risk to the residents of Rosterman and Eluche. The distance of residential houses from larval habitats played a major role in enabling the mosquito vector to access houses and thus sustain transmission. Studies in Kilifi, Kenya, in Morogoro and Dodoma; Tanzania, Yaoundé, Cameroon and in Southwest Sumba, Indonesia (Nixon *et al*., 2014; Mathania *et al*., 2020; Ngadjeu *et al*., 2020) have shown that the residential houses close to mosquito breeding sites heightened the risk of spread of malaria. This was principally by enabling the female *Anopheles* to readily get a blood meal from occupants of the nearest residential house before ovipositing its eggs in the nearest water habitat. However, Contrary to this finding, a study in India reported that availability and proximity to larval habitat was not a guarantee that the household would have malaria bouts more often than those far away from such habitats (Thomas *et al*., 2018). Knowledge of the risk posed by the proximity of a residential house to breeding sites for mosquitoes would significantly help in targeting immature forms of the vector in instituting malaria control strategies by policy makers.

House structure factors had a significant association with the risk of malaria transmission. Occupants in old, mud-walled, grass-thatched houses with eaves were more likely to suffer a malaria episode than those in new, concrete-walled, iron sheet-roofed houses without eaves. The absence of screens in doors and windows was also significantly associated with the malaria episodes as was a house with more than one room. These findings are congruent with other studies which reported that poorly constructed residential houses increased the chances of malaria transmission. Cracks in walls and roofs acted as entry points for malaria vectors as a study reported in Baringo county, Kenya and Korogwe, Tanzania which increased the abundance of mosquito vectors thus increased malaria transmission (Liu *et al*., 2014; Ondiba *et al*., 2018). Metal-roofed houses contributed to a reduction in malaria cases as was observed in a Gambian village. Another study in Nyabondo, Kenya and Yaoundé, Cameroon (Lindsay *et al*., 2019; Ng'ang'a *et al*., 2019; Ngadjeu *et al*., 2020) reported that the presence of eaves in the houses and the absence of screens in doors/windows sustained transmission.

Studies in Amhara and Oromiya; Ethiopia, rural towns and satellite villages in the Gambia, and in Assam, India (Kirby *et al*., 2008; Ayele *et al*., 2012; Thomas *et al*., 2018) observed that the number of rooms and the family size had been shown to have a significant association with malaria incidences. The number of occupants in a particular house had a significant association with the occurrence of malaria episodes in the surveyed populations. Studies in rural and urban Congo and two rural villages in Equatorial Guinea (Guerra *et al*., 2018; Ngatu *et al*., 2019) showed that many members residing in the smaller family house were an index of poverty. This made it relatively difficult to institute malaria control procedures such as using LLINs. A number of studies advocate for proper housing to act as an alternative to the available malaria mitigation methods. Such as in a study in the Nagongera sub-county, Uganda, Southern Tanzania, and in Nchelenge District, Northern Zambia (Lwetoijera *et al*., 2013; Pinchoff *et al*., 2016; Musiime *et al*., 2022). The information obtained from this study added to the existing need for proper house design and importantly enhanced EIC on the involvement of a household in mitigating malaria transmission.

Findings of this study indicated that bed net ownership was significantly higher than the Kenya national average. Status of the bed net was discussed here as a function of malaria transmission in the study sites. A study in Southern Tanzania reported that the presence of treated bed nets was meant to reduce human-vector contact.(Lwetoijera *et al*., 2013), thus reducing the risk of the occupants being bitten by the vector. The LLINs were to break human-vector interaction during sleeping completely. However, this study showed that the status of the LLINs significantly contributed to sickness due to malaria.

The holes in the bed net provided points of entry for the vector to bite those sleeping under the net. In Zimbabwe, it was reported that the status of the net significantly contributed to malaria transmission, particularly if the net was old, dirty, or needed re-treatment, while another one in 6 locations in Western Kenya found that loss of integrity of the net was a critical hindrance to forestalling malaria transmission (Kanyangarara *et al*., 2018; Sherrard-Smith *et al*., 2018). A study in Southern Malawi found that the older the net, the higher the frequency of *P. falciparum* in children and in Machinga District Hospital, Malawi found no association between holes in the net one year after distributing the nets and increased malaria infection odds (Minta *et al*., 2017; Andronescu *et al*., 2019). It can be concluded that the status of the bed net as a malaria prevention tool had a significantly higher risk of transmission of malaria in the two study sites.

This study reported that the respondents were able to identify and remove *Anopheles* vector breeding and resting sites through drainage of pools of water, removal of water holding artificial containers and clearing of bushes within the vicinity of their houses. This was done individually and communally to raise the success of the methods in malaria control. However, challenges abounded which put the residents at the risk of contracting malaria through improper environmental management. Their socio-economic activities – artisanal gold mining and sugarcane farming among others, increased the available and productive mosquito breeding habitats. Other studies in Dar es Salaam, Tanzania, in a cohort of children in Uganda in which in the spirit of IVM, proper environmental management greatly reduced cases of malaria within the population (Chanda *et al*., 2015; Sadoine *et al*., 2022) . An environment that surrounds homesteads is a principal predisposing factor to the occurrence of malaria episodes within the households. Proper environmental management calls on all stakeholders to come on board, including the political elite, religious, NGOs and individuals.

