UNIVERSITY OF NAIROBI

EFFECTS OF AN IRRIGATION SCHEME ON MALARIA BURDEN IN IRRIGATED AND NON-IRRIGATED AREAS OF HOMA BAY COUNTY, WESTERN KENYA.

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Declaration

I declare that this thesis is my original work and has not been presented elsewhere for examination or award of a degree. Where other people's work has been used, this has been properly acknowledged and referenced in accordance with the University of Nairobi's requirements

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ABSTRACT

Malaria control efforts in the Lake Victoria Basin region have been stepped up by combining indoor residual spray intervention with long-lasting insecticidal nets (LLINs) to reduce malaria transmission rates. However, ongoing irrigation projects in arid and semiarid areas may have a direct impact on malaria epidemiology and anemia. The objective of the study was to compare the prevalence of *Plasmodium* infection, malaria treatment behavior, prevalence of anemia in irrigated and non-irrigated areas in Homa Bay County, Kenya. The study used a two-stage sampling method to recruit participants aged six months and above. Cross-sectional surveys were conducted in February 2018, just before the introduction of IRS, and was repeated in June 2018, February 2019, June 2019, and July 2020, during IRS's annual application. The blood samples were collected via the finger-prick for microscopy and qPCR analysis of *Plasmodium* infection and to determine the level of hemoglobin among participants. In addition, passive case detection was conducted in ten public healthcare facilities to determine monthly malaria cases, methods of diagnosis and antimalarial drug availability. Structured questionnaires were used to determine malaria knowledge, treatment seeking behavior, and predictors of malaria treatment-seeking. During wet season, the prevalence of *Plasmodium* infection was significantly greater in irrigated areas (15.3%) than in non-irrigated areas (7.8%) ($\chi^2 = 8.7$, $p = 0.003$). However, during the dry season, the prevalence of *Plasmodium* infection was not significantly different between irrigated (1.7%) and non-irrigated areas (1%) ($p > 0.05$). During the wet season, anemia prevalence was found to be higher in communities living in non-irrigated areas (51.5%) than in communities residing in irrigated areas (38.9%) ($p = 0.001$). Similarly, during the dry season, non-irrigated areas had a higher prevalence of anemia (34.1%) than irrigated areas (25.2%) ($p = 0.007$). Prevalence of *Plasmodium* infections significantly reduced following the introduction of IRS. For example, just

before the start of IRS, the prevalence of *Plasmodium* infection was 18.5% (113/610) by microscopy. However, after the first IRS application, the prevalence of *Plasmodium* infection dropped to 14.2% (105/737) and 3.3% (24/720) ($p < 0.0001$). Similarly, after the second round of IRS applications, the prevalence of *Plasmodium* infections dropped to 1.3% (11/849) ($p < 0.0001$). The majority of local residents with fever (50.3%) purchased antimalarial drugs from a chemist. Key predictors of treatment seeking included access to healthcare facility (OR = 16.23, 95% CI: 2.74-96.12), and ability to pay hospital bill (OR = 10.6, 95% CI: 1.97- 57). Although irrigation scheme was linked to an increase in malaria transmission in the study area, the prevalence of anemia in irrigated areas were significantly lower than in non-irrigated areas. To reduce cryptic *P. falciparum* transmission, proper malaria management and adequate support for healthcare facilities to provide quality services are critical. Improved malaria control strategies should be considered to reduce the rates of asymptomatic and submicroscopic infections. The Ministry of Health should consider launching a community-based awareness campaign to educate the general public about the importance of seeking medical attention at a healthcare facility.

DEDICATION

I dedicate this thesis to my late parents Jacob Odhiambo Midigo and Elizabeth Achieng Midigo, who provided for all of my needs from when I was a child until now, and who, most importantly, provided moral support throughout my academic career.

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LIST OF ABBREVIATIONS AND ACRONYMS

MoH- Ministry of Health

MoPHS- Ministry of Public Health and Sanitation

RDT- Rapid Diagnostic Test

WHO- World Health Organization

LLIN- Long Lasting Insecticidal Nets

ITN- Insecticide Treated Nets

IRS- Indoor Residual Spraying

PMI- Presidential Malaria Initiative

MoRD- Ministry of Rural Development

GMPD- Geometric Mean Parasite Density

pLDH- Plasmodium lactate dehydrogenase

HRP-2- Histidine-rich protein

PCR- Polymerase chain reaction

qPCR- Quantitative Polymerase chain reaction

LAMP -loop-mediated isothermal amplification

DNA- Deoxyribonucleic Acid

RNA- Ribonucleic Acid

ELISA- enzyme-linked immunosorbent assay

IFAT -immunofluorescence antibody assay test

IgG -immunoglobulin G

IgM -immunoglobulin M

PBO -Piperonyl butoxide

ACT Artemisinins-based combination therapy

RBC- Red Blood Cell

HB – Hemoglobin

LIST OF PUBLICATIONS RELATED TO THE THESIS

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Collince J. Omondi, Wilfred O. Otambo, David Odongo, Kevin O. Ochwedo, Antony Otieno, Shirley A. Onyango, Pauline Orondo[2], Benyl M. Ondeto, Ming-Chieh Lee, Daibin Zhong, James W. Kazura, Andrew K. Githeko, Guiyun Yan (2022). Asymptomatic and submicroscopic *Plasmodium* infections in an area before and during integrated vector control in Homa Bay, western Kenya. *https://doi.org/10.1186/s12936-022-04288-2 Malaria Journal 21:272* : This work is presented in chapter four and covers objective three of thesis.

