SPATIO-TEMPORAL DISTRIBUTION OF *PLASMODIUM FALCIPARUM* INFECTIONS AND KELCH-13 ARTEMETHER-LUMEFANTRINE RESISTANCE IN MBALE TOWNSHIP, WESTERN KENYA

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A RESEARCH THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN PARASITOLOGY OF MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

AUGUST, 2024

DECLARATION

This research 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

We the undersigned certify that we have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a research thesis entitled **'Spatio-Temporal Distribution of *Plasmodium falciparum* Infections and Kelch-13 Artemether-Lumefantrine Resistance in Mbale Township, Western Kenya'**

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DEDICATION

The study is decidated to my late parents Mr.and Mrs. Ezekiel Eboyi Muzame and to my family of Mr. Abisay Lumosi Mudaki, Tracy Mudaki, Wancy Mudaki, Clancy Mudaki and Percy Mudaki for encouraging me to pursue further education.

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ABSTRACT

Malaria is the leading cause of mortality and morbidity in Sub Saharan Africa. Various intensive control strategies and measures have been put in place to reduce and ultimately eradicate malaria but it still exists at disproportionately high levels in many regions of Kenya. The study in Mbale town and its environs was carried out at Mbale Provincial Rural Training Health Center in Vihiga County, Western Kenya. The study determined spatio- temporal distribution of *Plasmodium* species by microscopy, effect of genotype of *P. falciparum* kelch 13 on efficacy of artemether-lumefantrine by *P. falciparum*'s DNA extraction from dry blood spots using Qiagen method, DNA genotyping by polymerase chain reaction and Sanger sequencing and influence of socio-economic factors on malaria prevalence done by structured questionnaire. A total of 768 malaria confirmed patients were purposively recruited from December 2019 to November 2020 and stratified according to demographic status and their geographical locations. Data was analysed using SPSS software version 17. Descriptive and inferential statistics were used to compare variables in the study population. In all tests p-value ≤ 0.05 was considered statistically significant. Out of 768 malaria patients recruited, 98.7% recorded *P. falciparum* positive, while *P. ovale*, *P. malariae* and *P. vivax* accounted for 1.3%. Edzava ward recorded all the four *Plasmodium* species. Lugaga/Wamuluma ward recorded more malaria patients than Edzava and Central Maragoli wards. Male malaria patients accounted for 49.3% recording all *Plasmodium* species while female constituted 51.7% without *Plasmodium ovale*. All age groups were diagnosed with *P. falciparum* while *P. malariae* and *P. ovale* were not identified among 14-18 years old age group. Children below 5 years old were not diagnosed with *P. ovale*. Multinomial regression analysis showed effect of wards, age and gender to malaria prevalence was ([F (3,764) = 1.854], < 0.136). Wards according to multinomial regression ($p = 0.034$) and Chi-Squared p-value = 0.036) were statistically significant to malaria prevalence as opposed to gender and age. Multiple linear regression analysis showed effect of time trend on malaria prevalence was ([F (3, 8) = 2.976], $p < 0.097$). Months however had a statistically significant effect on malaria prevalence ($p = 0.036$) as opposed to rainfall and temperature. Correlation between average temperature and months were statistically significant ($p = 0.013$). From the results obtained synonymous mutations identified in *P. falciparum* kelch 13 among study population did not compromise the efficacy of Artemether-lumefantrine in the study area. Chi- squared analysis showed influence of socio-economic factors on malaria prevalence in the study area as follows: - level of education (p-value = 0.014), wealth (p-value = 0.000), size of land (p-value = 0.035), house type (p-value = 0.006), salary (p-value = 0.043) and ventilation in the house (p-value = 0.000) were statistically significant to malaria prevalence as opposed to household size. Space, time trend and socio-economic factors influenced malaria prevalence in the study area as opposed to *P. falciparum* kelch 13. Malaria management bodies should strengthen malaria control strategies in relation to space, time trend, antimalarial efficacy and socio-economic factors at household level.

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ABBREVIATIONS AND ACRONYMS

ACPR	Adequate Clinical and Parasitological Response
ACR	Adequate Clinical Response
ACT	Artemisinin-Based Combination Therapy
AIDS	Acquired Immunodeficiency Syndrome
AL	Artemether-Lumefantrine
AMREF	The African Medical and Research Foundation
AQ	Amodiaquine
AS	Artesunate
AS+AQ	Artesunate + Amodiaquine Combination
AS+MQ	Artesunate + Mefloquine Combination
AS+SP	Artesunate + Sulfadoxine-Pyrimethamine Combination
CDC	Center of Disease Control and Prevention
CQ	Chloroquine
DEET	Diethyl-meta-toluamide
DEPA	Dimosia Epichirisi Paroxis Aeriou
DfID	Department for International Development
DHFR	Dihydrofolate reductase
DHDS	Dihydropteroate synthase
DOMC	Division of Malaria Control
ELISAs	Enzyme-Linked Immunosorbent-Assay
ETF	Early Treatment Failure
FY	Financial Year
HIV	Human Immunodeficiency Virus
IM	Intramuscular
IV	Intravenous

KEMRI	Kenya Medical Research Institute
KEMSA	Kenya Medical Supplies Agency
KNBS	Kenya National Bureau of Statistics
LCF	Late Clinical Failure
LPF	Late Parasitological Failure
LTF	Late Treatment Failure
ME	Monitoring and Evaluation
MIP	Malaria in Pregnancy
MIS	Malaria Indicator Survey
MOH	Ministry of Health
MQ	Mefloquine
NMS	National Malaria Strategy
PCR	Polymerase chain reaction
PMI	President's Malaria Initiative
QBC	Quantitative Buffy Coat
RDT	Rapid diagnostic test
SAA	Surface Active Agents
SP	Sulfadoxine–pyrimethamine
USAID	United States Agency for International Development
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Malaria is the main killer disease in Africa (WHO, 2018). Despite numerous global intervention strategies, malaria remains the leading cause of morbidity and mortality worldwide especially in sub-Sahara Africa (AMREF., 2020 & Zang *et al*, 2018). Female *Anopheles'* mosquitoes that are commonly found in Kenya include: - *Anopheles gambiae, A. arabiensis* and *A. funestus* which take in human blood meal as they innoculate a malaria parasite that kills a child every minute globally (Ndenga *et al.,* 2022). *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae* that cause malaria infection in humans vary in space, time trend, severity, microscopic appearance, ability to cause relapses (*P. vivax, P. ovale*) and ability to develop resistance to antimalarial drugs (CDC., 2019). About 99% of malaria globally is due to *P. falciparum* infection while *P. vivax, P. ovale* and *P. malariae* constitute 1% of human malaria infection (Awe., 2013).

Health is one of the 4 Agenda of Kenya vision 2030 ('Kenya Vision 2030') and also an agenda among African union agenda 2063 with malaria treatment and control being allocated a lot of funds in the ministry of health budget (African Union Agenda 2063). In 2017, a total of 445,000 malaria deaths were recorded globally, with African countries including Nigeria, Mali, Senegal, Ethiopia, Uganda and Kenya accounting for 92.6% (WHO., 2018). The democratic republic of Congo and Nigeria accounted for 84 million (54%) of total malaria deaths in the world in 2017 while India accounted for 4.5% (WHO., 2018). In 2019, the World Health Organization (WHO) reported that malaria prevalence and

mortality had been reduced significantly, mainly in developing countries, notably in Africa, and aimed at reducing the malaria burden globally to 10% by the year 2030 (Angelica *et al.*, 2020). A previous report on malaria elimination in the People's Republic of China indicated that the malaria burden had sharply declined due to improvements in malaria surveillance systems in the country (Zang *et al*., 2018). In 2020, malaria deaths in the world were 627,000 with 95% from Africa (WHO., 2020). Malaria prevalence in Kenya was 5.08 million in 2018 and 5.02 million in 2019 with Western Kenya carrying most of the malaria burden where more than 70% of the population was at malaria risk (Kenya National Malaria Strategy 2009-2017 & Kenya malaria indicator survey, 2020). Between January and March 2019, 28,786 patients across Vihiga County were diagnosed with malaria leading to high mortality among children below 5 years old (Wafula & Mulinya, 2018).

A study carried out in South-East Iran indicated that, geographical distribution of malaria parasites varied from region to region due to difference in climate, land use, drug resistance and topography (Minoo & Khodadad., 2019). Understanding patterns of malaria transmission in relation to movement of people, space and time trend is very important in effectively eliminating malaria according a spatio-temporal study findings from a spatial unit on the Colombian Pacific Coast (Angelica *et al.*, 2020). Studies in effect of geographical location (space) and time trend on malaria prevalence were also carried out in various parts of the world which included; Nepal (Dhimal *et al*., 2014), China (Zhang *et al*., 2018), South Zimbabwe (Tawanda *et al*., 2017), Ethiopia (Alemu *et al*., 2013) and Malawi (Chirombo *et al*., 2020). Study results obtained from these studies indicated that space and time affected

malaria prevalence and strategies for cost-effective implementation of malaria intervention strategies were strengthened accordingly.

Malaria zones in Kenya are based on climatic conditions which vary at different times (Kenya Malaria Indicator Survey 2020). Studies on how space and time trend influenced malaria prevalence were carried out in lowlands and highlands of Western Kenya (Omukunda *et al*., 2013 & Ndenga *et al*., 2016). Results from these studies indicated malaria prevalence was still high despite intensive malaria control strategies having been put in place (Kenya Malaria Indicator Survey, 2020). Study findings in Vihiga County indicated climate affected malaria prevalence (Wafula & Mulinya, 2018). Malaria parasites and mosquitoes have become resistant to drugs that are suppose to eliminate them (WHO., 2015). Genetic mutations in genes encoding for drug target proteins in malaria parasites such as *P. falciparum* kelch protein on chromosome 13 (*Pf*K13) may result in an unusual expression and folding of these proteins modifying the usual binding site of the drug which leads to antimalarial drug resistance (Makoah and Gabriel, 2013 & Wirichada *et al*., 2009).

Monitoring antimalarial efficacy is important in detecting antimalarial drug resistance which can spread in a population and increase malaria morbidity and mortality (Zurovac *et al.*, 2014). Reduced susceptibility to Artemether-Lumefantrine (AL) in Africa and Asia has been associated with polymorphisms in *P. falciparum* mixed drug resistance 1(PfMDR1) particularly the variant N86 and amplification of the *pfmdr1* encoding gene (CDC., 2019; Makoah & Gabriele, 2013). Antimalarial drug resistance increased malaria prevalence according to studies findings reported in; India, China, Brazil, Pakistan and Malaysia

(WHO., 2015 &Yadar *et al*, 2014). In Africa, antimalarial resistance increased malaria cases in 2018 (Willem Roper, 2020). Artemether–lumefantrine treatment failure in a *falciparum* malaria patient was reported.in Tunisia (Aouam *et al*, 2016). Antimalarial policy change in Kenya from sulfadoxine-pyrimethamine to artemether-lumefantrine was broadly implemented countrywide by 2008 with support from global fund (Wamae *et al*., 2019). Trends in drug codons in genes associated with enzymes in *P. falciparum* was reported in Kenya (Angela *et al*, 2015 & Dennis *et al*., 2012). At the coastal region of Kenya (Wamae *et al*., 2019) and in Western Kenya (Akala *et al*., 2011; Daibin *et al*., 2008 & Hemming *et a1*., 2018) *Pf*K13 arteminisinin resistance markers detected did not cause delayed clearance of malaria paraistes after artemether-lumefantrine.

Socio-economic factors refer to an individual's social and economic status in relation to other people in a given area (Degarege *et al*., 2019). Socio-economic factors determine mosquito bites which lead to malaria tramsmission and can also contribute towards antimalarial resistance (Ibor, U.W., & Okoronkwo, 2017: Makoah & Gabriel, 2013). A study carried out in tribal dominated Mandla district, Assam, India (Yadar *et al.*, 2014) indicated that malaria burden can be reduced by improving people's socio-economic status. A Kenya malaria indicator survey was carried out in all 47 Counties in Kenya, which associated malaria prevalence with socio-economic factors (Kenya malaria indicator survey, 2020).

1.2. Statement of the Problem

More than 70 % of the population in Western Kenya where the study area falls is at malaria risk (PMI., 2020). The challenges facing control and treatment of malaria in Kenya include: - misdiagnosis, lack of adequate modern diagnostic tools to detect mutations in malaria parasites, delay in seeking early treatment, povery, inadequate medication, poor infrastructure and lack of adheherance to medication (PMI., 2018). Spatio-temporal distribution of *Plasmodium* species among gender and age is important because they determine severity of malaria infection. *P. falciparum* malaria is the most severe type of malaria in Africa which occurs during short and long rain seasons and it is highly infectious (CDC., 2019). Malaria prevalence and distribution of *Plasmodium* species should be monitored in relation to effect of movement of people, space and time trend in order to apply cost-effective malaria control strategies accordingly (Andrea *et al*., 2019).

Genotypes of human malaria parasites determine whether the malaria parasite is sensitive or resistant to antimalarial drugs which may affect malaria burden in a given area. Factors contributing to the emergency of drug resistance include: poverty which makes it difficult for poor malaria patients to prevent and treat malaria, genetic mutations in genes encoding for targeted proteins in the malaria parasites and the prevalence of asymptomatic malaria infection (Makoah & Gabriele, 2013). Socio-economic factors including poverty were considered to be risk factors in malaria transmission because they influenced mosquito bites and played a very important role in attitude towards malaria, malaria control and management (Degarege *et al.,* 2019).

1.3. Justification of the Study

Vihiga County is a densely populated malaria holoendemic zone with poverty level of 62% (Kenya National Bureau of Statistics, 2020). Factors contributing to the malaria burden should be closely monitored because malaria is the main killer disease in Africa (WHO., 2018). In Vihiga County, 28,786 patients were diagnosed with malaria between January and March 2019, leading to high mortality among children below 5 years old (Wafula & Mulinya, 2018). The densely populated malaria holoendemic Mbale township remains scanty and poorly documented regarding studies on: spatio-temporal distribution of *P. falciparum* malaria and of kelch 13 on the efficacy of artemether-lumefantrine which is the first-line treatment for malaria cases, and the effect of socio-economic factors on malaria prevalence, hence the study as recommendation by Wafula & Mulinya, 2018 (Wafula & Mulinya, 2018).

It is important to determine spatial and temporal prevalence of human malaria parasites; *P. falciparum, P. ovale, P. malariae* and *P. vivax* for malaria control bodies to be able to effectively distribute adequate and correct antimalarial drugs, parasitology tools, health workers and malaria control materials such as insecticide-treated bed nets (Zang *et al*., 2018). Monitoring emergence of resistance of malaria parasites to antimalarial drugs using a combination of therapeutic efficacy studies and molecular markers is recommended by world health organisation (WHO) to prevent antimalarial drug resistance from spreading from one region to another via malaria transmission (Ishengoma *et al*., 2019). The *Pf*K13 gene mutation is associated with resistance of artemether-lumefantrine which is the first-line treatment for *P. falciparum* malaria and any resistance to it by malaria parasites is a threat to

elimination of malaria globally (Wamae *et al*., 2019). *Plasmodium falciparum* was chosen because it is the most common human malaria parasite as it constitutes 99% of malaria infection in Kenya and in the study area (PMI, 2020). Socio-economic factors influence malaria prevalence by determining mosquito bites and contributing towards antimalarial resistance (Makoah & Gabriele, 2013).

1.4. Objectives

1.4.1. General Objective

Spatio-temporal distribution of *Plasmodium* species, efficacy of Artemether-lumefantrine and influence of socio- economic factors on malaria prevalence in Mbale town and its environs, Vihiga County, Western Kenya.

1.4.2. Specific Objectives

1. To determine the spatio-temporal distribution of *Plasmodium* species circulating in Mbale town and its environs, Vihiga County, Western Kenya.
2. To determine the effect of *PfK*13 on efficacy of Artemether-lumefantrine (AL) in Mbale town and its environs, Vihiga County, Western Kenya.
3. To determine the influence of socio- economic factors on malaria prevalence in Mbale town and its environs, Vihiga County, Western Kenya.

1.4.3. Research Hypothesis

1. Spatia-temporal distribution of *Plasmodium* species is homogenous in Mbale town and its environs, Vihiga County, Western Kenya.

2. *Plasmodium falciparum* kelch 13 (*Pf*K13) does not affect efficacy of Artemether-lumefantrine (AL) in Mbale town and its environs, Vihiga County, Western Kenya.
3. Socio-economic factors do not influence malaria prevalence in Mbale town and its environs, Vihiga County, Western Kenya.

1.5 Significance of the Study

At a personal level, the study results will help malaria patients to understand when to visit hospitals for treatment on time when they observe clinical signs of malaria to prevent malaria deaths. Malaria patients should also complete the antimalarial drugs given and reduce mosquito bites by implementing malaria control strategies. Study results will assist health personnel in identifying if there is any antimalarial resistance in the study area that could compromise malaria treatment. Results obtained from the study will assist malaria case management bodies in planning, implementation, monitoring and evaluation of malaria control strategies in relation to when (time trend) and where (space) to: distribute health facilities close to people, improve socio-economic status, increase free malaria diagnosis and provide adequate antimalarial treatment in order to reduce malaria burden in the study area, Vihiga County, Kenya and globally.

Results from this study will assist relevant government agencies and other malaria case management bodies to frequently monitor antimalarial drug efficacy and guide study area and Kenya government on national antimalarial policy decision. The study will also assist malaria case management bodies to continue developing new malaria control strategies, antimalarial treatment and to improve the understanding of patterns of malaria prevalence in

relation to human movement, space, time trends, socio-economic factors and the genotype of *P. falciparum* that is associated with efficacy of Artemether-lumefantrine (AL) in study area.

1.6: Limitations of the Study

The research was conducted during the COVID-19 pandemic period. Most patients were reluctant to visit health facilities during this period for fear of being infected with COVID-19.

CHAPTER TWO

LITERATURE REVIEW

2.1 Spatial-temporal Distribution of Malaria Parasites

Malaria incidences are still high in Africa and Asia despite various global intervention strategies having been put in place (Zang *et al,* 2018). The geographical distribution of malaria parasites varies from region to region due to various factors that include; difference in climate, land use, drug resistance and topography (Muhammad *et al*., 2019 & Rumisha *et al*., 2019). Understanding malaria transmission patterns in relation to space, time trends and population distribution is very important in providing cost-effective implementation of malaria control strategies in order to effectively eliminate malaria (Angelica *et al*., 2020 & PMI, 2018). Malaria transmission risk is determined by the population of the adult mosquitoes which depend time trend (Wafula & Mulinya, 2018). The main malaria vectors in Western Kenya are: *Anopheles funestus, A. arabiensis and A. gambaiae* (Omukunda *et al*, 2013). Time trend and space affect economic activities and environmental factors because they determine monthly rainfall and temperature which influence mosquito breeding grounds and in turn affect malaria transmission (Chirombo *et al*., 2020; Minoo & Khodadad, 2019).

Results from Bangladesh (Ubydel *et al*., 2011) and at two high-risk districts of Nepal (Dhimal *et al.*, 2014) showed decrease in malaria prevalence because the study was able to predict malaria prevalence in relation to geographical location and time trends. Effect of space and time trend on malaria was also reported in a study carried out in: - China (Lin *et al*., 2009; Yonze *et al*., 2016 & Zhang *et al*., 2018) and at Tak Province in Thailand (Mercado *et*

al., 2019). Studies carried out in Colombia Pacific Coast and in Western Brazil also associated malaria with space and time trend (Angelica *et al*, 2020 & Kohara *et al*, 2016). In Africa studies on effect of space and time trends on malaria prevalence were carried out in: - a low endemic area of Eastern Rwanda (Rulisa *et al*., 2013), Gwanda district, Matabeleland in South Zimbabwe (Tawanda *et al*., 2017), urban Dares Salaam in Tanzania (Mlacha *et al*., 2017), Mvomera district in Tanzania (Rumisha *et al*., 2019) and Malawi (Chirombo *et al*., 2020) which showed decrease in malaria prevalence because the studies were able to identify geographical distribution and time trends of malaria cases and implemented effective malaria control strategies. Studies carried out in Northwest Ethiopia (Alemu *et al*., 2013 & Ayele *et al*., 2013) assessed whether malaria prevalence was increasing, decreasing or remained the same in different geographical regions, at different times and provided cost-effective implementation of malaria intervention strategies accordingly.