5.4 Malaria Control Measures Used in the Study Sites

This subsection brings out a poignant discussion of the results of the malaria prevention strategies that the residents of Rosterman gold mining and Eluche sugarcane growing regions used. There has been a general up-scaling of malaria control interventions recommended by WHO, PMI, and other agencies that advocate for a healthy citizenry. The most notable measure was the free provision of the ITNs and LLINs by the GoK and RBM initiative and IVM. The provision of the bed net has targeted every household, but particularly the vulnerable lot, pregnant women and children under the age of 5 years. Other stand-out calls to curtail malaria transmission included EIC and prompt treatment of those who fall sick with malaria (Klootwijk *et al*., 2019). The most recent malaria control strategy was the development of a vaccine to protect the naïve segment of the population – children under 5 years (El-Moamly & El-Sweify, 2023). Despite these up-scaled malaria control interventions, this study found that the disease was still a public health concern for the two communities. The implication of this finding was to re-evaluate the activities of individuals and the entire communities of Rosterman gold mines and Eluche which put them at significant risk for malaria transmission.

The people of Rosterman and Eluche had proper malaria prevention and control EIC. Proper EIC enabled them to make an effort at reducing the likelihood of the mosquito vector bite by possessing and using the net. Additionally, the study showed that the LLIN was the most available bed net that protected people in the two sites from bites by mosquitoes when asleep and hence prevent transmission of malaria. Bed net availability and ownership was above the national average. This finding was similar to the result of network coverage in a study in Kakamega county, Kenya, Lake Basin malaria endemic region and in a review on net ownership and use in SSA (Singh *et al*., 2013; Ng'ang'a *et al*., 2021; Mukabane *et al*., 2022).

The bed net played a significant role in reducing transmission. However, challenges to its use abounded. In this study, it was reported that the larger and the poorer the family was, the more challenges it faced in using the net on day to day basis. And therefore the bed net should be availed to families in proportion to the family size and socioeconomic status. Findings in Nigeria and in a setting in Southern Africa showed that socio-demographics influenced bed net availability and use (Auta, 2012; Kanyangarara *et al*., 2018). People who work at night were unable to use the net and this put them at risk of being bitten by the mosquito. This was true also for results in Ethiopia and Zimbabwe in which people working outdoors at night were unlikely to use a net and consequently had a higher malaria prevalence(Graves *et al*., 2011; Maseko & Nunu, 2020). Much as a lot of effort has been on protecting pregnant women and children below 5 years of age, a lot more has to be done to enlighten the other segments of the population on the consequences of improper bed net use. Alternatively, the agencies tasked with the passage of malaria control efforts through the use of the bed net should triple their effort, to rum the point to the “uncaring” lot.

Proper malaria prevention and control EIC enabled the residents to make an effort at preventing their loved ones from malaria infections. Primarily, the participants preferred using their LLINs, seeking treatment whenever a family member fell sick with malaria, and managing their surroundings well to reduce breeding and resting sites for mosquito vectors by draining stagnant water, clearing bushes near their homes, and keeping the house surrounding clean. This study showed that there was a significant association between socio-demographic factors and the choice of malaria control practice. Result of a review of studies in SSA showed that proper use of the bed net substantially reduced transmission, in Tanzania revealed that the bed net was considered a cultural practice, while in Zimbabwe where the status of the net had a significant association with malaria transmission and in Nigeria reported that socioeconomic issues influenced bed net availability and use (Singh *et al*., 2013; Makungu *et al*., 2017; Kanyangarara *et al*., 2018).

This findings on malaria treatment seeking behaviour were significant in reducing malaria morbidity and mortality. Other studies in Bangladesh and Tanzania reported that seeking treatment was a direct function of knowledge of the disease symptoms, while on the Thailand-Myanmar border, a study showed that socio-demographic factors such as tribe influenced how treatment for malaria was sought (Bashar *et al*., 2012; Chipwaza *et al*., 2014; Sonkong *et al*., 2015; Souza *et al*., 2016).

Participants preferred proper environmental management practices for malaria case reduction by reporting a strong relationship between the knowledge of malaria by the participants and the proper environmental management practices they used to control the transmission of malaria. Proper management of the surroundings in which the inhabitants lived, significantly reduced the proliferation of the mosquito vector. As a consequence reduction in malaria episodes within the households in the same area. Draining stagnant water and clearing bushes around their homesteads reduced the population of the anopheline larval stages and adult female *Anopheles*. This study finding supported earlier results of studies in Kenya and Tanzania in which combined effort by households and communities in removing natural and human-made habitats was key in reducing vector abundance. Bush clearing and hedge trimming reduced the available mosquito resting points (Adebayo *et al*., 2015; Alam *et al*., 2018; Nicholas *et al*., 2021; Mukabane *et al*., 2022) and thus thwarted the risk of contracting malaria. Lack of co-operation among members of communities would most likely defeat the effort to control malaria (*Appendix 10*). This notwithstanding effort should be made through continuous education that, as people go about their economic activities, they should beware of the risk of malaria.

Indoor vector control was critical to the declining malaria prevalence in the study sites. Besides the mosquito net, the residents knew and practiced other methods aimed at safeguarding the households against malaria. Indoor insecticidal spray and the use of repellents, though on a smaller, scale played a big role in reducing transmission. A review showed that indoor spraying reduced mosquito abundance. In another study, the use of vapor-phase and veterinary insecticides in homes with livestock living indoors resulted in reduced vector abundance (Killeen *et al*., 2017; Samuel *et al*., 2017). A study echoed the importance of indoor cultural practices in controlling mosquitoes in which innovative and environmentally friendly community-based strategies were highlighted. Body-applied repellents have been used to deter mosquito bites both outdoors and indoors (Karunamoorthi, 2011; Makungu *et al*., 2017). This study did not find significant use of available repellents by the residents.