Collince J. Omondi, David Odongo, Wilfred O. Otambo, Kevin O. Ochwedo, Antony Otieno, Ming-Chieh Lee, James W. Kazura, Andrew K. Githeko, Guiyun Yan (2023). Malaria diagnosis in rural healthcare facilities and treatment-seeking behavior in malaria endemic settings in western Kenya. *https://doi.org/10.1101/2023.01.05.23284237 PLOS Global Public Health*. : This work is presented in chapter five objective and addresses thesis objective number two.

LIST OF PRESENTATIONS RELATED TO THE THESIS

I. Oral presentation

Collince J. Omondi, David Odongo, Wilfred O. Otambo, Kevin O. Ochwedo, Antony Otieno, Ming-Chieh Lee, James W. Kazura, Andrew K. Githeko, Guiyun Yan. Malaria diagnosis in rural healthcare facilities and treatment-seeking behavior in malaria endemic settings in western Kenya. The 4th Kenya National Malaria Forum 2023 conference, 15th – 17th February 2023 at Safari Park Hotel, Nairobi.

II. Poster presentations

Collince J. Omondi, Harrysone Atieli, David Odongo, Andrew K. Githeko, James W. Kazura, Guiyun Yan. Impact of Indoor Residual Spray Program on the Prevalence of *Plasmodium* infections and Anemia in Western Kenya: An open cohort study. The 6th Pan-African Mosquito Control Association (PAMCA) Annual Conference, 23rd – 25th September 2019 at Hilton Hotel in Yaounde, Cameroon.

Collince J. Omondi, Harrysone Atieli, David Odongo, Andrew K. Githeko, James W. Kazura, Guiyun Yan. Impact of Indoor Residual Spray Program on the Prevalence of *Plasmodium* infections and Anemia in Western Kenya: An open cohort study. ASTMH 68th Annual Meeting, 20th – 24th, 2019 at the Gaylord National Resort and Convention Center in National Harbor, Maryland (adjacent to Washington, DC).

CHAPTER ONE:
INTRODUCTION

1.1 Background information

Malaria is caused by five recognized *Plasmodium* species infecting human; *Plasmodium falciparum, P. malariae, P. ovale*, *P. vivax* and *P. knowlesii* with the latter being recently identified to infect human (Antinori *et al.,* 2013). Globally, 90% of all malaria mortality is caused by *Plasmodium falciparum* (Garcia, 2010; Snow, 2015). *Plasmodium falciparum* is reported to cause 99.7% of all malaria cases in Sub-Saharan Africa (WHO, 2018). In Kenya, *Plasmodium falciparum* is reported to cause majority of all infections (MOPHS, 2009).. In western Kenya, *Plasmodium falciparum* remains the most abundant (92%) with sporadic reports of *P. malariae* (6%) and *P. ovale* at 2% (MOH, 2016c). *Plasmodium malariae* and *P. ovale*, which are regarded as causing milder infections than *P. falciparum*, are typically associated with low parasite densities (Nino *et al.*, 2016; Wongsrichanalai *et al.*, 2007). *Plasmodium malariae,* in particular, is linked to chronic low-grade infections that can result in anemia or nephropathy (Douglas *et al.*, 2013; Langford *et al.*, 2015). Furthermore, due to low parasite densities, detecting *Plasmodium malariae* and *P. ovale* by microscopy or malaria rapid diagnostic test kits (RDTs) is difficult, resulting in misdiagnosis and underestimation of these species (Lo *et al.*, 2017; Murray *et al.*, 2008).

Although malaria is a global health issue, Sub-Saharan Africa bears the lion's share of the burden (WHO, 2021). Due to the debilitating nature of this disease, three billion dollars have been set aside for the eradication of the malaria burden in 2019 (Feachem *et al.*, 2019; WHO, 2020). Despite massive investments in malaria control programs in malaria-endemic nations, the disease continues to claim a significant number of lives. In 2021, there were an estimated 241 million malaria cases worldwide, with 95% of these cases being reported in Sub-Saharan Africa (WHO,

2022). The 247 million cases resulted in 619,000 deaths worldwide, with Sub-Saharan Africa accounting for 593,000 of them. In Kenya, malaria remains a major health challenge with roughly 70% of the total population is still at risk of infection (MoH, 2021). Furthermore, 13% to 15% of all outpatient consultations in healthcare facilities are for clinical malaria (MoH, 2021). .