Studies on effect of space and time trend on malaria prevalence in Kenya were carried out in:- Rarieda, Bondo, Yala, Siaya and Karemo in Kisumu County which associated malaria infection to time trend and space (Nyaguara *et al*., 2012). Spatial -temporal prediction of malaria risk also carried out in Western Kenya (Gilbert *et al*, 2013). Studies on effect of space and time trend on malaria prevalence in Kenya were also reported at Kenyan Coast (Walker *et al*., 2013). Study findings on association between space, time trend and malaria were also carried out in lowlands and highlands of Western Kenya which included the following Counties; Homa bay, Siaya, Vihiga, Kisumu, Kericho, Nandi and Bomet (Gilbert *et al*., 2013; Munyekenye *et al*., 2005 & Ndenga *et al*, 2016). Results from these studies indicated malaria prevalence was still high despite intensive malaria control strategies

having been put in place. A study carried out on effect of climate on the spread of malaria in Vihiga County showed time trend had an impact on malaria prevalence (Ndenga *et al*., 2016; Wafula & Mulinya, 2018). Vihiga County lacks documentary evidence on spatio-temporal distribution of *Plasmodium* species yet this is important in guiding bodies involved in malaria case management.

There is an increase in malaria prevalence following long rains season and relatively high temperature which enables mosquitoes to breed in many sites of stagnant water, develope very fast and accelerate the development of malaria parasites in *Anopheles* mosquitoes according to studies that were carried out in Zimbambwe (Tawanda *et al*., 2017) and in Iran (Minoo & Khodadad, 2019). Increase of temperature by 1^0C tends to increase mosquito abundance and shorten incubation period of malaria parasites which increase malaria prevalence by 27% (CDC., 2019). High humidity which is as a result of heavy rainfall and high temperature accelerate the growth of malaria parasites in the *Anopheles* mosquitoes and enables the mosquitoes to live longer, increasing malaria transmission (WHO., 2020 & Omukunda *et al*., 2013). The effect of rainfall on malaria transmission is complex because too much rainfall can flush away mosquito breeding grounds while too little rainfall reduces breeding grounds for the mosquitoes leading to decrease in malaria prevalence (Nyanguara *et al*., 2012). The effect of rainfall on malaria prevalence is observed 1-2 months after the rainfall because the time taken from infective bite by *Anopheles* mosquitoes and appearance of the first symptoms of malaria is 7 to 30 days depending on the *Plasmodium* species ((Minoo & Khodadad, 2019).

The bodies in Kenya which monitor malaria case management include; President's malaria initiative, Kenya national malaria strategy, Republic of Kenya malaria indicator survey and world health organization. There are various malaria zones in Kenya which vary in geographical location, altitude, topography land use and climate which influence mosquito breeding and in turn affect malaria transmission (Ndenga *et al.,* 2016). In Kenya, malaria endemic areas are the Coastal and Lake Victoria regions (Kenya Malaria Indicator Survey, 2020 & PMI, 2020). There are two rainy seasons in Kenya with the long rains from April to June and the short rains from October to December while the highest temperatures are from February to March and the lowest from July to August (Kenya National Bureau of Statistics, 2020; Wafula & Mulinya, 2018) but this has been affected by global warming.

2.1.1: Association between Age, Gender and Malaria

It is not well understood why naturally acquired immunity against malaria develops slowly (CDC., 2015). Children below five years old and old people have low immunity level (WHO., 2015). Immunity to various diseases including malaria is high between people who are 10-50 years old (Gonzales *et al*., 2020). Children build up their acquired immunity against disease infections as they grow (Andrea, *et al.,* 2019). Immunity among children of 5-14 years old who are mainly nursery and primary school going children tend to be high compared to that of children below 5 years old (Andrea *et al.,* 2017). Children between 14-18 years old are mainly secondary and tertiary going children tend to experience more exposure to mosquito bites which lead to malaria infection and this makes them to develop more immunity to malaria infection (Gonzales *et al*., 2020).

Immunity of children above 5 years old is high and that is why their body immunity is able to fight malaria parasites (Kinyanjui, 2012). Children below 15 years old who include school-age children tend to acquire *falciparum* malaria easily as opposed to adults and children below 5 years old and they have less duration of malaria parasites in their bodies as opposed to people above 5 years old (Korir *et al.,* 2012). Malaria infection in female tend to be more than male due to socio-cultural norms that exposed female to mosquito bites (Gender and Malaria in Kenya, 2015).

2.1.2: Life cycle of Malaria Parasites

Malaria parasite life cycle consists of one phase in the mosquito and three in man (Figure 2.1) comprising of sporogonic; pre erythrocytic, erythrocytic and exo- erythrocytic respectively (CDC., 2015). In a mosquito macrogamete (female gamete) and microgametes (male gametes) fuse to form an ookinete which develops into an oocyst (Alemu *et al*., 2013). A mature oocyst with sporozoites raptures releasing sporozoites into salivary gland of a mosquito (WHO., 2015). When a mosquito bites man it releases sporozoites into human blood. Human blood is infected with sporozoites about 30 minutes after mosquito bite where sporozoites can either be destroyed by macrophages which are host's immune response or the sporozoites enter the host's liver where they multiply asexually (Makoah & Gabriele, 2013).

Plasmodium vivax and *P. ovale* sporozoites form parasites in liver (hypnozoites) where they remain dormant for a long period of time (CDC., 2015). *Plasmodium malariae* and *P. falciparum* sporozoites develop into pre-erythrocytic schizonts in the liver which takes 6-16

days post-infection. Merozoites in erythrocytes develop into trophozoites which develop into immature and finally develop into mature schizonts (WHO., 2019). Mature schizonts rupture releasing merozoites into human blood stream increasing malaria parasite density and causing fever which is used as the main clinical diagnosis of malaria (Awe *et al*., 2013). Merozoites may develop into gametocytes which is a sexual form of malaria parasite which when taken in by mosquito fertilizes to form ookinete that finally develop into sporozoites and the malaria cycle repeats (CDC., 2019).

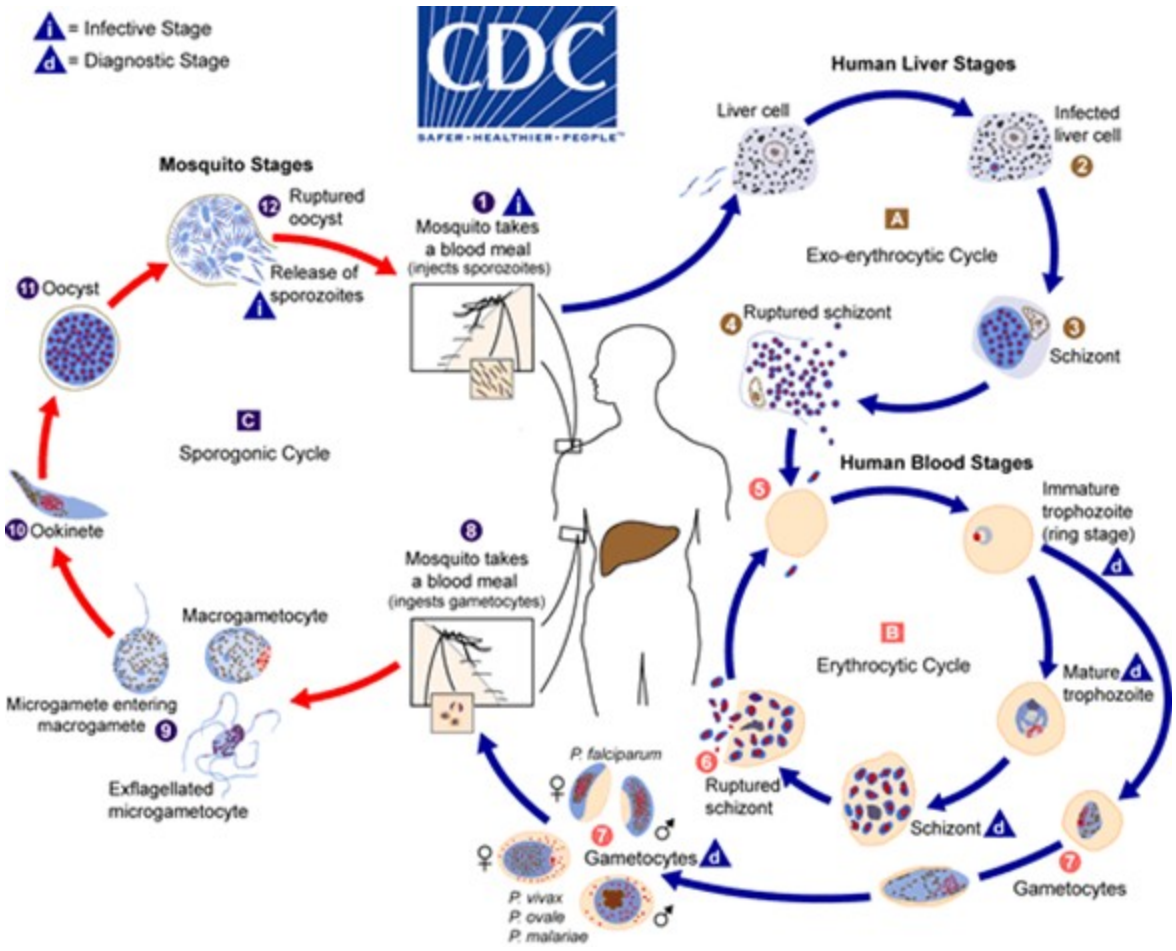


Figure 2.1: Life cycle of malaria parasite (*Plasmodium* species) (Source: CDC, 2019)

2.2 The Effect of Genotypes of Malaria Parasites on Antimalarial Drug Resistance

Factors contributing to the emergency of drug resistance include: - the prevalence of asymptomatic malaria infection, genetic mutations in genes encoding for targeted proteins in the malaria parasites and poverty which makes it difficult for poor malaria patients to prevent and treat malaria, (Makoah & Gabriele, 2013). Other causes of antimalarial drug resistance include: inadequate antimalarial treatment which can result into selective pressure for resistance, antimalarial drugs having a long half-lives and resistant malaria parasites being transmitted to other individuals by mosquitos' bites spreading antimalarial drug resistance

(Yeda, *et al*., 2016). It is important to assess genomic changes in malaria parasites which could be caused by drug pressure because it guides in auditing the efficacy of antimalarial drugs used in a given area (Rie *et al*., 2014). If an infected malaria host carries 10^{10} parasites in the body, 100 of the parasites will be drug-resistant whose number will keep increasing due to their selective genotype advantage over sensitive parasites (Kim & Schneider *et al.*, 2013).

Parasitaemia of asymptomatic malaria infections may lead to the formation of gametocytes harboring genomic mutations which confer resistance to drugs (Meera *et al*., 2016). A single point mutation is required to confer resistance in some antimalarial drugs, while for other drugs, more mutations are required to modify the phenotype of the malaria parasite (Srinivas *et al*., 2015). Antimalarial drugs are normally released in the blood circulation and given at a concentration high enough to kill the parasites but low to avoid serious adverse side effects in the patient (Rashad *et al*., 2014). Antimalarial drugs target various stages of the parasite life cycle: sporozoite, hepatic merozoites, sexual and asexual blood stages to prevent malaria parasites' growth to the next development stage (Wamae *et al*., 2019).

Antimalarial drug resistance study has been carried out in many parts of the world which include; India, China, Brazil, Pakistan and Malaysia (CDC., 2019; WHO., 2020 & Yeda *et al*., 2016). In Africa increased from 212 million in 2017 to 213 million in 2018 due to antimalarial drug resistance (Willem Roper, 2020). In Africa, antimalarial resistance study has been carried out in Nigeria, Senegal, Swaziland, and Guinea, Rwanda, Ethiopia, Sudan, Tanzania (Willem Roper., 2020). In Kenya, antimalarial resistance was carried out at the

coastal region and in Western part of Kenya at: - Kericho hospital, Kisii hospital, Kisumu hospital, and Chulaimbo hospital but resistance to artemisinin combined therapy was not reported in these regions (Akala *et al.*, 2011 & Hemming *et a1*., 2018).

Antimalarial drugs are classified as follows; artemisinin combined Therapy (ACT) such as artesunate, artemether and arteether which target blood and gametocyte stage, quinine and related compounds such as chloroquine, antifolate combination drugs such as sulfadoxine-pyrimethamine, proguanil and other sulfa drugs, antibiotics such as tetracycline, doxycycline and clindamycin, miscellaneous compounds such as halofantrine and atovaquone and combined therapy with antimalarial such as amodiaquine added to sulfadoxine-pyrimethamine which target blood stage of malaria parasite (WHO., 2018). Most currently used antimalarial drugs are closely related chemically and development of malaria parasites to drug resistance to one drug can facilitate development of resistance to others (Srinivas *et al*., 2015).

Laboratory methods for testing antimalarial drug resistance include; in vivo tests, in vitro tests, molecular genotyping, determination of drug levels, and animal models (Makoah & Gabriel, 2013**)**. It is very important to estimate the probabilities of gene resistance mutations at three stages of the parasite life cycle: sporozoite, hepatic merozoites and asexual blood stages (Wirichada *et al*., 2009). Molecular genotyping is used to differentiate between re-infection and recrudescence and provides an applicable approach in drug efficacy trials (Willem Roper., 2020). Molecular technique (PCR) is designed to detect molecular biomarkers of malaria parasites' drug resistance by determining the allelic diversity of

malaria parasites (Ines *et al*., 2011). Three *P. falciparum* genes, encoding the merozoites surface protein (MSP)-1, MSP-2 and glutamate-rich protein (GLURP), have been commonly used in clinical trials of antimalarial drug efficacy (Rashad *et al.*, 2014).

Genetic mutations in the genes encoding for target proteins in malaria parasites such as *P. falciparum* kelch protein on chromosome 13 (*Pf*K13) may result in an unusual expression and folding of these proteins modifying the usual binding site of the drug which leads to antimalarial drug resistance (WHO., 2015). Malaria parasites evade being destroyed by host's immune system by antigenic variation such as *P. falciparum* erythrocyte membrane protein 1 *(Pf*EMP1) which can cause antimalarial drug resistance (Ines *et al.*, 2011).

2.2.1 Treatment of Uncomplicated Malaria caused by *P. vivax*

Primaquine (PQ) is used under supervision as it prevents relapse, chloroquine with primaquine, ACTs plus primaquine and artesunate plus sulfadoxine-pyrimethamine (SP) are recommended for treatment of *vivax* malaria (WHO., 2019).

2.2.2 Treatment of Uncomplicated Malaria caused by *P. falciparium*

Antimalarial (first-line) treatment is 20mg of artemether and 120mg of lumefantrine (AL) given twice per day for 3 days depending on age, condition and weight of the patient (Sinclair *et al.*, 2009 & PMI, 2018). Second-line malaria treatment is ACTs, artesunate or quinine plus tetracycline or doxycycline or clindamycin and dihydroartemisinin-piperaquine (CDC., 2015). Artesunate can be given in the second and third trimester of pregnancy but the

recommended treatment in the first trimester of pregnancy is quinine and mixed malaria parasite species infections should be treated as *falciparum* malaria (WHO., 2020).

2.2.3 Treatment for Severe Malaria (*P. falciparum, P. vivax P. vivax* and *P. malariae*)

Treatment for *P. falciparum* malaria is considered as a medical emergency whose treatment is: artesunate intravenous infusion and intra muscular injection followed by artemether or quinine for both children and adult, followed by AL full dose and artesunate plus doxycycline or clindamycin (WHO., 2015). Treatment for severe malaria is the same for all the four *Plasmodium* species that cause human malaria and pregnant mothers are given artesunate (Rashad *et al*., 2014). There were improvements in the quality of malaria case-management in Kenya under the "test and treat" policy (Zurovac *et al*, 2014).

2.2.4 Effect of *Plasmodium falciparum Pf*K13 Genotype on Efficacy of Artemether-Lumefantrine Treatment

Malaria patients with antimalarial–resistant *Plasmodium* parasites are carriers of resistant gametocytes than those with antimalarial-sensitive parasites (Aouam *et al*., 2016; Kim & Schneider *et al.*, 2013). Antimalarial drugs inhibit the growth of malaria parasites by interfering with *Plasmodium* protein eventually killing the malaria parasites (Dennis *et al*., 2014 & Gardner *et al*, 2002). Artemether lumefantrine (AL) which is commonly known as coarten remains the first-line treatment for malaria treatment by targeting blood and gametocytes stages of malaria parasite and any resistance to it by malaria parasites is a threat to elimination of malaria (Eyasu *et al*., 2015). Artemisinin is a rapidly acting blood schizontocide antimalarial drug which is active against all human malaria parasites (Makoah & Gabriel, 2013).

Artemisinin combination therapy (ACT) consists of an artemisinin derivative co-formulated with another class of antimalarial such as Artemether-Lumefantrine (AL) is thought to reduce antimalarial drug-resistant parasites (Shah *et al*., 2015). ACTs offer the fastest malaria parasites clearance of all antimalarials currently used and act mainly on the trophozoite phase of malaria parasites in the host (Ishengoma *et al*., 2019). Artemisinin drugs tend to clear malaria parasites very fast depending on the developmental stage of the malaria parasite which is determined by decline in parasitaemia (Eyaso *et al*., 2015). AL has a half life of 3-5 days to destroy any residual malaria parasites in order to prevent recrudescence (CDC., 2019). Some malaria parasites may fail to be cleared by antimalarials and reappear when the concentration of the antimalarial reduces in the blood (Wamae *et al.,* 2019).

Mutations in the malaria parasites tend to alter regions on their surfaces which antimalarials bind on to destroy them hence causing resistance to these antimalarials (WHO., 2019). Mutation on kelch protein found on chromosome 13 of *P. falciparum* (*Pf*K13) is associated with artemisinin resistance (Ishengoma *et al*., 2019 & Gardner *et al*., 2002). *Plasmodium falciparum* genes which have shown evidence to cause resistance to arteminisin antimalarial are on protein kelch found on chromosome 13 (*Pf*k13) and on *P. falciparum* multidrug resistance transporter-1 (White Nicholas J., 2017).

Primers are short sequences of DNA nucleotides (around 20 nucleotides) used by all living organisms to provide starting points and provide energy for DNA synthesis and also used in DNA sequencing (Rie *et al*., 2014). Determination of resistance of malaria parasites to antimalarials can be done by molecular identification of anti-malarial resistance markers of *P. falciparum.* An enzyme called primase which is a type of RNA polymerase synthesizes

primers before DNA replication starts. RNA primers are used due to unavailability of DNA primers (CDC., 2019). Primers are used in polymerase chain reactions (PCR) to determine DNA of living organisms (WHO., 2015). PCR which relies on a thermostable DNA polymerase is used to make many copies of specific regions of interest in DNA *in vitro* or to amplify targeted areas of DNA (Srinivas *et al*., 2015).