Prompt and proper treatment forms a critical plank in breaking the malaria parasite life cycle. On the other hand, improper anti-malarial drug use was bound to culminate in treatment failure and the development of resistance by the parasite to conventional anti-malarial drugs. This study observed that the residents of Rosterman and Eluche sought medication whenever they felt/saw signs of malaria. Seeking medication though was two-pronged, those who went to health facilities and those who self-medicated by buying anti-malarial drugs from shops over the counter. The socio-economic status of the household did not determine treatment-seeking patterns and drug usage. This was similar to the results of studies in Bangladesh and in Tanzania in which seeking treatment was a direct function of knowledge of the disease symptoms (Chipwaza *et al*., 2014; Ngatu *et al*., 2019). However, in a study on the Thailand-Myanmar border, socio-demographic factors such as tribe influenced the seeking treatment for malaria (Sonkong *et al*., 2015). This study further reported that going to a health facility by the sick ensured proper diagnosis and subsequently proper medication. The findings in Nigeria showed that health facilities (private or public) ensured good diagnosis and treatment (Uzochukwu *et al*., 2018). It was observed from this study that they were differential times of seeking medication between adults and children. The adults would wait for one or two days after the onset of the signs before seeking medication while PSAC was immediately after the symptoms were detected.

The promptness of seeking treatment has been reported as a vital index in reducing malaria transmission Landier *et al*., 2016; Uzochukwu *et al*., 2018). Besides, attending health facilities for medication, this study observed that a section of the people preferred self-treatment. Findings of other studies reported that self-medication was a common practice around the world. However, among the ramification of this practice was treatment failure (Chipwaza *et al*., 2014; Ngatu *et al*., 2019). This finding led to the conclusion that the populace, through EIC, should seek early treatment from recognized healthcare-giving facilities and the repercussions of self-treatment.

This study found that AL was the most prescribed anti-malarial drug at the health centers or bought from drug stores, together with paracetamol/Panadol, as was according to the Kenya government ministry of health directive. This was an indicator that the drug was widely accepted by the people of Rosterman and Eluche as being effective, accessible, and cheap. Additionally, the knowledge of the anti-malarial drug use was a pointer of proper perception and practice and ability to follow GoK policy on primary health care. A study in Zambia reported that proper drug supply and management formed the main aspects of malaria treatment (Agarwal *et al*., 2013; Bruxvoort *et al*., 2015). Universal access to drugs promptly was a health facility priority and the use of ACT for the treatment of uncomplicated malaria around the world. In other jurisdictions, other anti-malarial drugs are still in use and effective (Uzochukwu *et al*., 2018; Winskill *et al*., 2019). This study also found that the respondents supervised and monitored drug use within their households (*Appendix 9*). A study in Afghan refugee camp in Pakistan found that a majority of rural community populations monitored drug use among members of a household (Bruxvoort *et al*., 2015; Klootwijk *et al*., 2019), through simple messages of encouragement even to the uneducated. Thus, it is affirmed that AL alongside a pain reliever was the most used drug to treat malaria. This was a significant step in breaking transmission.

Adherence to drug policy by the citizens was a very important step in malaria prevention, because they were consumers of government services, with health being one of the services. This study observed that a larger proportion of the participants had better compliance rates with drug use. Studies in Tanzania, Pakistan and in Brazil's Amazon Region showed that drug adherence was a way of evaluating therapeutic success and avoiding relapse of the malaria infection (Pereira *et al.*, 2011; Bruxvoort *et al*., 2015; Sambili *et al*., 2016). On the other hand, a segment of the study population failed to complete the AL dose that was prescribed to them.

Studies have further reported that drug non-adherence had major ramifications on malaria control strategies Souza *et al*., 2016; Winskill *et al*., 2019). There was no association between socio-demographics and non-adherence to drug regimens. This contradicted the finding of a study that privileged families had a higher likelihood of adhering to therapy than poor families (Simba *et al*., 2012; Hill *et al*., 2013). This group of non-adherent respondents although insignificant can have a far-reaching impact on relapse and the magnitude of infections in the population within these rural areas.

Failure to complete an anti-malarial regimen was attributed to various reasons. Whenever the sick felt better, they stopped/forgot to take the drugs among other reasons. Research in Tanzania and

Brazil had a similar outcome of failure to complete a dosage. Adverse effects, phobia of medication, discouragement by others, and having forgotten were reported as reasons of the discontinuation of anti-malarial drug use (Pereira *et al*., 2011; Sambili *et al*., 2016). Additionally, sharing drugs and lack of food to take during medication have been documented as other causes for failure to complete a dosage (Cohen *et al*., 2010; Gerstl *et al*., 2010). The failure to complete a malaria dose had the potential to confound malaria control efforts.

This study has demonstrated that socio-demographic factors do not have an association with decisions taken by participants who had contracted malaria, malaria treatment-seeking, drug usage, and failure to complete any particular dose. This research finding supported result of a study among children aged below 5 years in Kenya (Ogolla *et al*., 2013). However, this contradicted other research elsewhere in which socioeconomic factors contributed significantly to drug use patterns as a means of malaria prevention (Simba *et al*., 2012; Bruxvoort *et al*., 2015).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study was conducted to answer a set of four questions whose findings were to meet the general objective, to assess malaria entomological and parasitological indices among residents of gold mining and sugarcane growing areas in Western Kenya highlands. The parameters that were used to effectively achieve this objective were well analyzed and discussed, giving the likely implication to malaria control.