There is a renewed determination in Kenya to eradicate malaria by 2023 (MoH, 2020). As a result, expanded malaria control program, with a focus on malaria-endemic areas is being implemented. Indoor residual spraying, which was suspended in 2012 due to reports of pyrethroid insecticide resistance, was reintroduced in 2018, but with a different insecticide (an organophosphate, pirimiphos-methyl; Actellic® 300CS) (Bashir *et al.*, 2019; MoH, 2016b; Ochomo *et al.*, 2014; PMI, 2018). These interventions, along with long-lasting insecticidal treated nets (LLINs), which have been the main vector control measures in previous years, aim to significantly reduce malaria transmission and, if possible, achieve the goal of the country's malaria strategy for 2019-2023 (MoH, 2020). Concurrently, the government of Kenya has prioritized food security for the people. In some regions of western Kenya, agricultural food production has been inadequate due to unreliable rainfall, resulting in a perpetual food shortage (MoRD, 2006). Therefore, land classified as arid and semi-arid is being converted into irrigation systems in order to increase food production.

Irrigation schemes and dam construction have been implemented in Rangwe and Rachuonyo North sub-counties of Homa Bay County. In most developing countries, such projects are associated with economic prosperity and food security (Keiser *et al.*, 2005). However, disruption of natural ecology results in profound influence on emergence and increment of parasitic diseases (Brunner *et al.*, 2016). Construction of dams and irrigation systems may impact malaria transmission

patterns by creating more aquatic habitats necessary for malaria vectors' breeding thereby placing huge health burden to local communities (Keiser *et al.*, 2005).

The objective of present study was to determine the effect of a newly constructed irrigation scheme on malaria burden in Homa Bay County, western Kenya. Results of this study will guide policy makers in the health, agriculture and environment sectors with regard to malaria risk management in current and future irrigation schemes.

1.2. Statement of the problem

Development of dams and irrigation schemes are paramount to boosting economic prosperity and food security in developing nations (Keiser *et al.*, 2005). In spite of their valuable contribution to food production, they are again associated with negative public health repercussions like malaria and other water-related diseases (WHO, 2005). Development of irrigation schemes in different regions have impacted malaria transmission in variable ways (Kibret *et al.*, 2015; Sharma *et al.*, 2008). For instance, introduction of irrigation schemes in Northern Cameroon, Sudan and Tigray in Ethiopia led to upsurge in vector densities and malaria incidence (el Gaddal *et al.*, 1985; Robert *et al.*, 1992; Yohannes *et al.*, 2005). In contrast, studies within Ahero rice scheme and Mwea irrigation scheme in Kenya, reported lower malaria transmission within irrigated areas than in non-irrigated areas (Githeko *et al.*, 1993; Muturi *et al.*, 2008).

Water management methods and irrigation type are two significant elements that may influence malaria transmission in irrigated areas. Proper water drainage in irrigated areas, for example, may result in conditions unsuitable for vector breeding, lowering malaria transmission (Mutero *et al.*, 2000). Poorly managed irrigation schemes with leaking canals and clogged canals, on the other hand, may generate favorable environments for mosquito breeding, hence sustaining malaria

transmission all year (Kibret *et al.*, 2014). Flood irrigation employed in rice agriculture may increase the breeding of *Anopheles arabiensis* which prefers open water habitats (Haileselassie *et al.*, 2021; Sanchez-Ribas *et al.*, 2012). A concrete channel and gravity-driven water irrigation plan with various dams was built in Homa Bay county, western Kenya. The irrigation scheme is mostly used for rice farming, and water management is handled by members of the community. This type of irrigation may affect malaria transmission; however, no research has been conducted to establish the likely effects on prevalence of *Plasmodium* infection.

1.3. Objectives of the study

1.3.1. General objective

To compare the prevalence of malaria within irrigated and non-irrigated areas in Homa Bay County, western Kenya.

1.3.2. Specific objectives

i. To compare the prevalence of *Plasmodium* infections and anemia within the irrigation scheme and non-irrigated areas
ii. To evaluate knowledge, attitude and practice on malaria infection, control and malaria treatment seeking behavior.
iii. To determine the impact of indoor residual spray on asymptomatic and submicroscopic *Plasmodium* species infections

1.4. Hypothesis

Irrigation scheme affects the prevalence of *Plasmodium* infections within the study area.

1.5 Research questions

i. Does irrigation scheme affect the prevalence of *Plasmodium* infections?
ii. What is the effect of irrigation scheme on anemia?

iii. What is the effect of indoor residual spraying program on *Plasmodium* infections?

iv. What is the attitude or practice towards malaria and treatment seeking behavior?

1.6. Justification of the study

Surveillance and documentation of the parasitological profile of malaria parasite infection, *Plasmodium falciparum* parasite densities, among residents in endemic areas is critical for determining disease burden and evaluating the efficacy of existing control strategies (Bridges *et al.*, 2012). Along the lake region of Kenya, malaria transmission is perennial (MoH, 2016a), and environmental changes as a result of irrigation schemes may alter malaria infection rates, necessitating the incorporation of more competent control tools and prudent disease management. This can be accomplished through an elaborate revelation of malaria infection pattern. Previous malaria control programs resulted in significant reductions in community anemia (Korenromp *et al.*, 2004). As a result, the malaria burden or the efficacy of malaria interventions can be tracked by determining anemia rates in a community.