Different *Pf*K13 mutations have different effects on the malaria clearance phenotype (Rie *et al*., 2014 & WHO., 2015) but mutations in gene mixed drug resistance 1 (*mdr* 1) in *P. falciparum* (Kelch 13) encoded by *pfmdr* 1 has been seen to render AL resistance to uncomplicated falciparum malaria in Western Kenya and other parts of the world **(**Kim and Schneider, 2013 & Shah *et al*., 2015). Amino acids found on K13 of *P. falciparum* include: cysteine (C), glutamic acid (E), methionine, alanine (A), glycine (G), asparagine (N), tyrosine (T) aspartic acid (D)., isoleucine, (I) serine (S) and threonine (T). Any change in these amino acids interfers with the structure, function and quality of protein kelch (*Pf*K13) causing mutation that may enable the malaria parasite (*Plasmodium falciparum*) to evade being destroyed by antimalarials (Wamae *et al*., 2019).

Polymorphisms in the propeller domain of the *P. falciparum* Kelch protein which is found on chromosome 13, (*Pf*K13) can cause artemisinin resistance as identified by ACT genetic marker (Shah *et al*., 2015). ACT slows the spread of drug resistance to a partner drug, especially at high coverage rates in low transmission settings (Katia *et al*., 2014 & Wirichada *et al*., 2009). Main K13 resistance mutations include: E252Q, P441L, F4461, G449A, N458Y, Y493H, P553L, R561H, V568G, P574L, A578S, C 580Y, A675V, R539T and

I543T while less frequent variants are; MA476I, C469Y, A481V, S522C, N537D, G538V, M579I, D584V and H719N (Gardner *et al*., 2002 & WHO., 2015).

Antimalarial drug resistance studies have been carried out in many parts of the world which included; India, China, Brazil, Pakistan and Malaysia (CDC., 2019 & WHO., 2015). Artemisinin-based combination therapy (ACT) resistant malaria parasites showed decreased susceptibility in Western Kenya where resistant malaria parasites showed delayed clearance of *P. falciparum* after artemisinin treatment (Angela *et al*., 2015). This was as a result of mutations in PfATP6, a Ca++ ATPase enzyme, which when interacted with artemisinin killed the malaria parasites and any inhibition of this enzyme inhibits the action of artemisinin (Bhattacharjee & Shivaprakash, 2016).

In Africa, Artemether–lumefantrine resistance in *P. falciparum* malaria patients was reported.in Tunisia (Aouam *et al*., 2016). In 2016, results indicated there was no mutation in *Pf*K13 to have compromised AL failure in Kibaha, Mkuzi, Mlimba and Ujiji in Tanzania (Ishengoma *et al*., 2019). Studies on antimalarial resistance to *P. falciparum* have also been reported in Nigeria, Senegal, Swaziland, and Guinea, Rwanda, Ethiopia, Sudan, Tanzania which increased malaria cases from 212 million in 2017 to 213 million in 2018 (Willem Roper, 2020).

Ministry of Health in Kenya changed the first line of malaria treatment from chloroquine to sulfadoxine-pyrimethamine (Fansidar) in 1998 and officially changed the first-line drug to artemether-lumefantrine (Coartem) in 2004 (CDC, 2018). In Kenya, a study on trends in

drug resistance codons in *P. falciparum* dihydrofolate reductase and dihydropteroate synthase genes in Kenyan parasites from 2008 to 2012 was carried out with an aim of reducing malaria burden where resistance of *P. falciparum* to chloroquine, sulfadoxine-pyrimethamine and nonsynonymous mutation on *Pf*K13 was reported (Dennis *et al*, 2014). At the Coastal region of Kenya, *Pf*K13 arteminisinin resistance markers were not detected but other *Pf*K13 were observed in low frequencies which did not affect efficacy of arteminisinin (Wamae *et al*., 2019).

Some single nucleotide polymorphism (SNPs) in chromosome 13 were identified among study populations in Kericho hospital, Kisii hospital, Kisumu hospital, Chulaimbo hospital in Western Kenya (Akala *et al.*, 2011). No *Pf*K13 mutations affected efficacy of arteminisinin in the study populations in Western Kenya (Akala *et al.*, 2011; Angelica *et al*., 2015; Daibin *et al*., 2008 & Hemming *et a1*., 2018). Studies in Western Kenya suggested that efficacy of arteminisinin may be compromised in future because of the nonsynonymous mutations that were identified in *Pf*K13 which could be potential candidates for arteminisinin resistance (Akala *et al.*, 2011 & Shah *et al*., 2015). Study findings have shown that: - M476I, C580Y, Y493H, R539T, I543T and C469T within gene propeller region of Pfk13 can also cause resistance to artemisinin (Ines *et al*., 2011). Study findings in Nyando, Kisumu County Western Kenya identified novel non-synonymous mutations in *Pf*K13 a region associated with artemisinin resistance (Musyoka *et al.*, 2020). The absence of *Pf*K13 mutations associated with *P. falciparum* to AL was reported in the findings of a study that was carried out among children aged 5-59 months in Siaya County, Western Kenya from 2016-2017 (Chebore *et al*., 2020). A study on antimalarial resistance was carried out in

Kakamega which is a low malaria-transmission site, Bondo, Vihiga Counties and in Kombewa a high malaria-transmision site in Western Kenya from 2003 to 2015 (Hemming *et al*., 2018). Findings from this study indicated a molecular marker of artemisinin resistance linked at number 86 of mutations in the Kelch 13 -propeller domain (K13) did not delay parasite clearance rate among artemisinin-treated malaria patients in these regions found in Western Kenya (Hemming *et a1*., 2018 Chebore *et al*, 2020). AL achieved cure rates of up to 95%, in areas of multi-drug resistance and mutant alleles at codons 51 and 59 were detected at high frequencies without mutation detected at codons 16, 50, and 164 of *pfdhfr* in Western Kenya highlands (Daibin *et al*., 2008).

2.3: Influence of socio-economic Factors on Malaria Prevalence

Socio-economic factors refer to an individual's social and economic status in relation to other people in a given area (Ravendra *et al*., 2015). The following factors determine malaria transmission by influencing mosquito density (Omukunda *et al*., 2013) and frequency of mosquito bites include; salary, occupation, household size, level of education, age and sex of head of household, type of houses in the compound, economic status (income), residence (Humes *et al*., 2008) which can be in urban or rural areas, use of bed nets, socio-cultural practices, stagnant water, vegetation, farm use and the size of the farm, household size, time of fetching water for domestic use, nutrition which is linked to education and economic status, how far homes are from source of domestic water and sources of water for domestic use (Tusting *et al*., 2020 & Yadar *et al*., 2014).

A research on the effect of socio-economic factors on malaria occurrence was conducted in Northern India (Ravendra *et al*, 2020) and the results obtained showed that the type of house, income, knowledge and awareness about malaria and use of bed nets were positively associated with malaria occurrence (Nyamweya, 2012 & Yadar *et al.*, 2014). Effect of socio-economic factors on malaria infection was also reported in Pakistan (Muhammad *et al.,* 2019). Malaria burden in sub-Saharan Africa was reduced by improving socio-economic status of people in these regions (Degarege *et al.,* 2019 & Tusting *et al.,* 2017). In Nigeria, socio- economic factors influenced malaria incidences in Calabar region (Ibor, & Okoronkwo, 2017) where treatment and control of malaria attracted more financial and human resources (Usman *et al.,* 2011). A study carried out in: rural Mozambique (Humes et al., 2008) rural Uganda (Tusting *et al*., 2016) and in Western Ethiopia (Tefera *et al*., 2020) indicated that inequalities in socio-economics affected malaria prevalence. A study carried out in rural Western Kenya but not in study area showed that socio-economic factors led to malaria health inequalities and pro-poor intervention of malaria (Siekmansa *et al*., 2012 & Were *et al.*, 2018).

Socio-economic status influences malaria prevalence as they determine the individual's knowledge and attitude towards malaria infection and control as it was reported in India (Ravendra *et al*., 2015), Northern Tanzania (Luwasa *et al*., 2018) and in rural Western Kenya but not in the study area (Were *et al*., 2018). Socio-economic status influences malaria transmission as it determines the individual's knowledge and attitude towards malaria infection and control (Makoah & Gabriele, 2013). High levels of poverty and low education level in Kenya have led to malaria patients engaging in; self-antimalarial prescription

without laboratory test for malaria, failure to adhere to antimalarial treatment given and use of counterfeit drugs (Kenya National Bureau of Statistics, 2020). Use of counterfeit drugs can lead to antimalarial treatment failure that may be due to mutation in *Plasmodium* parasites resulting in spread of antimalarial drug resistance in the population that impact negatively to malaria control (Musyoka *et al*., 2020).

Inequalities in Socio-economic factors infuenced malaria transmission in sub-Saharan African countries and in India (Gabriel *et al.*, 2021 & Ravendra *et al*., 2021). Poverty can lead to malaria re-infection and even death if no antimalarial drug is taken by malaria patients on time (Were *et al*., 2018) or lack of knowledge towards malaria infection and adherence to antimalarial drugs prescribed by health workers (Kurumop *et al.*, 2013; Ibor & Okoronkwo, 2017). Perceptions and knowledge towards malaria infection and antimalarial compliance determines emergence of antimalarial resistance in malaria parasites (Ines *et al.*, 2011) which include failing to adhere to antimalarial drugs (CDC., 2015).

Anopheles mosquitoes that transmit *P. falciparum* malaria can gain entry into the house if there is no ceiling board in the house and through open windows and doors which also release insecticide sprayed to kill mosquitoes in the house (Atieli *et al*., 2009). *Anopheles* mosquitoes rest on vegetation within the home compound or on vegetation at the source of domestic water and bite in the evening causing malaria infection (CDC., 2019). House structure which seems to be linked to economic status and education level of a family determines mosquito bites because semi-permanent houses tend to allow mosquitoes to gain entry into the house even when doors and windows are closed (Tusting *et al*., 2017). The

head of the household's level of education and income tend to have an effect on malaria transmission because he or she may have some knowledge on malaria control (Ravendra *et al*., 2015). Positive attitude and correct perception towards malaria infection which include prevention and management of malaria and adherence to antimalarial drugs prescribed by health workers was reported in Northern Tanzania (Luwasa *et al.*, 2018).

Poor people cannot afford to go to health facilities and instead purchase drugs over the counters which might cause antimalarial failure and later antimalarial drug resistance (Degarege *et al.*, 2019 & Lucy *et al*., 2016). Poverty can also lead to people turning to use herbs due to high medical costs to be incurred and some visit traditional medicine people who might give them wrong medication (Usmam *et al*., 2011). Mosquito bed nets are supposed to cover beds yet most poor people do not have beds and it becomes difficult for them to use the nets available (Ndenga *et al*., 2016 & Ravendra *et al*., 2020). Treatment adherence and compliance to antimalarial drugs can be due to lack of adequate knowledge on its side effects which can be linked to poverty and education (Gabriel *et al*., 2021 & WHO.,2020). Most people in rural areas who are not well educated and not exposed to current information through media or through other means such as attending malaria days to be educated on management of malaria (Humes *et al*., 2008 & Republic of Kenya Malaria Indicator Survey, 2020). Some people tend to link malaria to being rained on or a curse or a punishment from God as opposed to being bitten by mosquitoes (Republic of Kenya Malaria Indicator Survey, 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study area was Mbale town and its environs (Figure 3.1 and Figure 3.2) which was identified by a global positioning systems (GPS) mapping at Vihiga County headquarters. Mbale town is the headquarters of Vihiga County, found within Vihiga and Sabatia sub-Counties, Western Kenya, along Kisumu-Kakamega Road. It is located at latitude 0° 0'54.0'N and longitude 34°43'17.0 E with an altitude of 1200 meters above sea level, rainfall of between 1800mm to 2000mm annually, highest in April (288 mm) and lowest in January 94 mm (Republic of Kenya, Vihiga County. First County Integrated Development Plan 2013-2017). Highest average temperature occurs in February (21.4^0 C) and lowest in July (19.1^0C) with an annual average temperature of 20.4^0 C (Wafula & Mulinya, 2018). Economic activities in the study area included; crop farming, livestock keeping, fishing, brick making, mining and trading (Kenya National Bureau of Statistics, 2020).

The study area is a malaria holoendemic zone as shown in figure 3.1 with high malaria mortality despite various malaria control strategies having been put in place (Wafula & Mulinya, 2018). The study area is resource limited with poverty level of 62%, high dependency ratio of 100.90 and high population of 60, 000 people (Kenya National Bureau of Statistics, 2020). A baseline survey was done to familiarize with mosquito breeding grounds in the study area before the study commenced. The study covered three malaria hotspot wards in Mbale town and its environs namely: - Central Maragoli, Edzava and Lugaga/Wamuluma Edzava wards.

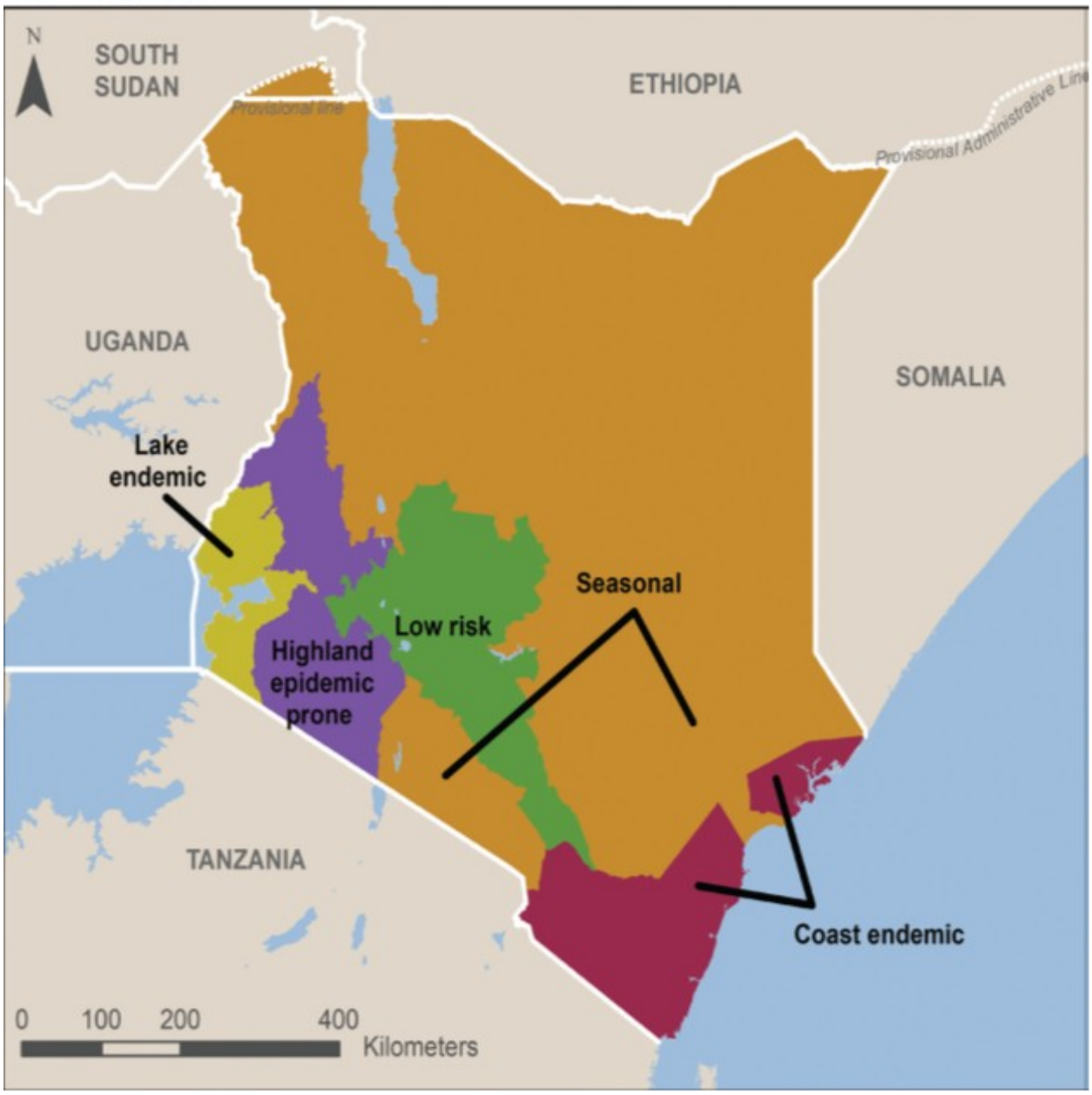


Figure 3.1: A Map of Kenya Showing Malaria Zones

Source: Kenya Malaria Indicator Survey, (2020).

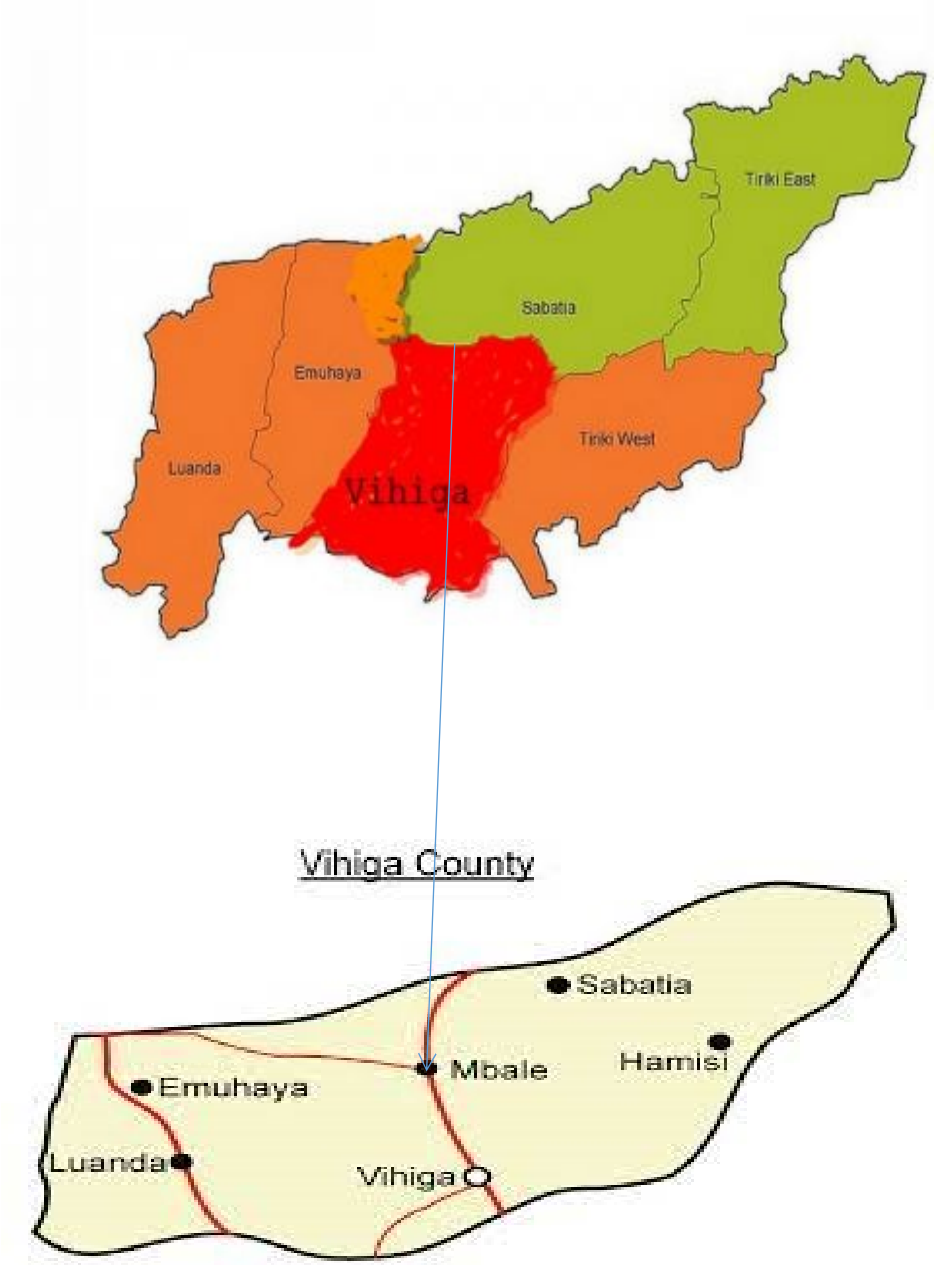


Figure 3.2: A Map Showing the location of Mbale town in Vihiga County, Western Kenya

Source: Vihiga County, Department of physical planning (2018)

3.2 Study Population

The Population of people in Mbale town and its environs was 60,000 according to population census that was carried out in 2019 (Kenya National Bureau of Statistics, 2020). The study was purposive to establish the distribution of *Plasmodium* species in different age groups in the study area. The study recruited 768 microscopically confirmed malaria patients from the study area who presented themselves to Mbale Provincial Rural Training Health Center. Sample size should have been 382 (Krejcie & Morgan, 1970) confirmed malaria patients but the research used 768 sample size to cover more malaria patients and to determine distribution of *Plasmodium* species in the densely populated malaria holoendemic study area. The sample population chosen volunteered to participate in the study and signed a written consent (Appendix I & II) before the study commenced as per WHO guidelines. For preparation of dry blood spots (Appendix III), blood from 384 confirmed *Plasmodium* falciparum malaria patients, 96 from each of the four age groups was used.