1. This study revealed that there was a significantly increased abundance and diversity of the *Anopheles* mosquito vectors in Rosterman gold mining and Eluche.sugarcane growing areas in Western Kenya.
2. This study showed that *P. falciparum* was the dominant parasite that caused the observed malaria cases among the residents. Much as the *Plasmodium spp* infections prevalence recorded was lower than for the entire endemic region, the asymptomatic malaria cases could act as a reservoir pool for malaria transmission.
3. There existed a wide range of risk factors in both study sites that predisposed the residents to malaria infections. These risk factors such as socio-demographic characteristics, house structure design, bed net status, and environmental management practices illustrated the dangers the residents had concerning malaria transmission.
4. This study illustrated that residents of the two sites had proper education, information and communication about malaria and practiced a range of malaria control strategies, both at the individual household level and communally. The use of LLINs was the most preferred malaria prevention method with very minimal use of IRS.

The conclusions from the findings of this study call upon all stakeholders to re-focus attention on the role of environmental modification due to artisanal gold mining and sugarcane farming in rural settings. This would enable participants and policy makers to come up with workable solutions that would enlighten the residents about the risk of malaria transmission posed by their on-going socioeconomic activities.

6.2 Recommendations

The recommendations arising from the study based are:

1. Prevalent and diverse mosquito breeding habitats within the study areas should call for alternative malaria control programs such as larviciding. Also when indoors they should be encouraged to do more on proper and consistent use of the bed net and complement it with other methods such as insecticide spraying.
2. Carry out studies to determine if there could be resistant markers in *Plasmodium* isolates from the people in the two study sites.
3. Residents of the two study sites ought to adhere more on safe socio-economic practices that would forestall malaria transmission and enhance public health awareness.

6.3 Suggestions for Further Research

This subsection gives suggestions for areas that require further research.

1. The role of water plants succession in the mosquito breeding habitats in the proliferation of the *Anopheles* vector.
2. The survivorship and the vectorial capacity of the mosquito species.
3. Carry out human blood index (HBI) of all local malaria vectors to directly link them to malaria transmission.
4. The blood immunity profiles of the symptomatic and asymptomatic malaria patients.
5. Relevance and success/failure of IRS in areas where houses are built using different materials.
6. Public health significance of anti-malaria drug non-compliance on the people of the two study sites.

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APPENDICES

Appendix 1: Consent Form – English Version

Consent Form to Access your Home/Land to Fill a Questionnaire, Collect Mosquitoes, and Get Blood from Members of your Family (English

PROJECT TITLE: **Entomological and Parasitological Indices of Malaria Transmission among Residents of Gold Mining and Sugarcane Farming Areas in Western Kenya**
Mukabane – Ph.D student- MMUST

: Registration Number – SMP/H/01/2016
: 0729416549; 0738481280
: Email: kipchomukabane@yahoo.com
: Dr Philip Ogutu- MMUST
:Dr Jackson Cheruiyot- MMUST

Mr Kipcho Mukabane is a PhD student at Masinde Muliro University of Science and Technology (MMUST) while Dr Philip Ogutu and Dr Jackson Cheruiyot are Senior Lecturers at MMUST. This research proposes to find out malaria indices among residents of Rosterman mines and Eluche in Kakamega County, Kenya. Both the malaria vector- *Anopheles* and the malaria parasite- *Plasmodium* will be obtained from the aquatic habitats, houses and residents in this areas respectively. The methods the residents use to protect themselves from malaria will also be determined. The findings will help the relevant authorities and locals in instituting appropriate malaria management programmes. The study personnel will explain (verbally) the details to you.

The purpose of this form is to ask you to allow us access your home or farm to collect both larval and adult mosquitoes and take a little blood from your finger and members of your family, help us fill a questionnaire and discuss about malaria in your area. The activity will be done during daytime from 7 a.m. to 4 p.m. Participants will be informed verbally of the purpose of the study and any questions answered before being involved.

Why do this study? The objective is to find out the prevalence, distribution and species of the malaria vector and *Plasmodium* parasite diversity in Rosterman mines and Eluche areas

The study site. The study will be carried in Rosterman mines and Eluche areas of Kakamega County, Kenya.

What is involved in the study? The study involves sampling of aquatic habitats for mosquito larvae, collecting adult mosquitoes in the field(s) by light traps and house by spraying or mouth aspiration, getting the parasite from blood of resident humans by finger prick and finding out how residents manage malaria.

Are there any benefits? There will be no direct financial benefits. However, those found to be sick with malaria will be referred to the nearest health centre for treatment. Vulnerable children and others will be counselled and handled by expert medical staff.

Will there be any form of disadvantages/discrimination? Those who will not participate in the exercise will not be disadvantaged/discriminated against before, during or after the study.

How long will the study last? The study lasts 3 years.

Can I stop you from accessing my home/house/farm? Yes, you can decide to deny us access to your property.