Previous research findings also point to an increase in the burden of asymptomatic and submicroscopic infections in malaria endemic areas (Diallo *et al.*, 2012; Lindblade *et al.*, 2013; Okell *et al.*, 2012). These infections have persistently posed a threat to current control strategies due to their insidious ability to sustain transmission (Nzobo *et al.*, 2015a). Effective parasite detection tools are required to mitigate further damage to existing control strategies resulting from the spread of asymptomatic and submicroscopic infections. As previously reported, the molecular techniques used in this study are capable of detecting submicroscopic malaria parasites (Jones *et al.*, 2012; Kuamsab *et al.*, 2012). Malaria disease management is also considered among the key pillars to malaria control and elimination (Nalinya *et al.*, 2022; WHO, 2020). Malaria treatment seeking, knowledge, attitude, and perception regarding malaria and its control measures are

important community factors that are likely to influence malaria management. Documenting such variables can guide policymakers in taking crucial measures to enhance malaria management and achieve the elimination goal.

CHAPTER TWO:
LITERATURE REVIEW

2.1. Malaria transmission pattern in Kenya

Kenya has been categorized into four distinct epidemiological zones based on varying malaria transmission intensities as shown in figure 2.1 (MoH, 2016a). These zones are classified based on altitude, temperature intensity, and rainfall pattern. Existing zones include endemic areas (such as the coastal region and the lake region of western Kenya) highlands epidemic vulnerable zones (such as the western highlands), seasonal malaria transmission zones (arid and semi-arid areas), and low-risk transmission areas such as Nairobi and the central part of Kenya (MoH, 2016b). Each transmission zone has specific temperature, precipitation, and humidity ranges that not only influence malaria parasite development, but also affect the survival and biting rate of mosquitoes, hence altering the malaria transmission pattern (Gage *et al.*, 2008; Githeko *et al.*, 2000).

Malaria endemic regions are characterized by lower altitudes and higher temperatures which is suitable for both parasite and mosquito growth and development leading to increased malaria transmission (Imbahale *et al.*, 2012; Kibret *et al.*, 2017) Higher temperatures also accelerate feeding rates of female mosquitos increasing the chance of malaria parasite transmission (Midekisa *et al.*, 2015). Rainfall on the other hand, influences malaria transmission by sustaining the breeding habitats of malaria vectors (Briet *et al.*, 2008). In malaria-endemic areas like Homa Bay County, suitable climatic conditions enhance mosquito survival rates, resulting in perennial malaria transmission (Elnour *et al.*, 2023; MoH, 2020). Malaria parasite prevalence along the lake region of western Kenya usually exceed 20% (Maniga *et al.*, 2022). Coastal region although classified as malaria endemic region, the prevalence rate is approximately 8% (Maniga *et al.*, 2022). Due to exposure to infective mosquito bites in endemic areas, older children and adults usually develop

immune response against malaria parasites reducing cases of severe malaria and epidemics (Laishram *et al.*, 2012)

Highland regions around western Kenya are usually characterized by lower temperatures which inhibits growth of malaria parasites and larval stages of anopheline (Aly *et al.*, 2009; Bousema *et al.*, 2011b; Elnour *et al.*, 2023; Githeko *et al.*, 2000). However, when temperatures rise to around 18 ^{0}C and above, malaria epidemics occur (Elnour *et al.*, 2023; MoH, 2020). Malaria transmission around western highlands is therefore seasonal depending on temperature variation (Elnour *et al.*, 2023). Natural immunity to malaria is weak in Kenya's western highlands, and when malaria transmission returns, epidemics are common, with a high risk of infections progressing to severe malaria (Hay *et al.*, 2002). Malaria prevalence around these regions is estimated to be approximately 3% (Maniga *et al.*, 2022).

In arid and semi-arid areas such as Samburu, Wajir and Turkana Counties, malaria transmission is seasonal especially during short rains (Elnour *et al.*, 2023). Since the temperatures in these areas are suitable to parasite development and mosquito reproduction, the creation of breeding habitats after short rains commences malaria transmission (Mala *et al.*, 2011). Like highland areas, people living in arid and semi-arid parts of Kenya, have poorly developed immune response towards malaria (Elnour *et al.*, 2023). Therefore, during periods of malaria transmission, epidemics usually occur. Malaria parasite prevalence is about 1% (MoH, 2020).

In central parts of Kenya, malaria transmission is minimal due to low temperatures (MoH, 2020). Low temperatures at higher altitudes significantly reduce malaria transmission, however, minor temperature increases can occasionally cause epidemics in populations with a poorly developed immune response to malaria parasite infection (Hay *et al.*, 2002).

Understanding the dynamics of malaria transmission is critical when developing an effective malaria control strategy (Hay *et al.*, 2006).