3.3: Inclusion and Exclusion Criteria

Malaria confirmed patients/guardians who voluntered to participate in the study were recruited. The study was purposive where equal number of 192 malaria confirmed patients were recruited for each of the following age groups; children below 5 years old, children between 5-14 years old, children between 14-18 years old and adults above 18 years old. Malaria patients with; allergy to antimalarial drugs, chronic infections and people who had been on antimalarial treatment within the preceding 2 weeks were excluded from the study as per world health organization regulations (WHO., 2020). The study also recruited 5 research assistants, parents and guardians of children who were chosen. Clinicians and laboratory

technicians from Mbale Provincial Rural Training Health Center diagnosed *Plasmodium* speies. Laboratory technicians from KEMRI Nairobi assisted with extraction of dry blood spots from *P. falciparum* and Inqaba Biotec in South Africa handled DNA sequencing.

3.4: Sample Size Determination

The sample size formula below was adopted from Krejcie and Morgan, (1970).

$S = X^2NP(1-P)/d^2(N-1) + X^2P(1-P)$

S =Desired sample size

X = Z-value = 1.96 for 95% confidence level

N = Population size (60,000)

P = Population proportion = 0.5

d = degree of accuracy = 0.05

$S = (1.96)^2(60,000)\ (0.5)\ (0.5)/\ (0.05)^2(59,999) + (1.96)^2(0.5)\ (0.5)$

S = 382

3.5: Study design

Descriptive study design was adopted (Mugenda and Mugenda, 2003).

3.6: Study Period

The study was carried out for a period of one year, from December 2019 to November 2020 to cover both long and short rain seasons in the study area.

3.7: Data Collection

At the triage room of Mbale Provincial Rural Training Health Center, anthropometric characteristics of the study population were recorded as per world health organization standards (WHO, 2020). Patients who were observed with signs of malaria by doctors at the health facility were screened by qualified laboratory technicians using microscopy and observation as per world health organization standards (WHO, 2020). Health workers interviewed malaria patients as they administered semi-structured questionnaires (Appendix VI and VIII) stratifying them into; age groups, gender, various socio-economic status and their residence (Kenya Malaria Strategy- 2009-2017).

3.7.1 Spatial - temporal Distribution of Malaria Parasite Species by Microscopy

Preparation of both thin and thick malaria films were prepared among patients who showed clinical observations of malaria which included: - headache, vomiting, fever, joint pains and fatigue using WHO standards (WHO, 2020). The area for finger-prick by a lancet to collect blood was sterilized with methylated spirit and allowed to dry before blood collection was done. A blood film was air dried, fixed with methanol (thick film was not fixed), dried for 1 minute using a fan heater, stained with 10% giemsa stain for 20 minutes, giemsa stain cleaned with tap water, put in a water buffer of pH 7.2, immersed in oil and observed under 100 x microscope objectives. Thin film was used to examine different *Plasmodium* species, thick film to quantify malaria parasites and results obtained recorded against the malaria patient's name (WHO., 2015).

Thick film

Malaria parasites counted

_____ X 8000 = number of parasites/ul

200 white blood cells

Thin film

Parasitized red blood cells

_____ X 5,000 000 = number of parasites/ul

4000 red blood cells

3.7.2: Effect of *P. falciparum Pf*k13 on Efficacy of Artemether-Lumefantrine

A drop of blood from the finger of dry blood spot were prepared from 384 confirmed *P. falciparum* malaria patients. Blood from *P. falciparum* malaria patients was transferred to circles on filter cards of dry blood spot kitties (DBSs) without touching the surface directly with the fingertip, done at Mbale Provincial Rural Training Health Center. The blood was allowed to soak into the texture of the filter by capillary forces only and dried at room temperature. For storage, the filter paper cards were put in single, gas-impermeable ziplock bags, containing 1 to 2 desiccant sachets to protect the specimens from moisture and stored at room temperature (21°C to 24°C). For filter cards initially kept at ambient temperature, a triple packaging system was used, which consisted of the zipper bag(s) as the inner container(s) as well as an inner and an outer envelope. The filter cards were bearing the patient's number which bears the name, age, gender, location (ward) and date of blood collection written on in individual plastic bag (Appendix III). After preparation of dry blood

spots (DBSs) at Mbale Provincial Rural Training Heath Center, the DBSs were transferred to KEMRI, Nairobi for DNA extraction using qiagen method as per instructions from the manufacturer (QIAampDNAminiand blood mini-Handbook- Qiagen). Genomic DNA was extracted from the dried blood samples at at KEMRI, Nairobi using QIAamp DNA blood mini kit-Qiagen following the manufacturer's protocol (QIAamp DNA blood mini kit-Qiagen Handbook). Three punched out circles from DBSs were placed at the bottom of well blocks and lysis Buffer ATL (contains sodium dodecylsulfate and EDTA) added. Buffer AL (which contain malic acid and guanidine hydrochloride 30-50%) was used to complete cell lysis and binding of DNA to the silica gel and Proteinase K digested proteins to free DNA from other cellular components through incubation. To allow DNA to precipitate in pellet form, 96–100% ethanol was added to the samples. Washing agents were AW1 (contains low concentration of guanidine hydrochloride) which removed proteins from DNA.

AW2 contained Tris-based 70% ethanol solution, removed salts from DNA. The QIAamp Mini spin column was carefully opened and 150 µL Buffer AE added (Elution Buffer AE contain10mM Tris.HCL; 0.5mM EDTA -Ethylene diamine tetraacetic acid; pH 9.0). It was incubated at room temperature (15–25°C) for 1 minute, and then centrifuged at 6000 x g (8000 rpm) for 1 minute. All the solution was removed and 70% ethanol was added to clean the pellets which were centrifuged for 2 minutes for spinning and DNA extracted was air dried. Extracted DNA was stored at - 20°C then shipped to Inqaba Biotec in South Africa on ice packs in cryo boxes. Total of 52 DBSs failed DNA extraction, 44 failed sanger sequencing and 288 DNAs were successfully sequenced (Appendix IV & V).

At Inqaba Biotec extracted DNAs were amplified using nested polymerase chain reaction. Primers for First PCR reaction were K13-1 and K13-4. This is where the 2 strands of a DNA double helix were physically separated at high temperatures of 94-95^{o}c for 15 to 30 seconds to melt (denature) DNA. Primers for second PCR reaction were K13-2 and K13-3. This is where the temperature was lowered to 50- 65^{0}c for 10 to 30 seconds to allow the DNA primers to attach to the template of DNA. Two primers were given sequences that made them bind to opposite strands of the template DNA just at the edges of the regions to be copied. Forward primers attached to the start codon of the template DNA while the reverse primer attached to the stop codon of the complementary strand of DNA in a 5' to 3' direction. In Sanger sequencing DNA fragments were separated to different sizes using gel electrophoresis within the sequencing machine and the unknown DNA sequence was identified.

Table 3.1: Primers and Gene Fragments used in Nested PCR for Evaluating AL Resistant *P. falciparum* Markers among Malaria Patients in the Study Area

Gene fragment (K13 Propeller)	PCR Primer Sequence (5'-3')
First Reaction Primers	
K 13-1	
CGGAGTGACCAAATCTGGGA	
K 13-4	
GGGAATCTGGTGGTAACAGC	
Second Reaction Primers	
K13-2 Forward primer	GCCAAGCTGCCATTCATTTG
K13-3 Reverse primer	GCCTTGTTGAAAGAAGCAG

Source: Research data

In addition to using PCR to determine efficacy of AL, the study also used microscopy to determine if *P. falciparum* parasitaemia had decreased or increased in the blood of malaria patient's day 3 after initiate on of AL treatment following WHO guidelines (Appendix IV). This was done with the assistance of community health workers who moved in the study area and using mobile phones to remind the study population to go to their nearest public health facilities to retest *P. falciparum* malaria (Sophia *et al*, 2014). This was to check if malaria had been cleared in their blood 3 days after AL treatment and if they still showed severe malaria symptoms after initiation of AL treatment following WHO guidelines (WHO, 2015). In some cases, malaria patients who felt they were not getting better after initiation of AL treatment, volunterly went back to the chosen medical facility for re-testing of malaria

parasites. If a malaria patient had responded effectively to AL treatment the temperature should have reduced to below 37.5^{o}c with reduced *P. falciparum* parasitaemia 72 hours after initiation of AL treatment as per WHO guidelines (Appendix VI).

3.7.3: Influence of Socio-Economic Factors on Malaria Prevalence

A questionnaire and face to face interview were used to collect data on effect of socio-economic factors on malaria prevalence following guidelines from Kenya National Malaria Strategy 2009- 2017 guidelines**.** The questionnaire was coded with the same code on the slide and filter paper for each patient. The questionnaire was administered after malaria patients and guardians signed the informed consent (Appendix VII & VIII).

3.8: Data Management and Statistical Data Analysis

Data was entered in spread sheets and analyzed using a SPSS software version 17. Descriptive and inferential data analyses were done. Results were presented in frequency tables and bar graphs to compare means of malaria parasite species' distribution in wards, age groups, gender, distribution of socio-economic factors in the study population. Linear regression and correlations were used to analyse time trends (temperature and rainfall). Chi-squared and multinomial regression were used to analyse distribution of *Plasmodium* species among wards, age groups gender. Chi-squared was also used to analyse effect of socio-economic factors on malaria prevalence in the study area. *P-value* ≤ 0.05 was considered statistically significant in all tests. Line graphs were used to determine effect of time trend and effect of socio-economic on malaria prevalence in the study area.

3.9: Ethical Approval

Since the study involved people, an ethical letter was obtained before data collection commenced. A written informed consent was signed by adult malaria patients and parents or guardians signed for malaria patients below 18 years old before the study commenced and their confidentiality was guaranteed (Appendix 1 & II). Masinde Muliro University of Science and Technology Institutional Ethical Review Committee granted an ethical review letter (IX & X) while Kenya National Commission for Science, Technology and Innovation provided permit (XI) for data collection.

CHAPTER FOUR

RESULTS

4.1: Spatial and Temporal Distribution of *Plasmodium falciparum*

According to the results of microscopic malaria diagnosis obtained (Table 4.1) the distribution of *Plasmodium* species in different geographical locations (ward) in the study area were as follows; out of 768 malaria patients who were recruited in the study, *P. falciparum* malaria constituted 98.7% while *P.* malariae 0.8%, *P. vivax* 0.4% and *P. ovale* constituted 0.1%. *Plasmodium falciparum* which is highly infectious accounted for 98.7% and it occured at the peak during and after rain seasons, while *P. ovale*, *P. vivax* and *P. malariae* accounted for 1.3%. *P. ovale* and *P. vivax* normally undergo relapse which could be the reason why it was rare. During rain seasons many mosquito breeding grounds were identified in Lugaga/Wamuluma ward, created by poor drainage systems, poor disposal of waste containers and many economic activities resulting to high malaria prevalence.

According to the results obtained from the study area, it was observed that distribution of *Plasmodium* species (malaria parasites) varied in geographical locations as follows; Central Maragoli ward recorded 143 malaria patients infected with *P. falciparum*, 1 with *P. malariae and P. ovale* and none with *vivax.* Edzava ward recorded 272 malaria patients infected with *P. falciparum,* 2 with *P. malariae, P. ovale* infected 1 malaria patient and *P. vivax* 3 patients. Lugaga/ Wamuluma ward recorded 343 *P. falciparum* malaria patients *P. malariae* 3 patients *and P. ovale* and *vivax* none (Table 4.1).

Table 4.1: Distribution of *Plasmodium* Species in the Study Area

Geographical distribution (Ward)	*Plasmodium* species				Total
	P. falciparum	*P. malariae*	*P. ovale*	*P. vivax*	
Central Maragoli	143 (99.3%)	1 (0.7%)	0 (0%)	0 (0%)	144
Edzava	272 (97.8%)	2 (0.72%)	1 (0.4%)	3 (1.1%)	278
Lugaga/ Wamuluma	343 (99.1%)	3 (0.87%)	0 (0)	0 (0%)	346
Total	758 (98.7%)	6 (0.78%)	1 (0.13%	3 (0.39%)	768

Results are presented as number (n) and proportions (%) of subjects

Immunity to various diseases including malaria is high between people who are 10-50 years old. Children build up their acquired immunity against infection as they grow. The study grouped the study population into different age groups because of difference in their immune systems. According to study findings on gender and malaria in Kenya, women seem to be infected more with malaria because of socio-cultural norms that exposed them to mosquito bites.

Results obtained from the study area showed that distribution of *Plasmodium* species (malaria parasites) varied with gender and age groups (Table 4.2). *Plasmodium falciparum* is found during both long and short rain seasons and this explains why it was the most common *Plasmodium* species in the study area.

The analysis obtained showed that prevalence of *Plasmodium* species varied with age in the study area. *Plasmodium falciparum* infection in the study area was associated with age. *Plasmodium falciparum* infection was the most common type of malaria that was identified

in all age groups. Children below 5 years old and adults recorded *P. falciparum*, *P. malariae* and *P. vivax* malaria but not *P. ovale.* Children aged 5-14 years old recorded *P. falciparum, P. malariae* and *P. ovale* malaria. Children aged 5-14 years old were the only age group that recorded *P. ovale* malaria but not *P. vivax* malaria. Children aged 14-18 years old unlike malaria patients in other age groups did not record *P. malariae* and recorded the highest *P. falciparum* malaria in the study area.

Results obtained showed that 14-18 years old malaria who were mainly secondary school going patients were likely to have been exposed to more mosquito bites than other malaria patients, recorded slightly more *P. falciparum* malaria cases than other age groups with no records of *P. malariae and P. ovale* malaria. The study aimed at determining if gender had any effect on malaria prevalence in the study area. There was slightly more malaria prevalence in female than male in the study area which was likely to have been due to socio-cultural norms that exposed female to mosquito bites. From the results obtained 49.3% malaria patients were male while 51.7% were female. Among female, *P. ovale* was not recorded while all *Plasmodium* species were recorded among male malaria patients.

Table 4.2: Distribution of *Plasmodium* species by gender and age groups in the study area

	Plasmodium species				Total
	P. falciparum	*P. malariae*	*P. ovale*	*P. vivax*	
Age, years					
0-5	188 (97.92%)	3 (1.56%)	0 (0%)	1 (0.52%)	192
5-14	189 (98.43%)	2 (1.94%)	1 (0.52%)	0 (0%)	192
14-18	191 (99.48%)	0 (0%)	0 (0%)	1 (0.52%)	192
Adults	190 (98.96%)	1(0.52%)	0 (0%)	1 (0.52%)	192
Total	**758** (98.70%)	**6** (0.78%)	**1** (0.13%)	**3** (0.39%)	**768**
Gender					
Male	366 (98.65%)	2 (0.54%)	1 (0.27%)	2 (0.54%)	371
Female	392 (98.74%)	4 (1.01%)	0 (0%)	1 (0.25%)	397
Total	**758** (98.70%)	**6** (0.78%)	**1** (0.13%)	**3** (0.39%)	**768**

Results are presented as number (n) and proportions (%) of subjects**.**

Chi-square results obtained showed effect of wards (p-value = 0.036) on malaria prevalence was statistically significant as opposed to gender (p-value = 0.339) and age (p-value = 0.669) in the study area (Table 4.3).

Table 4.3: Pearson Chi-Square test on the effect of wards, gender, age on malaria prevalence

Location and demographic factors	*p* - value
Ward	0.036
Gender	0.339
Age	0 .669

Statistically significant at *p* - value ≤ 0.05 level (2-tailed).
Dependent variable: Malaria prevalence
Independent variables: Ward, gender, age

From the multinomial regression analysis obtained in table 4.4, independent variables (wards, age and gender) showed the following effect on malaria prevalence ([F (3,764) = 1.854], $p < 0.136$). Individually wards (space) had a statistically significant effect on malaria prevalence (([F (3,764) = 1.854], $p = 0.034$) as opposed to gender ([F (3,764) = 1.854], $p = 0.321$), and age ([F (3,764) = 1.854], $p = 0.712$)

Table 4.4: Multinomial Regression Analysis on Effect of Wards, Gender and Age on Malaria Prevalence

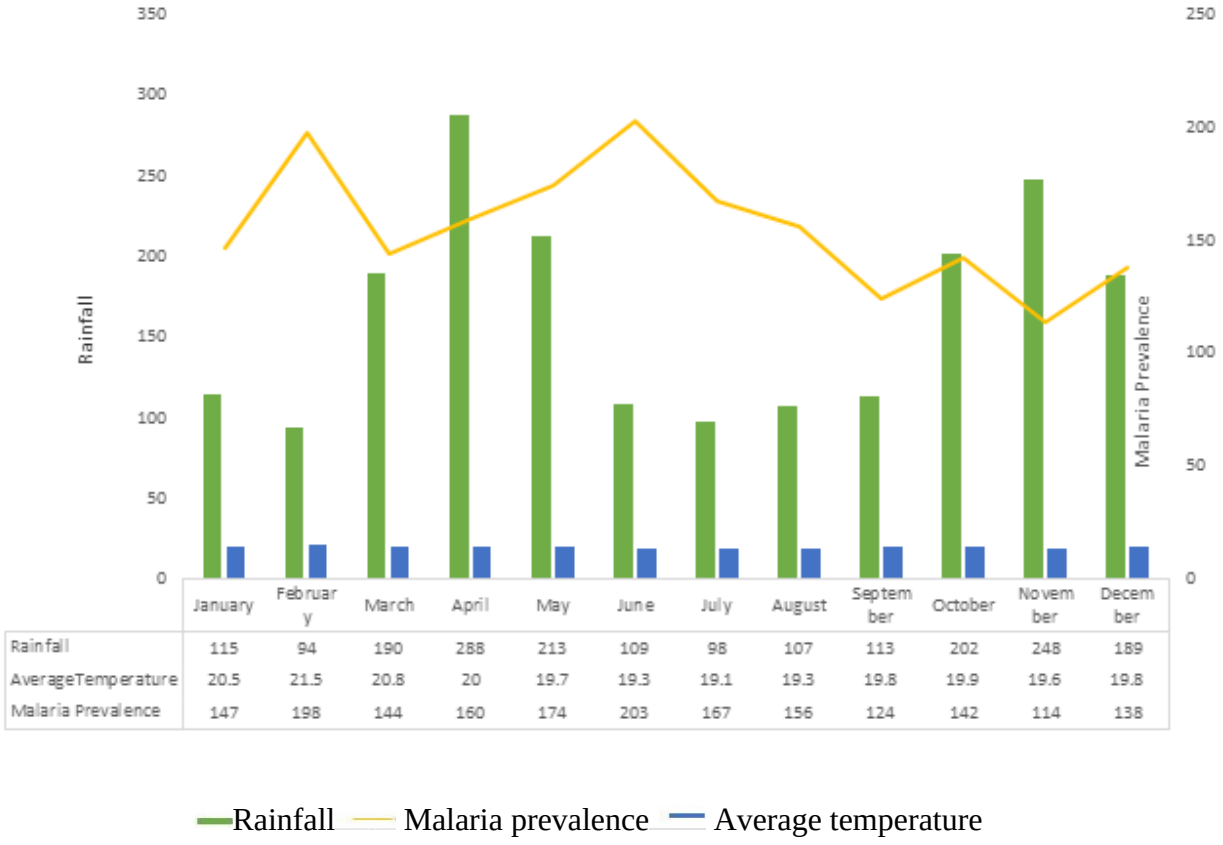
Coefficients[a]

Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**T**	**Sig.**	**95.0% Confidence Interval for B**	
		B	**Std. Error**	**Beta**			**Lower Bound**	**Upper Bound**
	(Constant)	351.207	39.242		8.950	<0.000	274.171	428.242
	Ward	22.452	10.600	0.076	2.118	0.034	1.643	43.260

	Gender	-15.893	16.006	-0.036	-0.993	0.321	-47.315	15.528 16.679
	Age	2.637	7.153	0.013	0.369	0.712	-11.404	

Results of regression analysis showing standardized and unstandardized beta coefficients, standard error, and 95% confidence intervals. Statistically significant at p - value ≤ 0.05 level (2-tailed). Malaria prevalence was entered in the model as the dependent variable, while independent variables were Ward, Gender, and Age.