Name of home/house/land owner
Thumb print of owner (if necessary)
Signature of home/house/land owner
Printed name of person obtaining informed consent
Signature of Principal Investigator
Printed name of Co-investigator
Printed name and signature of witness

Appendix 2: Consent Form – Luhya Version

Ibuchirira Khwinjira Hango/Mumukunda Khuchipi Marebo Kakhonya Limanya Kubulwale bwa Maleria, Khutoola Amana Ketsisuna nende Okhuhinia Amatsai Khubeinzu yoyo

ESHISINA: Likhabilitsa liu bulwale bwa Maleria mu-Rosterman Mines nende Wimikhonye Chirakungwa mu-Bikulu Bia mu-Kenya Yebukwe.

Mukhabilitsi: Kipcho Mukabane- Musomi wa PhD mu lisomelo lia Masinde Muliro.
Registration Number- SMP/H/01/2016
Inamba yisimu- 0729416549; 0738481280
Email: kipchomukabane@yahoo.com
Dr Philip Ogutu-MMUST
Dr Jackson Cheruiyot-MMUST

LICHOMO: LIKHABILITSA LIU BULWALE BWA MALERIA MU BAMENYI BA ROSTERMAN GOLD MINES NENDE WIMIKHONYE CHIRAKUNGWA MU-BIKULU BIA MU-KENYA YEBUKWE.

Omwami Kipcho Mukabane ni omusomi wa PhD mu lisomelo lia Masinde Muliro, naye Dr Philip Ogutu nende Dr Jackson Cheruyiyot ni abasomesia beikulu mwisomero liene yiro. Obukhabisi buno bwenya okhumanyirisia bulwale bwa maleria mu bamenyi ba Rosterman mines nende Eluche mu County ya Kakamega, Kenya. Tsisuna nende *Plasmodium* nibinyolekhe okhurula we tsibulaniranga, mutsinzu nende abhandu ba ebweneyo. Tsinjira tsia abhandu barumishiranga okhwikinga nende obulwale bwa maleria bwosi ni bukhabililwe. Aka nikanyolekhe nikakhonye abakali bahusiananga nende obulwale bwa maleria okhumeta amani khu khwikalira maleria. Abakholi ba obukhabilisi buno nibakhwibalire amanji.

Ifomu yino nokhureba iwe okhuhana obunyali bwa ohwinjira hango wuwo noho mumukunda kukwo okhusanya amana ke tsisuna nende tsisuna tsiene, okhubukulakhwo amatsai matutu okhurula khushitereshio nende shiabamenyani bobo, okhuchipa amarebo nende okhukhupa tsimbakha khuka obulwale bwene ibo. Nekhuchende okhurula etsisa 7 a.m okhula etsisa 4 p.m. aba ni babe nibahuchirire ni bebalirwe aka obukhabirisi buno nende amarebo kabwe okhuchipwa.

Mbona okhukhola obukhabilisi buno? Lichomo nokhukhabilisia obunyolekhi, okhwenea nende obwahukhani bwe tsisuna nende *Plasmodium* mu Rosterman nende Eluche.

Ewa obukhabilisi nibwikholekhe. Obukhabilisi buno ni bwikholekhe mu Rosterman nende Eluche mu County ya Kakamega, Kenya.

Ebia ni bikholekhe mubukhabilisi buno? Okhukhaba amana ke tsisuna mu matsi karerema, okhukhaaba tsisuna mutsinzu nende elwanyi khurumishiranga tsitaa, okhunyunyisia olunyasi, okhukhuna nende epaipu, okhunyoola Plasmodium mumatsai kabandu kokhuhinia mushiterenende shinga abandu baikaliranga okhunyola maleria.

Ne khuliho nende eshiakhubebwa? Shikhuliho nende obukhonyi bwa nonyoole tawe. Habula abalwale nibarumwe mwisirishiro liri ahambi. Abana nende abalenyalira tawe nibebalirwe mubwitsulu akenyekha nende akaruliramwo.

Ne khubeho nende ebiahulo? Tawe. Aba nibabule okhuba mubukhabilisi buno shinibasulumwe munjira yosiyosi tawe.

Ni mubukule luhono shi? Emiaka 3.

Ne enyala okhubekalira okhwinjira awanje/munzu/mukunda? Eeh, onyala okhukhwikalira okhwinjira mubindu bibio.

Eliria lia mwene bunyali
Okhufinya eshitere (kali shienyekha)
Esaini ya mwene hango/inzu/omukunda
Elira lia mundu uhebungwa obunyali
Elira lia mukhabilisi mukali
Elira lia mukhabilisi washie
Elira nende esaini ya mukobosheri

Appendix 3: QUESTIONNAIRE

Aspects of the questionnaire:

Part 1: Socio-demographic characteristics of participants

1. Gender
    a. Male [ ]
    b. Female [ ]
2. Age
    a. ≤19 [ ]
    b. 20-39 [ ]
    c. 40-59 [ ]
    d. ≥60 [ ]
3. Marital status
    a. Married [ ]
    b. Unmarried [ ]
    c. Widow/widower [ ]
    d. Divorced [ ]
4. Highest level of education completed
    a. No education [ ]
    b. Primary [ ]

c. Secondary [ ]
d. Tertiary qualifications [ ]

5. Main occupation
   a. Peasant (self-employed in agriculture) [ ]
   b. Small scale business [ ]
   c. Employed [ ]
   d. Housewife [ ]
   e. Unemployed [ ]
   f. Others (night guards, miners) [ ]
6. What is your average income per month?
   a. Below Sh 2,000 [ ]
   b. Sh 2,000 – 5,000 [ ]
   c. Sh 5,000 – Sh 10,000 [ ]
   d. above Sh 10,000 [ ]