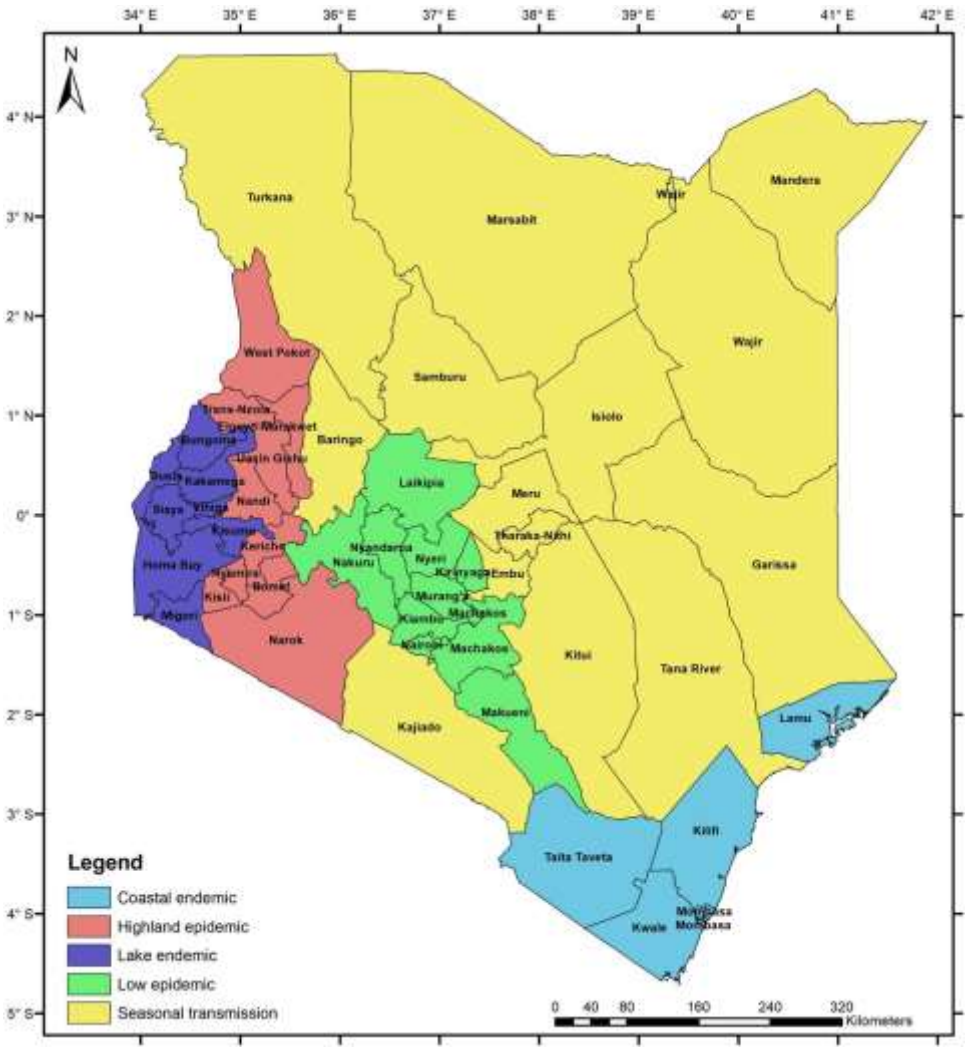

Figure 2.1 Map showing malaria epidemiological zones in Kenya (Source: (Elnour *et al.*, 2023))

2.2. Life cycle of *Plasmodium* parasites

Malaria parasites have a complex life cycle as they are able to develop both within and outside the cell niches of the host and vector (Aly *et al.*, 2009). The life cycle is composed of both sexual and asexual stages taking place in mosquito vector and human host respectively (Figure 2.2). During the blood meal, the mosquito picks both the female and male gametocytes. These gametes merge

to form a zygote and evolves into ookinete within the vector's midgut. Ookinete transforms to oocyst from which many sporozoites come forth and move to salivary glands via hemolymph. It's at this point that the sporozoites are transmitted to human beings when mosquitos bite (Cator *et al.*, 2014; Cator *et al.*, 2013; Paaijmans *et al.*, 2013; Smallegange *et al.*, 2013).

Within the human host, the parasite undergoes asexual phase both in the hepatocytes and red blood cells (Amino *et al.*, 2006; Lindner *et al.*, 2012; Yamauchi *et al.*, 2007). Sporozoites enter the liver cells and begin asexual multiplication (Antinori *et al.*, 2012). Sporozoites develop into schizonts, which then rapture, releasing merozoites into the bloodstream. Time taken for merozoites to invade blood cells differs among the malaria parasites. For instance, *Plasmodium falciparum* usually take shorter period (6-14 days) for merozoites to rapture into the blood stream (Mazier *et al.*, 2009). Clinical signs appear once the merozoites are discharged into the blood stream and parasite detection is possible during this stage of illness. However, people who reside in malaria endemic areas usually develop strong immune response to malaria parasites which may suppress the symptoms, resulting in to silent infections (Marsh *et al.*, 2006). Detection of silent infections is a challenge due to absence of symptoms and this further poses serious challenge to malaria control measures in malaria endemic areas (Laishram *et al.*, 2012).

Invasion of bloodstream by *P. falciparum* merozoites may trigger a rapid increase of parasite densities thereby influencing severe disease (Bousema *et al.*, 2014). Buildup of parasite densities consequently result in massive destruction of red blood cells, lowering hemoglobin level and resulting in anemia (White, 2018). A portion of merozoites discharged from infected red blood cells develop into sexual gametocytes both male and female. These gametocytes mature within the bone marrow for about 10-12 days (Eichner *et al.*, 2001), appear in the peripheral blood and can be ingested by mosquitoes when they take a blood meal (Bousema *et al.*, 2014; Garnham, 1988).

Studies indicate that the first produced gametocytes are likely to circulate at very low densities but can sustain transmission (Bousema *et al.*, 2011a). Therefore, to interrupt transmission, accurate quantification and clearance of gametocytes within a population is very important.