Effect of rainfall on malaria prevalence was observed 1-2 months after the rainfall (Figure 4.1). High rainfall observed in April in the study area might have led to increase in mosquito breeding grounds which might have led to high malaria prevalence that was observed in June. Low temperature and low rainfall in July was likely to have resulted to comparatively low malaria prevalence in September.



	January	February	March	April	May	June	July	August	September	October	November	December
Rainfall	115	94	190	288	213	109	98	107	113	202	248	189
AverageTemperature	20.5	21.5	20.8	20	19.7	19.3	19.1	19.3	19.8	19.9	19.6	19.8
Malaria Prevalence	147	198	144	160	174	203	167	156	124	142	114	138

Figure 4.1: Effect of time trend on malaria prevalence in study area in 2020

From the correlation results obtained, months (p-value = 0.074), rainfall (p-value = 0. 211) and average temperature (p-value = 0.836) had a statistically insignificant effect on malaria prevalence in the study area (Table 4.5).

Table 4.5: Correlation between Time Trend and Malaria Prevalence

Correlations					
		Month	Rainfall	Average temperature	Malaria
Month	Pearson Correlation	1	0.206	-0.690*	-0.534
	Sig. (2-tailed)		0.521	0.013	0.074
	N	12	12	12	12
Rainfall	Pearson Correlation	0.206	1	-0.103	-0.389
	Sig. (2-tailed)	0.521		0.750	0.211
	N	12	12	12	12
Average temperature	Pearson Correlation	-0.690*	-0.103	1	0.067
	Sig. (2-tailed)	0.013	0.750		0.836
	N	12	12	12	12
Malaria	Pearson Correlation	-0.534	-0.389	0.067	1
	Sig. (2-tailed)	0.074	0.211	0.836	
	N	12	12	12	12

Results presented are Pearson's correlation coefficient and the associated p-values. *. Correlation is significant at the 0.05 level (2-tailed).

From the multiple linear regression analysis obtained, time trend which varied in rainfall and temperature had the following effect on malaria prevalence (R^2 = 0.527, [F (3, 8) = 2.976], p < 0.097). Months had a statistically significant effect on malaria prevalence in the study area (R^2 = 0.527, [F (3, 8) = 2.976], p = 0.036) as opposed to rainfall ((R^2 = 0.527, [F (3, 8) = 2.976], p =0.310) and average temperature ((R^2 = 0.527, [F (3, 8) = 2.976], p = 0.137) according to results obtained in table 4.6.

Table 4.6: Multiple Linear Regression Showing Effect of Time Trend on Malaria Prevalence

Coefficients					
Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
	B	Std. Error	Beta		
Constant[a]	590.995	238.779		2.475	0.038
Predictors[b]					
Temperature	-18.768	11.366	-0.555	-1.651	0.137
Rainfall	-0.110	0.101	-0.269	-1.083	0.310
Month	-6.430	2.552	-0.862	-2.520	0.036

Results of regression analysis showing standardized and unstandardized beta coefficients, standard error, and 95% confidence intervals. Statistically significant at p - value ≤ 0.05 level (2-tailed). a. Dependent Variable: Malaria Prevalence. b. Predictors: (Constant), Month, Rainfall, Average temperature

The community health workers in Vihiga County where the study area falls have been trained to test for malaria using rapid diagnostic test (RDT) and administer antimalarial drugs to malaria patients in their homes. Most mothers and guardians preferred to have their children treated at home for malaria infection during COVID-19 pandemic period which reduced the number of children below 5 years seeking treatment in health facilities (Figure 4.2).

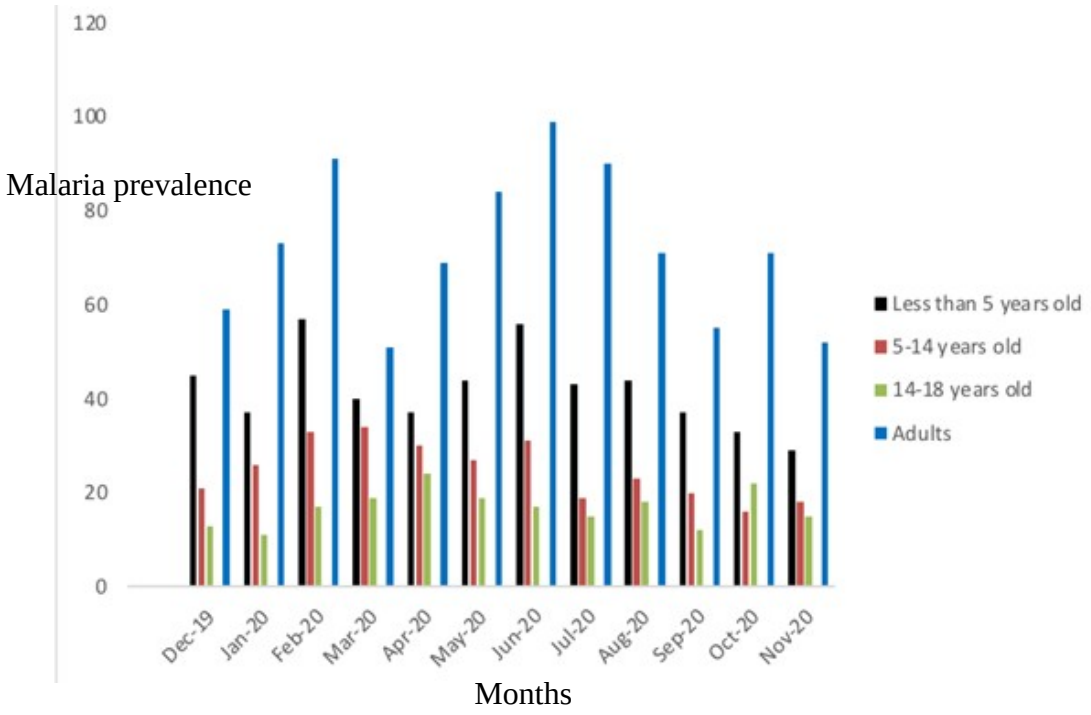


Figure 4.2: Malaria prevalence per month among different age groups in the study area from December 2019 to November 2020

4.2: *Plasmodium falciparum's* Genotype on Efficacy of Artemether- lumefantrine

Under close supervision all *P. falciparum* malaria patients in the study population recovered after taking AL as per the doctors' prescription. Delay of AL to clear *P. falciparum* was likely to have been caused by lack of compliance and adherence by *P. falciparum* malaria patients but not genotype of *P. falciparum*. None of the *Pf*K13 genetic changes (synonymous mutations) affected efficacy of AL in the study area. Transition and transverse mutation were identified in the DNA of *P. falciparum* found in dry blood spots collected from *P. falciparum* malaria patients in the study area (Table 4.7). In transition, there was interchange of one-ring pyrimidines (T, C) or two-ring purines (A, G) which involve bases of similar strucutres. In

transversion mutation, a single purine (Aor G) was exchanged for a pyrimidine (Tor C) and vise versa which involve bases of different shapes (Table 4.7). The 726 amino acids found on K13 of *P. falciparum* which include: cysteine (C), glutamic acid (E), methionine (M), alanine (A), glycine (G), asparagine (N) according to CDC, 2019.

Table 4.7: Pfk13 Polymorphism in Malaria Patients in the Study Area

Name	Minimum	Maximum	Length	Change	Coverage	Polymorphism Type	Variant Frequency
A	2128	2128	1	C -> A	1	SNP (transversion)	100.00%
T	2121	2121	1	C -> T	4	(transition)	25.00%
C	2100	2100	1	A -> C	6	SNP (transversion)	8.30%
G	1987	1987	1	C -> G	12	SNP (transversion)	4.20%
T	1953	1953	1	A -> T	12	SNP (transversion)	4.20%
C	1929	1929	1	A -> C	12	SNP (transversion)	4.20%
T	1862	1862	1	C -> T	12	SNP (transition)	4.20%
A	1546	1546	1	G -> A	12	SNP (transition)	4.20%
C	1529	1529	1	T -> C	12	SNP (transition)	4.20%
C	1491	1491	1	T -> C	12	SNP (transition)	4.20%
G	1438	1438	1	A -> G	12	SNP (transition)	4.20%
G	1436	1436	1	A -> G	12	SNP (transition)	4.20%
G	1288	1288	1	A -> G	6	SNP (transition)	50.00%

Results presented are proportions (%) of polymorphisms

Results obtained from blood samples of study population to diagnose *P. falciparum* by microscopy on day 3 after initiation of AL treatment showed 30 (10.8%) patients still tested positive for *P. falciparum* malaria. AL has a half life of 3-4 days to destroy any residual malaria parasites in order to prevent recrudescence. At the health facility chosen, it was observed that some *P. falciparium* malaria patients who had been treated with artemether-lumefantrine (AL) reported back with severe malaria. Their malaria parasitaemia had increased instead of decreasing on day 3 after initiation of AL treatment. This was detected using microscopes. Because of the severity malaria reported, these patients were admitted, put on quinine and later on AL treatment and they recovered. This suggested that AL antimalarial treatment failure was likely to have been caused by other factors such as to failure to adhere to treatment as opposed to genotype of *P. falciparum* kelch protein found on chromosome 13 (Kurumop *et al.,* 2013 & Siekmansa *et al*., 2012).

4.3: Influence of Socio-Economic Factors on Malaria Prevalence in the Study Area

Large number of assets were mainly recorded among head of households who had secondary and tertiary level of education as opposed to those with primary and no formal education (Figure 4.3). Level of education was likely to have affected a person's perception of malaria management.

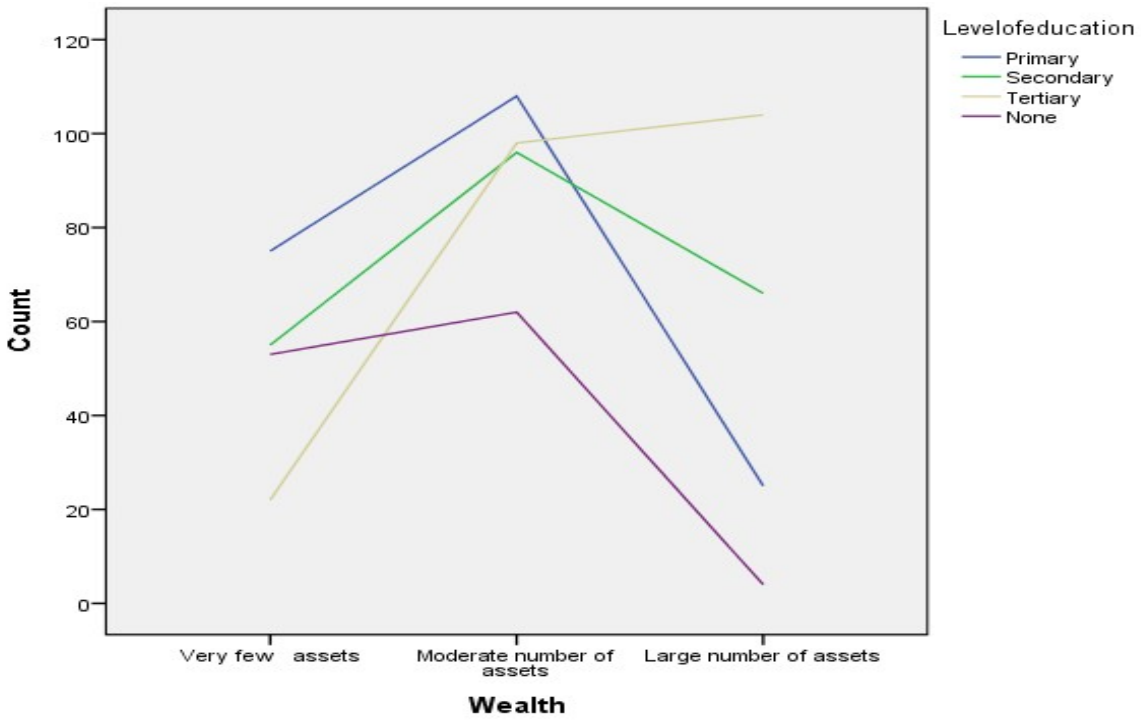


Figure 4.3: Relationship between wealth and level of education of homestead head in the study area

Head of households who recorded tertiary education were more in Lugaga/Wamuluma ward. Central Maragoli recorded more study population with secondary education while those who recorded primary education were more in Edzava wards (Figure 4.4). Head of households without formal education who did not seem to associate stagnant water and mosquitoes to malaria was relatively high in the three wards found in the study area. This might have influenced increased high malaria prevalence in the study area.

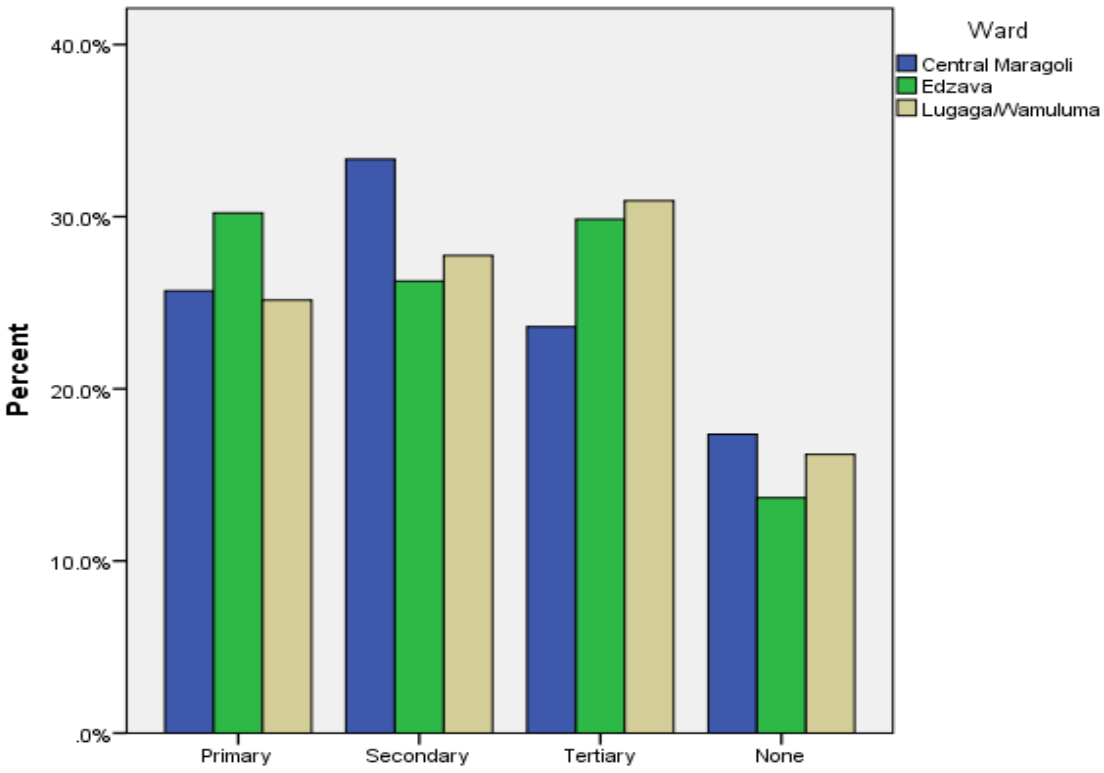


Figure 4.4: Distribution of head of the homestead's level of education in the study area.

Table 4.8: Size of Land for Homesteads and the Head of Household's Level of Education

	Frequency	Valid percent	Cumulative percent
Size of the land, acres			
< 1	237	30.9	30.9
	226	29.4	60.3
1.5– 2	120	15.6	75.9
> 2	61	7.9	83.9
Rented houses on <1 acre	124	16.1	100
Total	**768**	**100**	
Level of education			
Primary	208	27.1	27.1
Secondary	217	28.2	55.4
Tertiary	224	29.2	84.5
No formal education	119	15.5	100
Total	**768**	**100**	

Results are presented as number (n) and proportions (%) of subjects.

Most individuals in the study population occupied land that was less than 3 acres. The study area was an urban area and densely populated. This resulted to houses being very close to one another which enabled mosquitoes to freely move from one house to another spreading malaria. This might have contributed to malaria prevalence in the study area (Table 4.8).

Factors that determined salary included: education, experience and certification. Salary was considered to be one of the sources of income which determined: - malaria treatment, house designs, education and purchase of mosquito nets which influenced malaria prevalence to some extent. Distribution of salary among study population in the study area was considered to be high among individuals earning between Ksh. 10,000 to Ksh. 20,000 per month (51.4%) who were considered to be earning moderate salary, followed by those who were considered to have had low salary of below Ksh. 10,000 per month (27.9%), while those earning high salary of over Ksh. 20,000 per month accounted for 20.7 % (Table 4.9). Most homesteads recorded household size of more than 4 members (57 %). High household size with less income in a homestead might have compromised effective malaria treatment and usage of mosqiuto prevention nets to suppliment those given by the Kenya government resulting to high malaria prevalence.

Table 4.9: Salary Distribution of Head of Household and Household Size

	Frequency	Valid percent	Cumulative percent
Salary			
High	159	20.7	20.7
Moderate	395	51.4	72.1
Low	214	27.9	100
Total	**768**	**100**	
Household size			
Two	36	4.7	4.7
Three	86	11.2	15.9
Four	208	27.1	43
More than four	438	57	100
Total	**768**	**100**	

Results are shown as number (frequency) and proportions (%) of individuals. **Salary:** Low, below Ksh. 10,000/month; Moderate, Ksh. 10,000-20,000/month; High, over Ksh. 20,000/month

Permanent houses in the study area constituted 42.6 % while other types of houses constituted 57.4% (Table 4.10). Permanent houses with ceiling boards tend to have reduced entry of mosquitoes into the houses as opposed to houses without ceiling boards. Number of windows and doors in a house might have also influenced entry of mosquitoes into houses increasing malaria. The study population that was able to name only 2 mosquito breeding grounds accounted for 97.2% (Table 4.10). It's important for one to identify breeding grounds for mosquitoes in order to prevent and control malaria yet in the study area most participants identified less than 2 breeding grounds.