Part 2: Assessment of household's head knowledge

1. Have you heard of malaria?
   a. Yes [ ]
   b. No [ ]
2. What was your source of information?
   a. Home [ ]
   b. Radio/Television [ ]
   c. Health centre/dispensary/hospital [ ]
   d. Health workers [ ]
   e. Suffered from malaria [ ]
   f. Others [ ]
3. How is malaria transmitted?
   a. By bites of any mosquito [ ]
   b. By bites of mosquito which has bitten a malaria patient [ ]
   c. Others [ ]
   d. Do not know [ ]
4. What causes malaria?
   a. Germs [ ]
   b. Dirty stagnant water [ ]
   c. Mosquito bites [ ]
   d. *Plasmodium* organisms [ ]
   e. Does not know [ ]

5. Where do mosquitoes breed?
   a. Stagnant water [ ]
   b. Tall grass [ ]
   c. Bushes [ ]
   d. Others [ ]
6. Did a member of your house have a malaria bout in the last one month?
7. What age? …………..
8. What gender? …………..
9. Name common symptoms of malaria that make you know that you have contracted the disease.

....
....

Part 3: Malaria control practices

10. How do you prevent yourself or your household from malaria?
    a. Using insecticidal bed nets [ ]
    b. Using insecticide sprays [ ]
    c. Preventing breeding of mosquitoes and resting places [ ]
    d. Using mosquito coil/repellents [ ]
    e. Treatment [ ]
11. What preventive measure have you and your household used in the last 24 hours?
    a. Used insecticidal bed nets [ ]
    b. Used insecticide sprays [ ]
    c. Others [ ]
    d. No any method [ ]
12. What ways do you use to prevent mosquito from breeding?
    a. Cleaning of house surrounding [ ]
    b. Draining of stagnant water [ ]
    c. Clearing bushes around the house [ ]
    d. Others [ ]
    e. Do not know [ ]
13. Who possesses bed nets (treated or untreated)?
    a. Father [ ]
    b. Mother [ ]
    c. children [ ]
    d. Others [ ]

14. What type of net?
    a. ITN [ ]
    b. LLIN [ ]
15. Did those who own mosquito nets use them last night?
    a. Yes [ ]
    b. No [ ]
16. Who sleeps under bed nets daily?
    a. Mother and children [ ]
    b. Father and mother alone [ ]
    c. Children alone [ ]
    d. Everyone in the house [ ]
17. What is the reason that makes you use bed nets?
    a. Protect from malaria [ ]
    b. Protect from mosquito bites [ ]
    c. Protect children from malaria [ ]
    d. Habit [ ]
    e. Others [ ]
18. Why do you think you do not have a bed net?
    a. Expensive [ ]
    b. Not available [ ]
    c. Reduces surrounding air [ ]
    d. Cannot prevent malaria transmission [ ]
    e. Others [ ]
19. Do you re-treat your bed nets?
    a. Yes [ ]
    b. No [ ]
20. You re-treat your bed nets after how long?
    a. ≤ 1 month [ ]
    b. After 2 or 3 months [ ]

c. After 12 months [ ]
d. Do not treat [ ]
e. Others [ ]

✓ Which insecticide do you use to re-treat your net?
....

✓ What reason makes you not to use insecticide to re-treat your bed nets?
a. Expensive [ ]
b. Not available [ ]
c. Do not need re-treatment [ ]
d. Others [ ]

21. What is the source of the insecticide for re-treatment of bed nets?
a. Retail shops [ ]
b. Non-governmental organization [ ]
c. Donated by government [ ]
d. Others [ ]

Part 4: Indoor Residual Spraying

22. Have you heard of indoor residual spray campaign (IRS)?
a. Yes [ ]
b. No [ ]

23. What was the source of your information about IRS?
a. Radio [ ]
b. Television advertisement [ ]
c. Community health worker [ ]
d. I sprayed my house [ ]
e. Government campaign [ ]
f. Others [ ]

✓ Why did you use or accept IRS?
a. To kill mosquitoes [ ]
b. To kill mosquitoes and other insects [ ]
c. To protect the family from malaria [ ]
d. Others [ ]

24. Why did you not use or accept IRS?
a. Insecticides have bad smell [ ]
b. Insecticides may kill my children [ ]
c. Insecticides may kill my animals [ ]
d. Insecticides may cause infertility [ ]
e. Others [ ]

Part 5: Treatment seeking behaviour

25. When you feel that you have malaria what action do you take?
a. Self-treat [ ]
b. Seek herbalist/traditional healer [ ]
c. Go to health centre/dispensary/hospital [ ]
d. No treatment [ ]
e. Others [ ]

26. What drugs do you use to treat malaria when you fall sick?
....

27. Any side effects of the drug you use to treat malaria?
....

28. What challenges do you get when using your antimalarial?

....

N/B

- ✓ Household head- the person who was perceived by household members to be the primary decision maker in the family.
- ✓ Household- individuals living together and taking meals from a common cooking facility

Appendix 4: Institutional Ethics Review Committee (IERC) Approval

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

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Institutional Ethics Review Committee (IERC)

Ref: MMU/COR: 403012 vol2 (59)

Date: 29th October, 2019

Kipcho Davis Mukabane
Masinde Muliro University of Science and Technology
P.O. Box 190-50100
KAKAMEGA

Dear Mr. Mukabane

RE: Malaria Indices among Residents of Gold Mining and Sugarcane Farming areas in Western Kenya Highlands -MMUST/IERC/090 /19

Thank you for submitting your proposal entitled as above for initial review. This is to inform you that the committee conducted the initial review and approved (with minor revisions) the above Referenced application for one year.