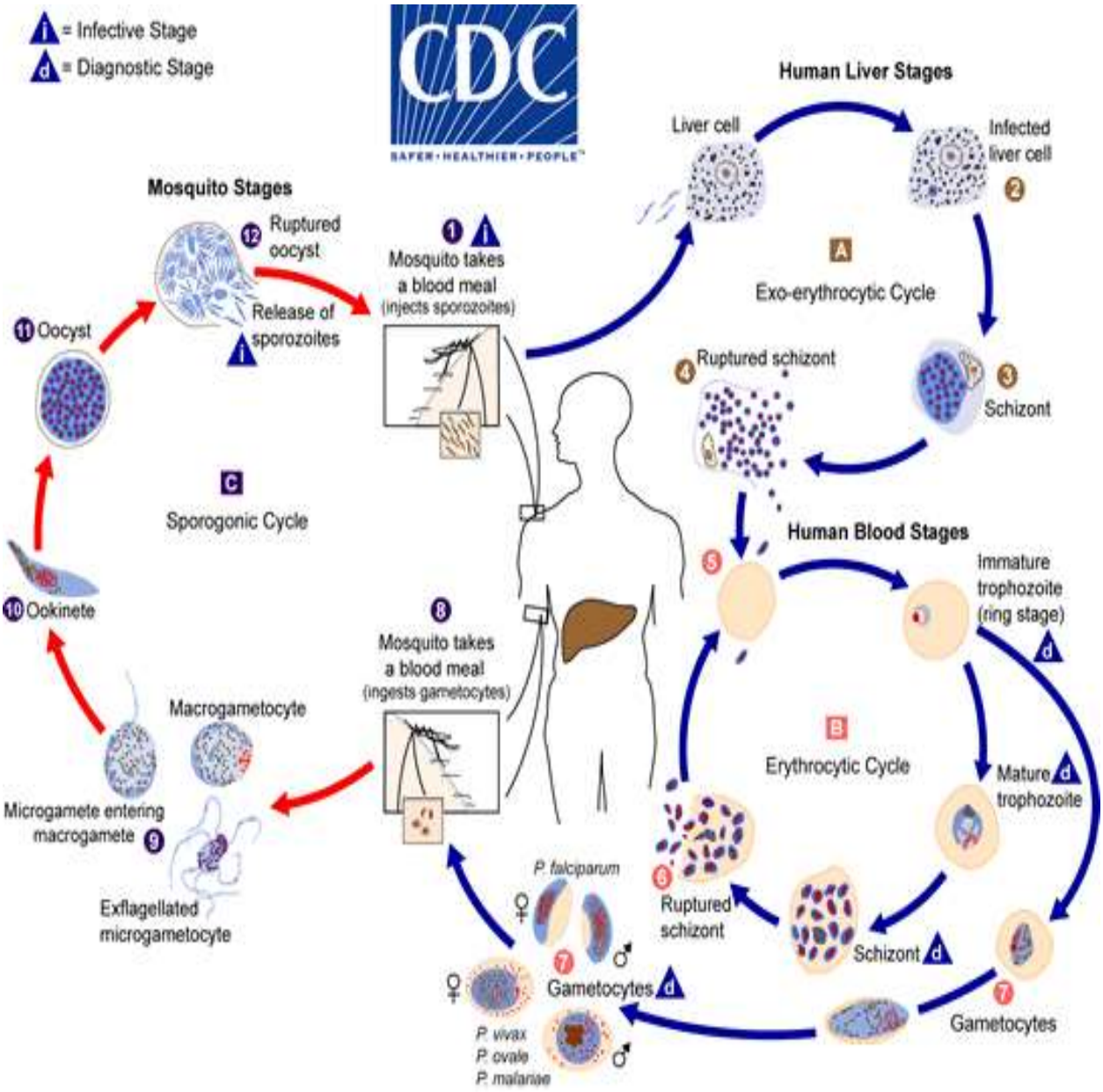

Figure 2.2 The life cycle of a *Plasmodium* parasite. (Source: Centers for Disease Control and Prevention, http://www.cdc.gov/malaria/about/biology/index.html.)

2.3. Malaria pathology and clinical manifestations

Clinical symptoms emerge as soon as the malaria parasites infiltrate the red blood cells (B. M. Greenwood *et al.*, 2008; Omer *et al.*, 2003). Both severe and uncomplicated malaria manifests with common clinical symptoms such as chills, fever, headache, and vomiting, sweating and joint pains. As the parasites multiply and grow within the red blood cells, they rapture the infected erythrocytes and waste products and toxic factors are discharged into the blood which consequently stimulates the action of the immune system. Temperatures above 37.5^0C, headache, and rigors, have been adversely linked to the action of cytokines against these parasite and red cell membrane products (Clark *et al.*, 2006). While *P. falciparum* infection has been vastly linked to a majority of severe malaria and related mortality, increasing body of evidence indicate that non-*falciparum* infections can lead to complications. Recent studies reported cases of severe infection including death due to *P. vivax* and *P. knowlesi* infections (Anstey *et al.*, 2009; Anstey *et al.*, 2007; Cox-Singh *et al.*, 2010; Daneshvar *et al.*, 2009; Kochar *et al.*, 2005) .