Table 4.10: Type of Houses and Identification of Mosquito Breeding Grounds

in the Study Area

House Type	Frequency	Valid Percent	Cumulative Percent
Permanent (Cemented floor/ bricks stone wall/iron/ceiling board)	327	42.6	46.2
Semi-permanent (Mud floor)	309	40.2	82.8
Semi-permanent (Cemented floor)	73	9.5	92.3
Others	59	7.7	**100**
Total	**768**	**100**	
Mosquito's breeding grounds			
1 Breeding ground named correctly	355	46.2	46.2
2 Breeding ground named correctly	397	51.7	97.9
3 Breeding ground named correctly	14	1.8	99.7
More than 3 Breeding ground named correctly	2	0.3	**100**
Total	**768**	**100**	

From the pearson Chi-Squared results obtained the following individual socio-economic factors were statistically significant to malaria prevalence in the study area: - level of education (*p*-value = 0.014), wealth (*p*-value = 0.000), size of land (*p*-value = 0.035), house type (*p*-value = 0.006), salary (*p*-value = 0.043) and ventilation in the house (0.000) while household size (*p*-value= 0.823) statistically insignificant to malaria prevalence in the study area (Table 4.11).

Table 4.11: Pearson Chi-Square Test

Showing Influence of Socio-Economic Factors on Malaria Prevalence in the Study Area

Socio-economic factors	***p* – value**
Level of education	0.014
Salary	0.043
Wealth	0.000
Size of the land	0.035
Household size	0.823
House design	0.006
Ventilation in the house	0.000

Statistically significant at p - value ≤ 0.05 level (2-tailed).

CHAPTER FIVE

DISCUSSION

The research hypothesis 'Spatia-temporal distribution of *Plasmodium* species is homogenous in Mbale town and its environs, Vihiga County, Western Kenya' was rejected. The study findings showed *Plasmodium falciparum* malaria was the most common type of malaria species among all age groups, wards and gender of the study population. *P. falciparum* accounted for 98.7% while *P.ovale, P. vivax* and *P. malariae* accounted for 1.3% in the study area. This was contrary to study findings from most parts of the World where *P. falciparum* accounted for 99% while the other types of *Plasmodium* species accounted for 1% (CDC., 2019). *P. falciparum* occurred throughout the year, during rainy seasons and after rain seasons and it is highly infectious. A study carried out on *Plasmodium* species occurrence, temporal distribution and interaction in a child-aged population in Burkina Faso indicated that *P. falciparum* prevalence was higher than *P.ovale, P. vivax* and *P. malariae* (Awe *et al*., 2013) which was consistent with findings in the study area.

The three wards found in the study area varied in terms of mosquito breeding grounds, altitude, land use, topography and socio-economic factors which are likely to have influenced differences observed in malaria prevalence. Fish ponds, pits (tyre tracks) and old vehicle tyres found at car washing areas which provided many breeding grounds for *Anopheles* mosquitoes were common in the three wards. High malaria prevalence in Lugaga/Wamuluma ward was likely to have been due to poor drainage system and poor disposal of empty containers which encouraged collection of water providing breeding grounds for mosquitoes. In Lugaga/Wamuluma ward there was Ehedwe valley with low and

gentle slopes, streams, bushes, swamps, pits formed by brick making and gold mining which might have encouraged breeding of mosquitoes resulting to high malaria prevalence. In Edzava ward, high malaria prevalence was likely to have been caused by rain water that collected in the burrow pits formed by brick-making and gold mining which provided breeding grounds for mosquitoes. Central Maragoli ward was comparatively well drained and had few rocks which explained why malaria prevalence was fairly low compared to Lugaga/Wamuluma and Edzava wards.

Geographical locations (wards) which recorded more mosquito breeding grounds in the study area also recorded high malaria prevalence similar to study findings in various parts of the Kenya and Sub-Saharan Africa (PMI., 2018 & WHO., 2018). Malaria prevalence in the study area was influenced by space (geographical location) and time trend which was consistent with study findings from: - highland and lowland regions of Western Kenya, (Omukunda *et al*., 2013), Amhara Region State, Ethiopia, (Alemu *et al*., 2013) and in Kerman, South East of Iran (Minoo & Khodadad, 2019) and other parts of the world (WHO., 2020). In the study area, effect of rainfall on malaria prevalence was observed 1-2 months after the onset of rainfall because of the time taken for developmental stages of *Anopheles mosquitoes* and development of malaria parasites in the *Anopheles* mosquitoes similar to study findings on the relationship between climatic factors and malaria incidence in Kerman, South East of Iran (Minoo & Khodadad, 2019).

The study findings obtained showed *P. falciparum* malaria was the most common malaria among all age groups because it occured during both low and high rainfall seasons which was

similar to studies that were carried out in other parts of Kenya and the world (PMI., 2018). *Plasmodium* species occurrence and *P. falciparum* infection varied by age in the study area, which was consistent with previous study findings in other parts of the world (Andrea *et al*., 2019). Spatio- temporal study findings on childhood malaria case incidence in Malawi between 2004 and 2017 also linked distribution of malaria parasites to age supporting results obtained in the study area (Chirombo *et al*., 2020).

Children below five years old and old people have low immunity levels and that is why they are easily infected with diseases including malaria (Gonzales *et al*., 2020 & WHO., 2015). Children aged 5-14 years who were mainly nursery and primary school going children recorded all *Plasmodium* species apart from *P. vivax* while adults and children below 5 years old did not record *P. ovale*. Children below 15 years old who included school-age children tend to acquire *P. falciparum* malaria easily according to study findings from Burkina Faso (Awe *et al.,* 2013) but it was contrary in the study area because there were more *P. falciparum* malaria patients recorded among children between 14-18 years old group compared to other age groups.

Children aged 14-18 years who were mainly primary and secondary school going children recorded high *P. falciparum* malaria compared to other age groups because they were more exposed to mosquito bites which was consistent with findings from other parts of the world (Andrea *et al*., 2019). There was slightly more malaria prevalence in females than males in the study area, which was likely to have been due to socio-cultural norms that exposed

females to mosquito bites, consistent with study findings that were carried out in Kenya on gender and malaria (Gender and Malaria in Kenya, 2015).

Temperature and rainfall had influence on malaria prevalence in the study area by providing more breeding grounds for mosquitoes during moderate temperatures and heavy rainfall which was similar to study findings carried out in Vihiga County (Wafula & Mulinya, 2018), in highland and lowland regions of Western Kenya (Ngenga *et al*., 2016), Malawi (Chirombo *et al*., 2020), and in Kerman, South East of Iran (Minoo & Khodadad, 2019). As monthly temperature increased the incidence rate of malaria increased significantly in the study area because increase in temperature tended to accelerate the development of malaria parasites in the *Anopheles* mosquitoes which was consistent with findings in other parts of the world (Angelica *et al*., 2020).

There was high malaria prevalence following long rains season and relatively high temperature in the study area which enabled mosquitoes to breed in many sites of stagnant water, developed very fast and accelerated the development of malaria parasites in *Anopheles* mosquitoes. This was similar to findings carried out in Vihiga County (Wafula & Mulinya, 2018), other regions in Western Kenya (Omukunda *et al*., 2013), Tanzania (Rumisha *et al*., 2019) and Colombian Pacific Coast (Angelica *et al*., 2020). In the study area, malaria prevalence varied in different months due to changes in amount of rainfall received and temperatures which was consistent with findings from other parts of Kenya and other regions in the world (PMI.,2020 & WHO., 2020). In the study area, effect of rainfall on malaria prevalence was observed 1-2 months after the rainfall because time taken from

infective bite by *Anopheles mosquitoes* and appearance of the first symptoms of malaria is 7 to 30 days depending on the *Plasmodium* species similar to findings from Iran and other regions in the world (CDC., 2019, Minoo & Khodadad, 2019).

In the study area, malaria prevalence was high in the month of June which was likely to have been due to the heavy rainfall that was observed in April, which increased breeding grounds for mosquitoes leading to increased malaria transmission which was consistent with study findings from Amhara Region State Ethiopia and in Iran (Minoo & Khodadad, 2019). In the study area, malaria prevalence was low in September which was likely to have been due to less rainfall that was observed in July which decreased breeding grounds for mosquitoes similar to study findings carried out in Tak Province, Thailand in rural Western Kenya (& Nyaguara *et al*., 2012), Malawi (Chirombo *et al*., 2020) and China (Zhang, *et al*., 2018). The month of July also recorded the lowest temperature which could have reduced time taken for *Anopheles* mosquitoes and malaria parasites to develop resulting to low malaria prevalence that was observed in September. Effect of climate on malaria prevalence in the study area was similar to study findings carried out in Tak Province, Thailand (Mercado *et al*., 2019).

The Research hypothesis '*P. falciparum* kelch 13 (*Pf*K13) does not affect efficacy of Artemether-lumefantrine (AL) in Mbale town and its environs, Vihiga County, Western Kenya' was accepted. Transition and transverse mutation which were likely to have been introns were identified in the DNA of *P. falciparum* collected from *P. falciparum* malaria patients in the study area. These mutations did not affect efficacy of AL because all the *P. falciparum* patients with delayed clearance of *P. falciparum* recovered when they were put

on AL treatment under supervision. The absence of *Pf*K13 mutations associated with *P. falciparum* to AL in the study area was in consistant with findings of a study that was carried out among children aged 5-59 months in Siaya County,Western Kenya from 2016-2017 (Chebore *et al*., 2020).

A study carried out in Kakamega which is a low malaria-transmission site and in Kombewa a high malaria-transmision site in Western Kenya from 2003 to 2015 indicated absence of mutation in *Pf*K13 to have compromised resistance of *P. falciparum* to AL treatment (Hemming *et a1*., 2018) similar to findings in the study are. This indicated that failure of AL to clear *P. falciparum* in the study area was likely to have been caused by host- defence mechanism among malaria patients and lack of compliance and adherence to AL treatment. This study supported study fidings carried out on improving adherence to malaria treatment guidelines in Papua New Guinea (Kurumop *et al*., 2013) other than genotype of mutation in *Pf*K13. This study finding was also in consistent with study findings reported in Kibaha, Mkuzi, Mlimba and Ujiji in Tanzania which indicated there was no mutation in *Pf*K13 to have compromised AL failure (Ishengoma *et al*., 2019). It is very important to understand factors affecting clearance of malaria parasites after post-antimalarial treatment which include; sequestration, severity of malaria infection, effect of antimalarial, compliance and adherence and host-defence mechanism (White Nicholas J., 2017).

The research hypothesis ‘Socio-economic factors do not influence malaria prevalence in Mbale town and its environs, Vihiga County, Western Kenya’ was rejected. Socio-economic factors were found to be important determinants of malaria transmission. Knowledge on

stagnant water as breeding ground of mosquitoes plays a vital role in malaria prevention. More than half of the study participants were unable to correctly name more than one mosquito breeding grounds in the study area which was likely to have been due to their wrong perception towards causes of malaria. Most study participants without formal education found it difficult to link malaria to stagnant water. High poverty level in the study area might have compromised malaria prevalence in the study area. Most homesteads in the study area were very close to one another due to small sizes of land. This might have encouraged transmission of malaria from one house to another.

Study participants with high household size and where the head of the homestead earned low income might have experienced challenges in purchasing adequate mosquito prevention bed nets which might have exposed them to mosquito bites according to results obtained in the study area. Semi- permanent houses which lacked ceiling boards and with more ventilation through windows and doors in the study area to some extent allowed entry of mosquitoes into the house which might have increased malaria prevalence in the study area. High level of education in the study area seemed to have enabled individuals to understand the causes, management and prevention of malaria as opposed to perception of individuals with low or no formal education. From the results obtained in the study area, some of the study participants who either dropped at primary school level and those without any formal education tended to link malaria to feeding on first harvest of maize and beans, curse from God and being rained on supporting malaria beliefs study findings from Kenya Malaria Indicator Survey, (2020). This misconception led these study population to use mosquito nets to cover their fish and vegetables thus increasing malaria prevalence in the study area.

Socio-economic factors had a strong effect on malaria prevalence in the study area. This was similar to study findings carried out in sub-Saharan Africa which indicated that improvement in socio-economic status of people in these regions reduced malaria prevalence (Degarege *et al.,* 2019) and associated socio-economic inequalities to malaria risk in rural Uganda (Tusting *et al.,* 2016). A study carried out on mapping socioeconomic inequalities in malaria in sub-Sahara African countries indicated that socio-economic factors influenced malaria which was consistent with results obtained in the study area (Gabriel *et al*., 2021). Influence of socio-economic factors on malaria in the study area was inconsistent with study carried out on: effect of socio-enviromental factors on malaria infection in Pakistan (Muhammad *et al*., 2019), socio-economic factors influence on malaria incidence in Calabar, Cross river state in Nigeria (Ibor, U.W and Okoronkwo, 2017), socio-economic and household risk factors of malaria in tribal areas of madhya Pradesh, central India (Ravendra *et al*., 2015), socioeconomic health inequality in malaria indicators in Western Kenya (Were *et al*., 2018).

House designs in the study area influenced entry of mosquitoes into the houses supporting study findings that were carried out in house design modifications reduced indoor resting malaria vector densities in rice irrigation schemes areas in Western Kenya (Atieli *et al*., 2009) and on housing improvements reducing malaria risk in sub- Saharan Africa (Tusting, *et al*., 2017). Poverty level in study area was high which was likely to have made it difficult for study population to engage effectively in malaria management which was consistent with study findings on poverty influences malaria in rural Uganda (Tusting *et al*., 2016). Wealth was considered to be a source of the income from the salary if employed and quantity and

quality of assets owned by the head of the household which was statistically significant to malaria prevalence.

One can be wealthy without necessarily being well educated. Wealth affected malaria prevalence in the study area similar to the study finding that was carried out in rural Uganda by Lucy *et al*., (2016) which linked malaria to poverty. Due to high level of poverty, most individuals were able to:- purchase adequate insecticide-treated mosquito nets for all household members for effective malaria prevention which was consistent with study findings from Western Kenya highlands and other parts of the world (Andrea *et al*., 2017& Ndenga *et al*., 2016), built houses with ceiling board to prevent entry of mosquitoes into the houses consistent with study findings in other regions in Western Kenya (Atieli *et al*., 2009) and sub- Saharan Africa (Tusting *et al*., 2017), spray their houses regularly with insecticides, purchase original antimalarial drugs as counterfeits drugs can cause unavoidable malaria deaths and availability of money enabled such individuals to take their household members for malaria treatment early enough supporting study findings that were carried out in Kenya (Kenya Malaria Strategy- 2019-2023).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

5.0: Conclusions

1. The study findings showed *Plasmodium falciparum* malaria was the most common malaria among all age groups, wards and gender. *Plasmodium falciparum* malaria prevalence seems to have reduced slightly while *P. ovale*, *P. vivax* and *P.* malariae malaria seem to have slightly increased. Malaria prevalence in the study area was heterogeneously distributed spatially depending on: - economic activities, topography and behavioural characteristics of the population at risk. Space and time trend influenced more mosquito breeding grounds in the study area which increased the population of *Anopheles* mosquitoes and fast development of malaria parasites in the vectors. This increased malaria prevalence in the study area.
2. There were no mutations in *Pf*K13 found in the samples tested that compromised efficacy of artemether- lumefantrine in the study area. However, transition and transverse substitution mutations were identified in the DNA of *P. falciparum* (*Pf*K13) found in dry blood spots collected from *P. falciparum* malaria patients in the study area. These mutations which were likely to have been introns (likely to have been synonymous mutations) did not seem to have affected the efficacy of AL because all malaria patients recovered after AL medication. Artemether lumefantrine (AL) still remains the first-line treatment for *P. falciparum* malaria for all age groups in the study area and there is no need to change it as recommened by WHO and Kenya malaria treatment policy. The delayed clearance of malaria

parasites that was observed in some patients was likely to have been caused by host-defense mechanism and lack of adherence to antimalarial drugs given.

3. Socio-economic factors influenced malaria prevalence in the study area as they determined malaria transmission. Inequalities in socio-economic status contributed towards variation in malaria prevalence recorded in different wards in the study area. Malaria prevalence was high among individuals from poverty-stricken homesteads. Individuals especially in poverty-stricken areas used torn nets which allowed mosquito bites while others did not use given nets at all due to poverty and low education level which might have increased malaria prevalence in some regions in the study area. Community health workers in Vihiga County are playing a very important role in malaria management.

5.1: Recomendations

1. In order to achieve ‘a malaria-free Kenya’ according to Kenya Malaria Strategy-2017-2023 the following recommendations should be considered: - Malaria management bodies should closely monitor and predict malaria prevalence patterns in relation to geographical regions and time trend when implementing malaria control strategies in order to reduce malaria prevalence. More research should be carried out on causes of antimalarial treatment failure in the study area, Kenya and the world, paying attention to: - mutation in malaria parasites. severity of malaria infection, distribution of *Plasmodium* species, efficacy of antimalarial, host-defense mechanism, adherence and compliance in order to eliminate malaria. To limit the spread of antimalarial resistance, policy makers should ensure counterfeit

antimalarials are eliminated and development of alternative antimalarials is in the process in case AL develops resistance.

2. There should be appropriate improvement of economic and social interventions in low- income and poverty- stricken individuals in the study area, Kenya and the whole world in order to reduce and eliminate malaria burden. Education on usage of insecticides-treated bed nets and other malaria control strategies should be monitored regularly at household level. The government should provide free malaria treatment in public health facilities where most poor individuals get their treatment and allow more health personnel to reach remote areas to educate people on malaria treatment and control. The Kenya government and the world at large should also put more emphasis on use of modern technology and laboratories to eliminate circulating counterfeit drugs from the market in order to prevent spread of antimalarial resistance which might increase in malaria deaths.

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APPENDICES

Appendix I: Written Consent for Human Investigation Studies

Masinde Muliro University of Science and Technology/ Health Facilities in Vihiga Sub-County/ Kenya Medical Research Institution

Study title: Malaria parasites distribution, genotype of *P. falciparum* and effect of socio-economic factors on malaria infection in Mbale town environs, Western Kenya

Purpose: To determine; spatial and temporal distribution of malaria parasite species, effect of *P. falciparum's* genotype on efficacy of artemether-lumefantrine (AL) and effect of socio-economic factors on malaria infection in Mbale township and its environs in Vihiga County, Western Kenya

Procedure: The study recruited health workers who administered questionnaires to recruited 768 malaria patients in Mbale Provincial Rural Training Health Center. The malaria patients or any authorized persons such as parents and guardians were requested to orally answer questions asked by the research assistants. However, the study volunteers were also free to personally fill the questionnaire.

Confidentiality: A study number was assigned to the recruited 768 sample population to preserve confidentiality where a database linking personal identifiers to the study number was kept by the principal investigator and relevant key personnel. Other people assisting the investigators were blinded to the true identity of the study participants.

Summary of Your Rights as a Participant in a Research Study: You are freely volunteering to participate in this research study and refusing to participate will not alter your usual health care or involve any penalty or loss of benefits to which you are otherwise entitled to. You are free to withdrawal at any time from the study for any reason and if information generated from this research is published or presented, your identity will not be revealed.

Consent: I volunteer to participate in a research project conducted by Beatrice Aleyo Muzame from Masinde Muliro University of Science and Technology. I understand that the research is designed to gather information on malaria prevalence among the study sample. I will be one of those people who will be interviewed for this research. My participation in this project is voluntary. I understand that I will not be paid for my participation. I may withdraw and discontinue participation at any time without penalty. If, however, I feel uncomfortable in any way during the interview session, I have the right to decline to answer any question or to end the interview. The interview will last approximately 20 minutes. Notes will be written during the interview. Photographs may be taken by researchers. I understand that the researcher will not identify me by name in any reports using information obtained from this interview, and that my confidentiality as a participant in this study will remain secure.