This approval is valid from **29th October, 2019 through to 29th October, 2020.** Please note that authorization to conduct this study will automatically expire on **29th October, 2020**. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the MMUST IERC by **29th September, 2020.**

Approval for continuation of the study will be subject to submission and review of an annual report that must reach the MMUST IERC secretariat by **29th September, 2020**. You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to MMUST IERC prior to implementation.

Please note that any unanticipated problems or adverse effects/events resulting from the conduct of this study must be reported to **MMUST IERC.** Also note that you are required to seek for research permit from **NACOSTI** prior to the initiation of the study.

Yours faithfully,

Dr. Gordon Nguka (PhD)
Chairman, Institutional Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)
- DVC (A & F)

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

Tel: 056-31375
Fax: 056-30153
E-mail: ierc@mmust.ac.ke
Website: www.mmust.ac.ke

P. O. Box 190-50100
Kakamega, Kenya

Institutional Ethics Review Committee (IERC)

Ref: MMU/COR: 403012 Vol 3 (01)

Date: 15th February, 2021

Kipcho Davis Mukabane
Masinde Muliro University of Science and Technology,
P.O. Box 190-50100,
Kakamega.

Dear Davis,

RE: Malaria indices among residents of gold mining and sugarcane farming areas in Western Kenya highlands- MMUST/IERC/166/2021

Thank you for submitting your proposal entitled as above for continual review. This is to inform you that the committee conducted the continual review and approved (with no further revisions) the above Referenced application for one year.

This approval is valid from **15th February, 2021** through to **15th February, 2022**. Please note that authorization to conduct this study will automatically expire on by **15th February, 2022.** If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the MMUST IERC by **15th February, 2022.**

Approval for continuation of the study will be subject to submission and review of an annual report that must reach the MMUST IERC Secretariat by **15th February, 2022.** You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to MMUST IERC prior to implementation.

Please note that any unanticipated problems or adverse effects/event resulting from the conduct of this study must be reported to MMUST IERC. Also note that you are required to seek for research permit from NACOSTI prior to the initiation of the study.

Yours faithfully,

Dr. Gordon Nguka (PhD)
Chairman, Institutional Ethics Review Committee

Copy to:

- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)

Appendix 5: Research Permit/Licence

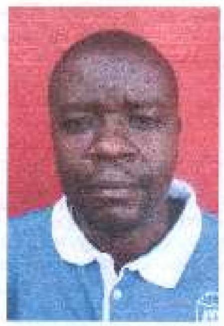
REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 846846

Date of Issue: 09/January/2020

RESEARCH LICENSE



This is to Certify that Mr.. Kipcho Mukabane of Newcastle University, has been licensed to conduct research in Kakamega on the topic: Malaria Indices among Residents of Gold Mining and Sugarcane Farming areas in Western Kenya Highlands for the period ending : 09/January/2021.

License No: **NACOSTI/P/20/3215**

846846

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR
SCIENCE,TECHNOLOGY &
INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document,
Scan the QR Code using QR scanner application.

Appendix 6: Mosquito Larval Stages Sampling Data Sheet

LARVAL DISTRIBUTION SURVEY FORM

Larvae No:………………

Site: [ ] Rosterman [ ] Eluche

Date (DD/MM/YY): / /20….

Habitat #	Habitat Type	Veg cover %	Length (M)	Width (M)	Land use Type	GPS			Number of individual species												
						Lat (E)	Long (N)	Elev	*An. coustani*			*An. gambiae*			*An. fun*	*An. squam*	*An. implex*	Culex	Aedes	Other	
									L1-L2	L3-L4	pupae	L1-L2	L3-L4	Pupae							

#Habitat Type (A – K)

A Drainage ditch D Pond (not 1)G Container K Other

B Footprint E Swamp H Rockpool

C Gold mine F Tire track I Fish pond

#Land use Type (1, 2….)

1 Farmland 2 (Pasture) 3 Swamp 4 River/Stream

5 Forest 6 Shrub 7 Road 8 Compound/Home

9 Water body 10 A forest…..11 Other

Investigator ……………………………. Supervisor ……………………………………………

Appendix 7: Adult Mosquito PSC Data Collection Sheet
Mosquito Population Dynamic Survey

Investigator…………………………………………
Supervisor……………………………………………..

Site ……………………………………………………

Survey Date: | | | |

House No…………………… New……… Old…….

Latitude……………………. Longitude……………. Elevation ………………………..

Number of people ……………….. Age………………..

Number of bed nets …………………………

Number of holes……………………………..

Mosquito preventive methods used [ ] IRS [ ] Repellents [ ] Other

Number of adult mosquitoes ………….

	Stage	*An. gambiae*	*An. funestus*	Culex	Aedes	other
Male						
Female	Empty					
	Blood fed					
	Half gravid					
	Gravid					
	Total					

Appendix 8: Blood Sample Collection and Entry Sheet

Blood Sample Collection and Entry Form

Name	Code	Site	Age	Sex	Total samples	Parasite Load (/200 wbc)	RDT				Microscopy				PCR			
							Pf	*Pv*	*Pm*	Mixed	*Pf*	*Pv*	*Pm*	Mixed	*Pf*	*Pv*	*Pm*	Mixed

Investigator ………………………………………………. Clerk …………………………………………

Supervisor ………………………………………………..