Majority of fatal cases caused by *P. falciparum* result from cerebral malaria or anemia. However variable symptoms which differ in severity also exist based on the organ involved and the health-seeking behavior (Autino *et al.*, 2012). Parasite sequestration which may lead to organ failure or malfunction has been linked to the occurrence of severe clinical symptoms and cerebral malaria (Grau *et al.*, 2012). In western Kenya, *P. falciparum* accounts for about 92% of total malaria infections (MoH, 2016b), hence it is essential to ascertain the spectrum of disease severity within the study site to design appropriate interventions.

2.4. Diagnosis of malaria

Malaria diagnosis entails screening blood for malaria parasites or antigens. To cut down malaria-related morbidity and mortality, diagnosis must be precise and timely (Tangpukdee *et al.*, 2009).

With breakthroughs in parasite identification, the World Health Organization now advices that all suspected malaria cases undergo parasite-based diagnosis prior to taking antimalarial medication (WHO, 2015a). Accuracy in malaria diagnosis is critical not only for reducing rising drug resistance, but also for implementing cost-effective treatment, especially with the arrival of expensive artemisinin-based combination therapy (Amexo *et al.*, 2004; Barnish *et al.*, 2004; Zurovac *et al.*, 2006). Case confirmation further avoids inappropriate antimalarial drug use and ensures that patients with fever who do not have malaria receive the necessary therapy (MoH, 2016d). Furthermore, proper and prompt diagnosis is likely to reduce treatment failure, parasite recurrence, and, all of which are commonly linked with misdiagnosis of non-*falciparum* parasite primary infection (Savargaonkar *et al.*, 2014; A. Smith *et al.*, 2011).

In recent years, several diagnostic techniques are being considered to aid in the accurate and rapid detection of malaria parasites. Today's malaria diagnostic tools consist of rapid diagnostic test kits, microscopy, and polymerase chain reaction assays (Mbanefo *et al.*, 2020). Clinical diagnosis was widely used prior to advances in parasite detection, as well as in some malaria endemic areas (Berzosa *et al.*, 2018; Tangpukdee *et al.*, 2009). Furthermore, clinical diagnosis has been widely used and antimalarial medications have been prescribed in regions with poorly equipped laboratories that cannot provide microscopy-based diagnosis (Bardaji *et al.*, 2008; Endeshaw *et al.*, 2008; Juma *et al.*, 2011). Because malaria symptoms are generally non-specific and frequently overlap with symptoms of bacterial or viral diseases, this type of diagnosis is considered inaccurate (Tangpukdee *et al.*, 2009). For example, a study in western Kenya on children with clinical symptoms similar to malaria discovered that the majority of them had upper respiratory viral infections, such as influenza A virus and rhinovirus infections, rather than malaria (Waitumbi *et al.*, 2010). The overlap of these symptoms complicates diagnosis, lowering clinical diagnosis

accuracy and potentially leading to antimalarial drug overuse (Mwangi *et al.*, 2005; Reyburn *et al.*, 2004). As a result, microscopy and rapid diagnostic test kits (RDT) are widely regarded as the most preferred diagnostic tools capable of significantly contributing to malaria control (Berzosa *et al.*, 2018; Wongsrichanalai *et al.*, 2007).

2.4.1 Microscopy diagnosis

Microscopy-based diagnosis has been ideal for diagnosing malaria because it is readily available and inexpensive to acquire (Makler *et al.*, 1998; Mukry *et al.*, 2017; Ndao *et al.*, 2004). Numerous health facilities have adopted microscopy due to its capacity to facilitate simple differentiation of malaria parasites using thin smear and estimation of parasite density using thick smear (Feleke *et al.*, 2017; Oyegoke *et al.*, 2022). However, due to unreliable power supplies or a lack of properly established infrastructure, microscopy-based diagnosis is not feasible in most remote and resource-limited areas (Kumar *et al.*, 2020). Although the sensitivity of microscopy is approximately 50-500 parasites/µl, when performed by incompetent personnel, its sensitivity is drastically reduced and becomes extremely low (Berzosa *et al.*, 2018; Moody, 2002). Other obstacles to microscopy include poor slide quality, sub-microscopic infections, and mixed species infections, all of which can significantly reduce the sensitivity of microscopy, resulting in poor parasite detection (Bisoffi *et al.*, 2010; Oyegoke *et al.*, 2022). To reduce misdiagnosis, the majority of healthcare facilities with trained personnel always have two or three experts read slides before declaring samples negative or positive (Berzosa *et al.*, 2018).

Considering the usefulness of microscopy in malaria management, governments and stakeholders need to invest in health-care facilities to ensure reliable microscopy diagnosis. Previous research indicates that the majority of health facilities, particularly in developing nations, do not meet operational standards due to inadequate training, a dearth of quality reagents, and erratic electricity

supply (Harchut *et al.*, 2013; Kahama-Maro *et al.*, 2011). Regular quality assessments in health care facilities are therefore critical to encouraging accurate microscopy-based diagnosis, as recommended by the World Health Organization (WHO, 2016).