Subsequent uses of records and data will be subject to standard data use policies which protect the anonymity of individuals and institutions. This precaution will prevent my individual comments from having any negative repercussions. I understand that this research study has been reviewed and approved by the Institutional Review Board (IRB) for Studies Involving Human Subjects at Masinde Muliro University of Science and Technology. For

research problems or questions regarding subjects, the Institutional Review Board may be contacted through Beatrice Aleyo Muzame telephone number 0721872323.

I have read and understand the explanation provided to me. I have had all my questions answered to my satisfaction, and I voluntarily agree to participate in this study.

I have been given a copy of this consent form.

Name_____Signature----------Date----------

Witness: Name---------- Signature---------- Date----------

Appendix II: A Malaria Patient Consent Form

Masinde Muliro University of Science and Technology/ Health Facilities in Vihiga Sub-County/ Kenya Medical Research Institution Consent for Human Investigation Studies.

Study Title: Malaria distribution, genotype of *Plasmodium falciparum* and effect of socio-economic factors on malaria infection in Mbale town environs, Western Kenya

Purpose: To determine; spatial and temporal distribution of malaria parasite species, effect of *P.falciparum's* genotype on efficacy of artemether-lumefantrine (AL) and effect of socio-economic factors on malaria prevalence in Mbale township and its environs in Vihiga County, Western Kenya.

Malaria can be cured if it's diagnosed early enough and effective treatment given on time. Malaria morbidity and mortality can be reduced and even be eradicated from the population by effectively eradicating both malaria parasites and female *Anopheles* mosquito which is a vector for malaria parasite. AL's efficacy should be monitored to identify if malaria parasites are resistant or sensitive to them in order to reduce malaria mortality and morbidity.

Procedure: The study recruited health workers such as doctors, clinical officers, nurses, pharmacists and laboratory technicians in the study area. The blood samples from clinically malaria diagnosed patients was taken by finger pricking and placed on slide, dried, stained with giemsa or field stains, oil emulsion added to it and examined under microscope to identify malaria parasite species and malaria density in the chosen patients. Any other malaria parasitological methods that were available were also be used. The health workers recruit 768 confirmed malaria positive patients who presented themselves to Mbale

Provincial Rural Training Health Center for various treatments. Patients who were found to be malaria positive were treated.

Long-term Storage and future usage:

I agree to have my blood sample stored by Muliro Masinde University of Science and Technology, KEMRI and Mbale Provincial Rural Training Health Center and it should be under lock and key and only authorized to be accessed by principal investigator and research assistants and only used for the purpose the participant consented to. I understand I have a right to withdraw any agreement for future research any time for any reason. I may also request that my blood sample be used for certain future medical testing. To do this, I will request Beatrice Aleyo Muzame the study principal investigator of my request and she will inform other investigators future testing not described here.

Tick YES if agreed or NO if you don't agree YES NO

Signature…….Date…….

Witnessed by……..Date……

A person can sign or verbally state his/her consent in the presence of a witness who will the sign or put a thumb impression.

Risks and Benefits: Collection of blood samples for malaria diagnosis from suspected malaria patients may lead to; a little bleeding, pain, bruises and rarely infections which are a minimal risk and may occur in very few people. Malaria testing and treatment will be beneficial to the patient because it will be free. Malaria can cause severe sickness and even

death and patients may react differently to these medications. Incase such cases arise; clinicians will be contact for advice and necessary action to be taken.

Confidentiality: A study number (1- 768) will be assigned to blood that will be used to preserve confidentiality where a database linking personal identifiers to the study number will be kept by the principal investigator and relevant key personnel. Other people assisting the investigators will be blinded to the true identity of the study participants.

Summary of Your Rights as a Participant in a Research Study: You are freely volunteering to participate in this research study and refusing to participate will not alter your usual health care or involve any penalty or loss of benefits to which you are otherwise entitled to. You are free to withdrawal at any time from the study for any reason and if information generated from this research is published your identity will not be revealed. In case you experiences any unusual feeling physical injury or illness as a result of participating in this research study, contact Beatrice Aleyo Muzame 0721872323.

Signature: Signing and the information given to you to make a fully informed and free decision about your participation in the study. You do not waive any legal rights, the investigators are not relieved of any liability they may have and you can withdraw from the study any time. A copy of this consent form will be provided to you.

Name……. Date…….

Signature……

This form indicates that you have been informed about the research study in which you voluntarily agree to participate; that you have asked any questions for clarity about the study.

Appendix III: Dried Blood Spots Preparation

Dried blood spots provide an easy and inexpensive way to collect and store peripheral blood specimens from infants, children and adults and are especially important in low-resource settings where storing and shipping frozen plasma are difficult. Dried blood spots are prepared by applying a small amount of peripheral blood to filter paper cards from heel sticks for infants, finger sticks (from adults), or anticoagulated whole blood.

Reagents and Materials

1. Materials for all methods of specimen collection

2. Alcohol swab 55-DriedBloodSpots-LTC-SOP-v2.0-19Mar2012 4 of 20

.3. Gas-impermeable storage bag (Whatman #10548232; Fisher Scientific #50853570)

4. Desiccant pack (Whatman #10548234; Fisher Scientific #50853571)

5. Humidity indicator Cards (Multisorb Des Manufacture #MS200032; Fisher Scientific #NC9511648 or equivalent)

6. Whatman card drying rack (VWR catalogue #89015-592; Whatman #10537173; Whatman # 10539521 or equivalent)

7. Gloves, preferably powder-free

8. Waterproof marker

9. Glassine Envelopes, 3 1/4 inches x 4 7/8 inches, 100-pack (Whatman #10548236; Fisher Scientific # 50853572); optional

Materials specifically needed for heel stick method

1. Unistick 2 device (Fisher Scientific #22-0227)

2. Dry sterile gauze pads

Materials specifically needed for finger stick method

1. Lancet/needle

2. Materials specifically needed for venous blood method using Vacutainer® Tubes

3. Vacutainer Evacuated Blood Collection Tubes

4. Tourniquet

5. Bandage/plaster **Source:** CDC, 2019

Appendix IV: Molecular Markers for Drug Resistance

Drug	Molecular Marker
Quinine	**-pfnhe**: microsatellite ms4670, msR1, ms3580 **-crt**: M74I, N75E, K76T, H97Q, A220S, Q271E, N326S, I356T,C350S, and R371I R **-mdr1**: N86Y, Y184F, S1034C, N1042D, D1246Y
Chloroquine	**-crt**: M74I, N75E, K76T, H97Q, A220S,Q271E, N326S, I356T,C350S, and R371I **-mdr1**: N86Y, Y184F, S1034C, N1042D, D1246Y **-mrp-1**: Y191H, A437S
Mefloquine	amplification of Pfmdr1 gene
Proguanil	**dhfr**: A16V, S108T or N51I, C59R, I164L, S108N
Sulfadoxine +	**-dhps**: S436A/F, A437G, K540E, A581G, A613S/T
Pyrimethamine	**-dhfr**: N51I, C59R, S108N, I164L or C50R, N51I, S108N, I164L
Atovaquone	**cyt b**: Y268S/N
Artemisinin	-Amplification of **Pfmdr1** -Mutation of **PfATPase6** **-C580Y** mutation in **K13-Propeller domain** with increased **P13-P** has been confirmed.

Primer for MDRI 5’ is - ATGGGTAAA GAGCAGAAAGA-3

MDR2 5’ is AACGCAAGTAATACATAAAGTCA.

Main K13 resistance mutations include; E252Q, P441L, F4461, G449A , N458Y, Y493H, P553L, R561H, V568G, P574L, A578S, C 580Y, A675V ,R539T and I543T while less frequent variants are; MA476I, C469Y, A481V, S522C, N537D, G538V, M579I, D584V and H719N (Rie *et al*, 2014 and WHO, 2016). *Pfdhps* PCR has two primers *Pfdhps –F 5-ATGATTCTTTTTCAGATG-3 and* primer *Pfdhps-5*-CCAATTGTGTGATTTGTCCAC-3 to amplify 747BP of the area showing mutations to sulfadoxine resistance. Primer *pfdhps-F1* (5-GTTGAACCTAAACGTGCTG-3) and *pfdhps-RI* (5 ATTACAACATTTTGATCATTC-

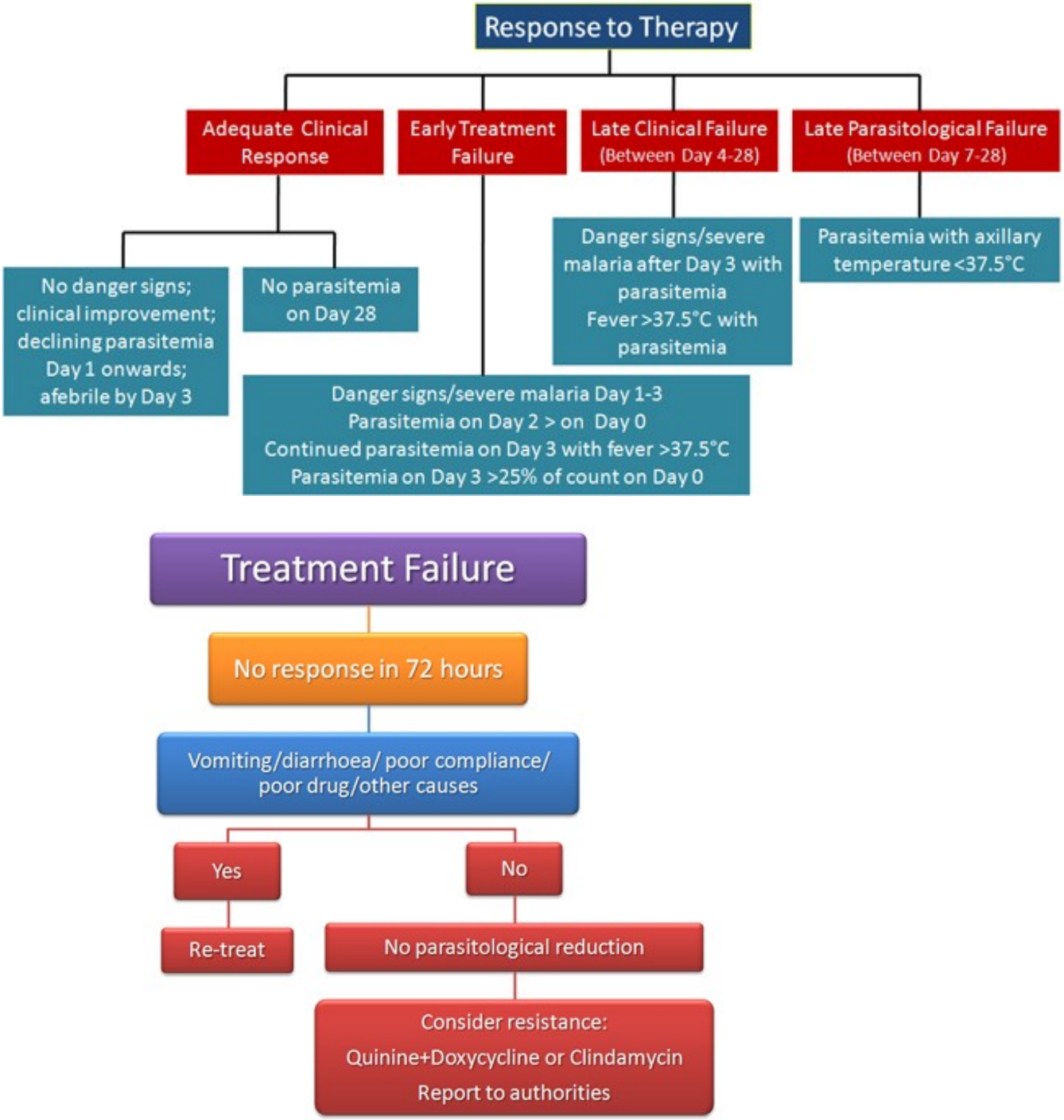
3). PCR and RPLP assay for (SERCA) *PfATPase*6, primers are 2307FW 5’ – TGA GCA TGG CAC AAG TIT 3’; 2307EV-5’ TCA ATA ATA CCT AAT CCA AAA TA-3’ for primary amplification and primers for nested PCR are FW-EN 5’ –TGA GCA TGG TAG AAG TTT T-3’ and RV-EN 5’ –TCA TCT GTA TTC TTA ATA TTT AAA TCT GTA CTA-3 **Source:** Srinivas (2015).

Appendix V: Antimalarial Drugs, Targeted Malaria Parasite Stage, Mode of Action, Resistance Duration and Genes Involved in Resistance

Drug	Type	Treatment recommendation	Target	Mode of action	Resistance	Genes involved in resistance
Quinine	Quinoline	Severe malaria	Blood stage (2)	Inhibits FP detoxification	rare	N/A
Chloroquine	Quinoline	*vivax* malaria	Blood stage (2)	Inhibits FP detoxification	Since 1950s	*pfcrt, pfmdr1*
Amodiaquine	Quinoline	Uncomplicated malaria, as ACT	Blood stage (2)	Inhibits FP detoxification	Since 1990s	*pfcrt, pfmdr1*
Mefloquine	Quinoline	Uncomplicated malaria, as ACT	Blood stage (2)	Inhibits FP detoxification	Since 1990s	*pfmdr1*
Piperaquine	Quinoline	Uncomplicated malaria, as ACT	Blood stage (2)	Inhibits FP detoxification	Since 1980s	unclear
Primaquine	Quinoline	*vivax* and *ovale* malaria; as gametocytocidal component for *falciparum* malaria	Liver stage (1) and gametocytes (3)	Maybe Mitochondrial electron transport	Unclear	N/A
Lumefantrine	Acryl alcohol	Uncomplicated malaria, as ACT	Blood stage (2)	Inhibits FP detoxification	Not reported	N/A
Atovaquone	naphthoquinone	Uncomplicated malaria, in combination with the antifolate proguanil	Liver and blood stage (1, 2)	Mitochondrial electron transport	Unclear	N/A
Sulfadoxine/ Pyrimethamine	Antifolate	Uncomplicated malaria, as ACT	Blood stage (2)	Inhibits folate metabolism	Since 1970s	*pfdhps/pfdhfr*
Artemisinin derivative	Artemisinin	Main component of ACTs	Blood stage (2) and gametocytes (3)	unclear	Unclear	unclear
Doxycycline, Tetracycline	Antibiotic	*falciparum* malaria	Blood stage (2)	Inhibits apicoplast functions	Not reported	N/A

Source:Makoah, N.A and Gabriele, P., 2013

Appendix VI: Response to Antimalarial Treatment



Source: Srinivas (2015).

Appendix VII: Malaria Patient/Guardian/Parent Study Questionnaire

Malaria distribution, genotype of *Plasmodium falciparum* and effect of socio-economic factors on malaria prevalence in Mbale town environs, Western Kenya

The study involves malaria patients who included minors where their parents or guardians will provide the needed information on their behalf.

NB Tick the answer you have chosen √

SECTION A: PERSONAL INFORMATION

A.1. Study number/ Patient's OPD number ----------

A.2. Date of interview ----------

A.3. Interviewer ----------

A.4. Villages surrounding Mbale town 1. ------ 2.------3----------4---------5----6

7----------8---------9---------10----------

A.5 1. Rural 2. Urban

A..6. Name of the assistant Chief ----------

A. 7. Marital status 1. Married 2. Not married

SECTION B: EFFECT OF SOCIO-ECONOMIC STATUS ON MALARIA INFECTION AND ANTIMALARIAL ADHERENCE AND COMPLIANCE BY MALARIA PATIENTS

B. 1. Gender 1. Male 2. Female

B. 2 Age 1. 0-5 years old 2. 5-14 years old 3. 14-20 years old

4. Adults a. Male b.. Female 1. Pregnant 2. Not Pregnant

B.3. Highest level of education: 1. Primary 2. Secondary 3. Tertiary 4. None.

B. 4. Salary of head of household 1. High 2.Moderate 3.Low

Low (below 1000ksh per month), Moderate (5000ksh-10,000ksh/month) High (over 10,000ksh per month).

B.5. Wealth/Ownership of assets (Consider housing, materials, number and quality of livestock and poultry kept, radio, TV, electricity, refrigerator, motorcycle, car, piped water, flush toilets)

1. Very few assets 2. Moderate number of assets 3. Large number of assets

B.6. Size of the land.

1. Less than one acre 2.1-3 acres 3. 4-5 acres 4. More than 5acres 5. Rented house

B.7. How many people are there in your household, that is, people whose main residence this is and who share at least one meal a day, or share living accommodation with you?

1. Two 2.Three 3.Four 4.More than four.

B.8.Which of the following best describes your house?

1. Permanent cemented floor/bricks/stone wall/ iron/tile roof

2. Semi-permanent (mud floor/mud/cement wall/ iron roofed

4. Traditional hut (mud floor/mud wall/grass thatched roof)

D. Other types of housing----------

B.9. How many doors/windows are there in the main house? ---------

1. One/One 2.Two/Two 3.Three/three 4.More than three /More than three

B.10. How many doors/ windows does the kitchen have? ----------

1. One/One 2.Two/Two 3.Three/Three 4.More than three/More than three

B.11. Are there other ventilations in the houses 1. Yes 2. No

B.12. Information on mosquito breeding grounds 1. Correct 2. Incorrect

(correct – stagnant water such as disposal of empty container water, ponds, ditches, uncleared vegetation etc.)

SECTION C: INFORMATION ON KNOWLEDGE AND ATTITUDE ABOUT MALARIA

C.1. Where do members of your household get medical services when they fall sick?

1. Health centers/self 2.Traditional doctors 3.Self-medication/chemist 4.Others

C.2. Whom do you trust with health information? 1. Parents 2. Shopkeepers 3. chemists 4. Health workers

C.3. Who among your family member fall sick more frequently? 1. Children below 5 years old 2. Adults

C.4. Do you know the disease you or your child is suffering from (1) Yes (2) No

C.5. Are you or your child having fever, headache and shivering? (1) Yes (2) No

C.6. Was diagnosis for malaria done? (1) Yes (2) No

C.7. Was any drug given before coming to the health facility? (1) Yes (2) No

C.8. If yes, when was the drug taken? 1. Less than 2 weeks ago 2. More than 2 weeks ago

C.9. Anti-malaria drugs to give/take: 1. (AL) 2. (SP) 3. (Quinine)

4. (Others)

C.10. Do you have a problem with taste and colour of antimalarial drugs? 1. Yes 2. No

C.11. Do you react badly to any antimalarial drug?

1.Yes 2. No

C.12. If yes how do you react 1. Severe 2. Less severe (Reaction can be; vomiting, rashes, stomach ache etc)

C.13. How many times do you take antimalarial drugs and how many drugs a day? (1) Correct (2) Not correct

C.14. At what time do you take antimalarial drugs 1. Correct 2. Not correct

C.15. For how long will you take antimalarial given? (1) Correct (2) Not correct

C.16. How many antimalarial drugs were you given/bought? (1) Correct (2) Not correct

C.17. How long will you give or take the antimalarial drugs? (1) Correct (2) Not correct

C.18. When will you return the child for checkup? (1)After completing medication

(2)Unless he/she falls sick again

C.19. Are satisfied with care of service in this facility? (1) Yes (2) No

C.20. Suggest how services can be improved in this facility ……

C.21. Overall assessment on adherence and compliance?

(1) Bad (2) Fair (3) Good (4) Excellent

C.22. How often do your family members get malaria?