Appendix 9: Publications

Scientific African 15 (2022) e01140

Contents lists available at ScienceDirect

Scientific African

Journal homepage: www.elsevier.com/locate/sciaf

Bed net use and malaria treatment-seeking behavior in artisanal gold mining and sugarcane growing areas of Western Kenya highlands

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ARTICLE INFO

Article history:
Received 17 March 2021
Revised 4 January 2022
Accepted 25 February 2022

Editor: DR B Gyampoh

Keywords:
Western Kenya Highlands
Bed net
malaria treatment
Antimalarial drug
Dose completion

ABSTRACT

Provision of the bed net in Sub-Saharan Africa has substantially resulted in the decrease in malaria incidences in the region. However, malaria still ravages these regions causing significant deaths. This situation has been attributed to socio-economic inequalities that reduce access to the net, failure to seek treatment, and poor antimalarial drug use. Information about these factors in malaria control in Western Kenya highlands is not clearly documented. This study sought to find out bed net availability and use, treatment-seeking, and antimalarial drug use behavior in two rural communities. The study was a qualitative-quantitative baseline cross-sectional survey carried out in two rural villages in Western Kenya highlands in November – December 2018. Focus group discussion, a semi-structured questionnaire, and in-depth interviews were conducted in 236 households to determine: socio-demographic characteristics; bed net availability and use; action taken when sick with malaria, and antimalarial drug usage. Socio-demographic characteristics and the responses of the participants were expressed as percentages. Dependent and independent variables were cross-tabulated for calculation of OR and association was tested using Pearson Chi-square at 95% CI and a $p \leq 0.05$ was considered significant. Ninety-three percent of the respondents had used the net the previous night, females (56.50%) constituted the majority of respondents; going to health facility (84.26%) was the preferred action taken when a person had malaria, Artemether Lumefantrine (30.19%), and a pain killer was the most cited drug, 22.96% of the responders reported to have failed to complete antimalarial dose saying that they had recovered from the malaria bout and 9.80% reported to re-use the remaining part of the dosage. This was the first study in mining and sugarcane growing communities in Western Kenya Highlands that has shown that there was no association between socio-demographic parameters and bed net use, action taken when having malaria, and adherence to drug use. The two rural communities have the bed net and know its use. They have good knowledge of malaria and seek treatment whenever they saw malaria signs. The study also reported that the action the sick person took when they had a malaria bout, the drug they got from the health facility, or failure to complete an AL dosage regimen was not associated with socio-demographic characteristics of the respondents. These findings raise the need for further qualitative studies in other settings to accept

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https://doi.org/10.1016/j.sciaf.2022.e01140

Assessment of knowledge of malaria and its control practices in mining and sugarcane growing regions of Western Kenya highlands

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Abstract

Background: Despite upscaled control efforts, deaths and hospitalization due to malaria remained high in counties of western Kenya highlands.

Objectives: This study assessed the knowledge of malaria in two rural communities, the control strategies they use, and their capacity to integrate the available control programs.

Methods: A cross-sectional survey was carried out in two rural villages in November – December 2018. Focus group discussions and a questionnaire survey were carried out in 736 households. Frequencies and proportions were used for descriptive analysis while the Chi-square test was used to determine factors that were associated with knowledge of malaria at $p \leq 0.05$

Results: Ninety-seven percent of the respondents had knowledge of malaria and this was associated with the level of education attained ($\chi 2 = 30.108$; $p > 0.0001$). Bed net ownership was at 86% and 92% correctly identified its use. Draining stagnant water (53.9%) was the most cited environmental management practice.

Conclusion: There was awareness of the risk factors of malaria transmission in the study sites. The local communities must be mobilized and empowered through EIC for the control practises to bear fruit against malaria transmission. However, more sensitization needs to be done to optimize the use of malaria control practices.

Keywords: Malaria, control practices, Kenya highlands, Mining.

DOI: https://dx.doi.org/10.4314/ahs.v22i2.23

Cite as: *Mukabane DK, Kitungulu N, Ogutu PA, Korir JC, Mulama DH. Assessment of knowledge of malaria and its control practices in mining and sugarcane growing regions of Western Kenya highlands. Afri Health Sci. 2022;22(2): 194-203. https://dx.doi.org/10.4314/ahs.v22i2.23*

Introduction

Malaria is still reported in many areas around the world even with concerted efforts to control the transmission. In 2016 Sub-Saharan Africa (SSA) reported 90% of the cases[1]. In Kenya, the control practices are widespread in malaria-endemic zones where malaria prevalence remains high[2]. Despite these control efforts, deaths from malaria and hospitalization due to the disease are still high especially in highlands west of the Rift Valley[3].

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This suggests that there could be a stagnation in the fight against the malaria scourge. This calls for newer and more effective control tools and practices to shore up the existing ones without reversing the gains already made. The focus has been more on the long-lasting insecticidal nets (LLINs) and IRS. Other strategies can be incorporated and implemented at the individual household level to significantly limit human-vector interaction[4]. Besides environmental management and bed net use, perceptions and knowledge of the people enhance efforts to eliminate the vector[5].

The socio-economic abilities of individuals and populations have a crucial role to play in the malaria cycle[6]. Poverty is a major cause of unnecessary deaths due to malaria and other public health issues[7]. Economic status determines the success or failure of a program by an individual or government since impoverished house-