1. Very often (monthly) 2.Less often (after 2 months) 3.Rarely (after 3 months).

C.23. Where are empty containers in your homesteads thrown?

1. Latrine/burry 2.Burn 3.Thrown on composite pit 4.Others/N/A

C.24. How frequent do you spray your house with insecticides?

1. Weekly 2.Monthly 3.Quaterly 4.Others/never

C.25. Do all members of your household sleep under treated bed nets?

1. Yes 2. No

C.26. If no how many of your household members don't use bed nets at night?

1. One 2.Two 3.Three 4. More than three./N/A

C.27. Do pregnant women use insecticide treated mosquito nets? 1. Yes 2. No

C.28. How frequent do you wash your bed nets with insecticides?

1, Monthly 2.Quaterly 3.Yearly 4.No washing /others

C.29. Do you normally finish taking malaria medication you are given when you fall sick? 1. Yes 2. No

C.30. Do you normally use mosquito repellants to drive away mosquitoes in your home? 1. Yes 2. No .

(Kenya Malaria Strategy- 2019-2023)

Appendix VIII: Translation of Questionnaire and Consent from English to Kiswahili:

Maswali ya mahojiano juu ya ugonjwa wa Malaria, watunzi na wazazi

Sahihisha jibu kwa kutumia alama √

Ueneaji wa Malaria, aina ya mbuna athari za kijamii na uchumi katika kueneza malaria katika mji wa Mbale na vitongoji vyake mkoani magharibi.

Uchunguzi wajumuisha wagonjwa wa Malaria aidha Watoto wachanga ambao wazazi wao watatoa habari kwa niaba yao.

SEHEMU A: HABARI KUNIHUSU

A1. Nambari ya uchunguzi/ nambari ya mgonjwa

A2. Tarehe ya mahojiano

A3. Anayekuhoji

A4. Kijiji 1.------2-------3-----4------5------6-----7----8------9------10

A5. 1. Mashambani

2. Mjini

A6. Jina la Naibu Chifu

A7. Habari juu ya ndoa

1. Umeoa/ Umeoleka
2. Hujaoleka/ Hujaoa

SEHEMU B: ATHARI ZA KIJAMII NA KIUCHUMI KUTOKANA NA MALARIA NA NI KIVIPI WAGONJWA WA MALARIA WAMEATHIRIKA NA DAWA ZA MALARIA.

B1. Jinsia?

1. Kiume
2. Kike

B2. Umri (Miaka)?

1. 0-5
2. 5-14
3. 14-20
4. Wakubwa a) Kiume

   b) Kike 1)Waja wazito

   2) Si waja wazito

B3. Kiwango cha juu cha elimu?

1. Shule ya Msingi
2. Shule ya Sekondari
3. Chou Kikuu
4. Hakuna

B4. Mshahara wa mkuu wa Familia?

1. Juu (>10,000)
2. Kadri (5000-10,000)

3. Chini (<1000)

B5. Mali/ vitu ulivyonavyo zingatia nyumba, bidhaa, nambari ya Wanyama na kuku, radio, televisheni, nguvu za umeme, Rufiji, pikipiki, gari, maji ya mfereji, choo ya maji?

1. Mali kidogo
2. Mali kadri
3. Mali nyingi

B6. Kiwango cha shamba?

1. Chini ya hekari moja
2. Hekari 1-3
3. Hekari 4-5
4. Juu ya hekari 5
5. Nyumba ya kukodi

B7. Kuna watu wangapi katika familia hasa wanao kaa hapo, wanao kula hapo japo mara moja ama mnatumikia vitu pamoja?

1. Wawili
2. Watatu
3. Wanne
4. Zaidi ya wanne

B8. Kati ya haya lipi linazungumzia juu ya nyumba yako?

1. Nyumba ya kudumu ya matofali/ mawe/ mabati/ tiles juu
2. Nyumba isiyo ya kudumu samadi chini/ ukuta wa matope/ ukuta wa kudumu/ mabati juu
3. Nyumba ya kiasili/ samadi chini/ ukuta wa matope/ juu nyasi

4. Aina zingine za nyumba

B9. Kuna milango na madisrisha mangapi kwa nyumba kuu?

1. Moja
2. Mbili
3. Tatu
4. Zaidi ya tatu

B10. Milango na madirisha mangapi yamo kwenye jikoni?

1. Moja
2. Mbili
3. Tatu
4. Zaidi ya tatu

B11. Kuna sehemu zingine za kuingiza hewa katika nyumba yako?

1. Ndio
2. La

B12. Habari juu ya sehemu ya kuzalisha mbu?

1. Ni sawa
2. Si sawa

Ni sawa: Sehemu kama ile ya maji kusimamia, mikebe iliyotupwa, vichaka visivyokatwa.

SECTION C: HABARI JUU YA UFAHAMU NA NAMNA UNAVYOCHUKULIA MALARIA

C1. Watu wa jamii yako wanapata wapi huduma ya afya?

1. Zahanati
2. Daktari wa kitamaduni
3. Mwenyewe/ duka la dawa
4. Zinginezo

C2. Unamwamini nani na habari za kiafya?

1. Mzazi
2. Muuzaji
3. Duka la dawa
4. Wafanyakazi wa kiafya

C3. Ni nani katika jamii yako huwa mgonjwa mara kwa mara?

1. Watoto chini ya miaka mitano(5)
2. Mtu mkubwa

C4. Unajua ugonjwa unaougua na ule wa Watoto wako?

1. Ndio
2. La

C5. Unachemka mwili ama Watoto wako, kuumwa na kichwa na kutetemeka kwa mwili?

1. Ndio

2. La

C6. Uchunguzi wa Malaria ulifanyika?

1. Ndio 2. La

C7. Ulipewa dawa yoyote kabla kuja kituo cha afya?

1. Ndio
2. La

C8. Kama ndio, lini ulimeza dawa?

1. Chini ya wiki mbili
2. Wiki mbili zilizopita

C9. Ulimeza dawa gani ya Malaria?

1. AL
2. SP
3. Quinine
4. Zinginezo

C10. Unashida na ladha na rangi ya dawa ya Malaria?

1. Ndio
2. La

C11. Unaathirika na dawa ya Malaria yoyote?

1. Ndio

2. La

C12. Kama ndio unaathirika vipi?

1. Vibaya sana
2. Si vibaya sana (kupitia kutapika, tumbo kuuma, n.k)

C13. Unameza mara ngapi dawa ya Malaria na dawa ngapi kwa siku?

1. Ni sawa
2. Si sawa

C14. Ni wakati gani unameza dawa?

1. Ni sawa 2. Si sawa

C15. Kwa muda gani unameza dawa ulizopewa?

1. Ni sawa
2. Si sawa

C16. Ulipewa dawa gani za Malaria/ ulinunua?

1. Ni sawa
2. Si sawa

C17. Utapewa kwa muda gani ama kumeza dawa?

1. Ni sawa
2. Si sawa

C18. Utamrejesha mtoto kwa uchunguzi lini?

1. Baada ya kumaliza matibabu
2. Wakati akiwa mgonjwa

C19. Umerithika na huduma katika kituo hiki?

1. Ndio
2. La

C20. Toa maoni yako namna kituo hiki kinaweza kuimarika

……

……

C21. Maoni ya jumla juu ya matibabu?

1. Mbaya
2. Wastani
3. Vyema
4. Vyema sana

C22. Ni mara ngapi jamii yako hupata Malaria?

1. Mara kwa mara
2. Si mara kwa mara baada ya miezi mbili
3. Kwa nadra baada ya miezi tatu

C23. Ni wapi mikebe mitupu hutupwa nyumbani mwako?

1. Chooni

2. Huchoma
3. Kwenye taka
4. Zingine/ hakuna

C24. Ni mara ngapi unanyunyuzia nyumba yako na dawa ya kuua wadudu?

1. Kila wiki
2. Kila mwezi
3. Baada ya miezi minne
4. Hakuna (nyingine)

C25. Kila mtu hulala kwa neti zilizotibiwa?

1. Ndio
2. La

C26. Kama ‘la’ ni watu wangapi wajamii hawatumii neti wakati wa kulala?

1. Moja
2. Mbili
3. Tatu
4. Zaidi ya tatu

C27. Akina mama waja wazito wanatumia neti zilizotibiwa?

1. Ndio
2. La

C28. Ni mara ngapi unaosha neti kwa kutumia dawa za kuua wadudu?

1. Kila mwezi
2. Baada ya miezi minne
3. Kila mwaka
4. Hakuna kuosha

C29. Ukiwa mgonjwa unamaliza dawa ulizopewa?

1. Ndio
2. La

C30. Unatumia njia mbadala (zingine) kufukuza mbu wasikufikie?

1. Ndio
2. La

RUHUSA YA MGONJWA WA MALARIA

Chuo Kikuu cha Sayansi na Teknolojia cha Masinde Muliro, vitengo vya Afya katika kaunti ya Vihiga, chuo cha utafiti cha matibabu Kenya chatoa ruhusa ya utafiti kwa mujibu wa masomo ya binadamu.

MADA YA UCHUNGUZI

Usambazaji wa Malaria “genotype of *plasmodium falciparum*” na athari za kijamii na kiuchumi katika mji wa Mbale na viungo vyake katika eneo la magharibi mwa Kenya.

LENGO

Kupata suluhisho la muda juu ya usambazaji wa Malaria “parasite species” athari za “genotype *plasmodium falciparum* ” kwa utumiaji bora wa “artemether-lumefantrine (AL)”. Athari za kijamii na kiuchumi za madawa katika mji wa Mbale na viunga vyake katika kaunti ya Vihiga magharibi mwa Kenya. Malaria yanaweza kutibiwa kama imegunduliwa mapema na matibabu kupewa kwa wakati ufaao. Idadi ya wagonjwa wa Malaria na vifo vinaweza kupunguzwa kumaliza Malaria ‘parasites’ na mbu wa kike,’Anopheles’. Dawa za Malaria kama AL, SP na Quinine ni sharti kuchunguzwa ili kubainisha ikiwa Malaria ‘parasites’ zimekolea ama hapana, kwa nia ya kupunguza vifo na kiwango cha maambukizi.

MPANGILIO

Utafiti utahitaji kuwafunza watafiti watano (5) na wafanyakazi wa afya kama tabibu , afisa wa kiliniki Nasi , mtaalamu wa dawa na mwana teknoligia wa mahabara katika eneo la utafiti . Sampuli ya damu itatolewa kwa mgonjwa kupitia kidole chake. Itawekwa kwa kifaa cha kupimia itakaushwa itawekwa “staine” na “giemsa” mafuta ya “emulsion” yataongezwa na kuangaliwa ukitumia “microscope” kubainisha malaria “parasite species” na uzito wa malaria kwa mgonjwa . Njia badala ambazo zitakuwepo zitatumiwa . Hawa watafiti watapata waginjwa 500 wa malaria wengine 100 waja wazito ambao hawana malaria. Watapelekwa katika kituo cha afya cha “Mbale Provincial Rural Training Health Centre.” Wagonjwa watakao patikana na malaria watatibiwa na uchunguzi mwingine wa malaria kufanywa kwa kutumia “Microscopy” na “Polymerase chain reaction”

Nimesadiki sampuli ya damu yangu kuhifadhiwa na chuo kikuu cha sayansi na teknoligia cha Masinde Muliro, KEMRI na “Mbale Provincial Rural Training Health Centre. Itakuwa

chini ya ulinzi bora. Wadadisi wakuu wa naibu wachunguzi pekee ndio watakuwa na usemi Juu yake. Itatumika tu kwa lengo kusudiwa. Nina haki yake kutoendelea na makubaliano wakati wowote kwa hoja yoyote. Aidha ninaweza kuomba damu yangu kutumika kwa vipimo vya baadaye kwa kutekeleza haya ninaomba Beatrice Aleyo Muzame aliye mchunguzi mkuu , adadisi ombi langu na atajulisha wadadisi wengine vipimo vingine ambavyo havimo kwenye Nakala hii .

Chagua Ndio kama umekubali ama la kama hukubali

Ndio _____ La _____

Sahihi _____ Tarehe _____

Shahidi _____ Tarehe _____

Unaweza katia sahihi ama kusema kwa mdomo mbele ya shahidi mwenye anatia kidole gumba na sahihi.

MADHARA NA MANUFAA

Kutoa sampuli kutoka kwa mgonjwa wa malaria Inaweza kusababisha haya , kutokwa na damu , uchungu , maambukizi kwa nadra . Haya yote ni madhara madogo na yanaweza kuwapata watu wachache. Kupima na kutibu malaria itakua na manufaa kwa wagonjwa kwa kuwa itakuwa bure . Malaria inaweza kumfanya mtu kuwa mgonjwa na hata kusababisha kifo na pia wagonjwa wanaweza kuathirika kwa njia aina mbalimbali. Jambo hili likitendeka wahudumu wa afya waweza kuitwa kupeana ushauri na kuchukua hatua maridhawa kama namna ya kushughulikia wagonjwa.

UHIFADHI WA HATI

Namna ya uchunguzi (768) itatiwa kwa damu itakayo tumiwa kwa nia ya kuhifadhi hati. Mfanyakazi wa “database” inayounganisha mwenye kufanya utafiti na mkuu wa utafiti. Wengine wasaidizi wataunganishwa.

RUHUSU NAKILIWA JUU YA UTAFITI KWA MJIBU WA BINADAMU

Chuo Kiku cha sayansi na teknologia cha Masinde Muliro, vitengo vya afya katika kaunti ndogo ya Vihiga na Chuo cha utafiti cha matibabu Kenya .

MADA YA UTAFITI

Ueneaji wa Malaria “genotype of plasmodium falciparum” na athari za kijamii na kiuchumi za Malaria katika uambukizi mjini Mbale na viunga vyake Magharibi mwa Kenya.

MPANGILIO

Utafiti utajumiusha watafiti watano, ambao watatumia ratiba ya maswali kusajili washiriki 768 kutoka “Mbale Provincial Rural Training Health Center “ Wagonjwa wa Malaria au watu wote waliopewa mamlaka wataombwa kujibu maswali yanayoulizwa na mtafiti . Hata hivyo watafiti wakujitolea wataruhusiwa kujaza ratiba ya maswali.

UHIFADHI WA HATI

Nambari ya utafiti itapewa wahusika 768 wa kufanya sampuli kwa nia ya kuhifadhi hati. “Database” itatumika kuunganisha mtu anayefanya utafiti na mkuu wa utafiti na wahusika wengineo. Watu wengine wanaokusaidia watafiti pia watapewa hati.

MKUTASARI WA HAKI ZAKO KAMA MHUSIKA KATIKA UTAFITI

Umejitolea kushiriki katika utafiti huu. Kukataa kushiriki hakutaathiri huduma zako za afya. Hauta adhibiwa na hauta poteza manufaa yako. Unahaki ya kujitoa katika shughuli ya utafiti wakati wowote kwa sababu yoyote. Ikiwa habari kutoka kwa utafiti huu imechapishwa ama kutolewa, siri yako haitatolewa.

RUHUSA

Ninajitolea kushiriki katika mradi wa utafiti wa Beatrice Aleyo Muzame wakutoka chuo kikuu cha sayansi na teknologia cha Masinde Muliro. Ninaelewa kuwa utafiti ni juu ya kupata habari kuhusu malaria “parasite species, “antimalarial drug resistance, adherence to and compliance with malaria medication among the study sample. Mimi ni moja wapo ya wale watakao hojiwa kwenye utafiti huo.

ushiriki kwangu ni kwa kujitolea . Nimeelewa fika kuwa sitalipwa. Naweza kujiondoa wakati wowote pasipo kuadhibiwa. Nikijihisi siko sawa kwa njia yoyote ile wakati wa mahojiano, nina haki kukataa kujibu maswali na kutamatisha mahojiano. Muda wa mahojiano ni takribani dakika (20) ilani itatolewa wakati wa mahojiano, picha huenda zikachukuliwa. Ninaelewa kuwa watafiti hawawezi kunitambua kwa Jina. Katika ripoti wakitumia habari kutoka kwa utafiti siri yangu kama mshiriki itahifadhiwa.

Utumiaji wa rekodi na data utazingatia sera za utoaji data ambazo hulinda haki za kibinafsi na za taasisi. Ilani hii itanizuia kusema kinyume na matarajio ya utafiti. Ninaelewa wazi kua utafiti huu umeidhinishwa na tume ya “Institutional Review Board (IRB)” utafiti unahusisha Binadamu na chuo kikuu cha sayansi na teknologia cha Masinde Muliro. Kwa utafiti na shida za utafiti tume ya “Institutional Review Board” kupitia kwa Beatrice Aleyo Muzame No:

0721872323. Nimesoma na kuelewa yote yaliyopewa mbele yako, maswali yote yamejibiwa na nimeridhika, nimesadiki bila kulazimishwa kuhusishwa katika utafiti huu.

Nimepewa nakala ya Ruhusu kutoka kwa;

Jina _____Sahihi _____

Shahidi Jina _____ Sahihi _____ Tarehe _____

(Kenya Malaria Strategy- 2009-2017).

Appendix IX: ETHICS LETTER FOR RESEARCH

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 vol2 (70) **Date:** 4th December, 2019

Beatrice Aleyo Muzame
Masinde Muliro University of Science and Technology
P.O. Box 190-50100
KAKAMEGA

Dear Ms. Muzame

RE: Malaria distribution, genotype of plasmodium falciparum and effect of socio economic factors on malaria infection in Mbale Town Environs, Western Kenya-MMUST/IERC/095 /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 no further revisions) the above Referenced application for one year.

This approval is valid from **4th December, 2019 through to 4th December, 2020.** Please note that authorization to conduct this study will automatically expire on **4th December, 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 **4th November, 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 **4th November, 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)

Appendix X: APPROVAL OF PROPOSAL

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY (MMUST)

Tel: 056-30870
Fax: 056-30153
E-mail: dps@mmust.ac.ke
Website: www.mmust.ac.ke

P.O Box 190
Kakamega – 50100
Kenya

Directorate of Postgraduate Studies

Ref: MMU/COR: 509099 **Date:** 17th September, 2019

Beatrice Aleyo Muzame,
PST/H/02/2015,
P.O. Box 190-50100
KAKAMEGA

Dear Ms. Muzame,

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your Ph.D. Proposal entitled: ***"Malaria Distribution, Genotype of Plasmodium falciparum and Effect of Socio-Economic Factors on Malaria Infection in Mbale Town Environs, Western Kenya"*** and appointed the following as supervisors:

1. Dr. Omukunda Elizabeth – SONAS, MMUST
2. Dr. David Mulama - SONAS, MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director of Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Natural Sciences Graduate Studies Committee; Chairman, Department of Biological Sciences & Departmental Graduate Studies Committee. Kindly adhere to research ethics consideration in conducting research.

It is the policy and regulations of the University that you observe a deadline of two years from the date of registration to complete your Ph.D. thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours Sincerely,

Prof. John Obiri
DIRECTOR, DIRECTORATE OF POSTGRADUATE STUDIES

Appendix XI: NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

REPUBLIC OF KENYA COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION Ref No: 640839 Date of Issue: 03/November/2019

RESEARCH LICENSE

This is to Certify that Ms.. BEATRICE MUZAME of Masinde MuUro University of Science and Technology, has been licensed to conduct research in Vihiga on the topic: MALARIA DISTRIBUTION, GENOTYPE OF Plasmodium falciparum AND EFFECT OF SOCIO-ECONOMIC FACTORS ON MALARIA INFECTION IN MBALE TOWN ENVIRONS, WESTERN KENYA for the period ending : 03/February/2021.

License No: NACOSTI/P/20/3379

Director General

APPLICANT IDENTIFICATION NUMBER 640839

NATIONALCOMMISSION FOR SCIENCE.TECHNOLOGY & INNOVATION