2.4.2 Malaria rapid diagnostic tests (RDT)

Malaria rapid diagnostic tests are used to detect the antigen of malaria parasites in blood (Jang *et al.*, 2020). *Plasmodium* species that cause human infections express antigens like *Plasmodium* lactate dehydrogenase (pLDH), *Plasmodium* aldolase, and Histidine-rich protein (HRP-2) (Cunningham *et al.*, 2019; Mouatcho *et al.*, 2013). RDT kits that can detect only HRP-2 can diagnose *P. falciparum* infection, whereas the ones that can detect either pLDH or aldolase antigen can identify infections caused by parasites other than *P. falciparum* (Wanja *et al.*, 2016). Mostly detected *Plasmodium* antigen are pLDH and HRP-2 (Mouatcho *et al.*, 2013). RDTs are simple to use, take less time to display test results, require no power supply, and have a sensitivity of 100-200 parasites/µl of blood (Laban *et al.*, 2015; Moody, 2002). Rapid diagnostic test kits were introduced in endemic settings, particularly in areas with few microscopists and a lack of adequate infrastructure for microscopy diagnosis (Lubell *et al.*, 2007; Ogunfowokan *et al.*, 2020). Malaria parasite antigen's propensity to persist in the blood many days after the parasites have been eradicated is one of the major obstacles and typically results in false-positive RDT results (Yan *et al.*, 2013). The sensitivity of RDT kits may also be affected by the high temperature, humidity, and deletion of gene (Berhane *et al.*, 2017; Yan *et al.*, 2013).

Since the introduction of RDTs in the 1990s, more than 200 different RDT kits from various manufacturers have been developed for malaria diagnosis (Cunningham *et al.*, 2019; Kavanaugh *et al.*, 2021). Although the majority of RDTs target *Plasmodium falciparum*-specific antigens, others can target both pan-specific antigens (pan malaria or aldolase) and *Plasmodium falciparum*-

specific antigens(Tangpukdee *et al.*, 2009). In addition, some RDTs have been developed to adequately diagnose *P. vivax* (Kim *et al.*, 2008; T. S. Park *et al.*, 2006) and *P. knwolesi* (McCutchan *et al.*, 2008). Over a 9-year period beginning in 2010, approximately 1.9 billion RDTs were distributed, with Africa receiving 84% of them (WHO, 2020). The parasitological testing of suspected malaria cases increased from 36% in 2010 to 87% in 2016 as a result of widespread distribution of RDT, primarily in African countries (WHO, 2017a).

2.4.3 Molecular diagnosis

Proper malaria infection management necessitates the use of high-accuracy parasite detection tools, particularly in areas with low parasite density among asymptomatic individuals (Cook *et al.*, 2015). Asymptomatic infections are characterized by submicroscopic parasitemia, which is frequently undetectable by RDT or microscopy (Morris *et al.*, 2015). Polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) are the most precise forms of molecular tools for malaria examination (Tambo *et al.*, 2018). The two diagnostic techniques can detect parasites in samples with extremely low parasite concentrations, as well as mixed infections (Morassin *et al.*, 2002). When compared to microscopy and RDT, PCR and LAMP are much more sensitive, with a detection threshold of 1-5 parasites/μl of blood (Berzosa *et al.*, 2018; Tambo *et al.*, 2018).

Three modified forms of PCR, reverse transcription PCR, nested PCR, and real-time PCR, have vastly improved parasite detection in the majority of epidemiological surveys (Imwong *et al.*, 2008; Mlambo *et al.*, 2008; Swan *et al.*, 2005). However, the most accurate in detection of parasites causing malaria is nested PCR (Banoo *et al.*, 2006). During diagnosis, PCR can target a variety of genes, including, *AMA1* genes (Garg *et al.*, 2007), 18S ribosomal RNA genes (Imoukhuede *et al.*, 2007; E. Kamau *et al.*, 2011; Okell *et al.*, 2009), *cytochrome b* (Farrugia *et al.*, 2011; Hwang *et*

al., 2012)(), and transfer RNA (*tRNA*) (Beshir *et al.*, 2010). Among the four genes listed, 18S rRNA is utilized in the majority of studies (Adams *et al.*, 2015; Imwong *et al.*, 2014). They are being considered for diagnosis in most laboratories in endemic countries due to their high detection of both parasites and gametocytes (Andrade *et al.*, 2010; E. Kamau *et al.*, 2011). These molecular detection techniques are particularly useful in areas where malaria transmission is decreasing as a result of long-term control interventions, resulting in infections with low parasite densities that are likely to be missed by microscopic diagnosis. Despite their most valuable contributions to malaria diagnosis, PCR diagnostic methods are extremely complex and must be operated by highly trained personnel. Furthermore, PCR machines and reagents are very expensive, making them difficult to acquire and deploy in health care facilities in resource-constrained countries (Makanjuola *et al.*, 2020; Mens *et al.*, 2008).

The loop-mediated isothermal amplification technique can amplify nucleic acids under isothermal conditions (Ocker *et al.*, 2016). The technique is relatively simple to use, yields results more quickly, and has a parasite detection threshold comparable to nested PCR (Cook *et al.*, 2015; Hopkins *et al.*, 2013; Ocker *et al.*, 2016). Furthermore, the LAMP technique is less expensive than PCR and thus may be accessible in resource-limited areas (Aonuma *et al.*, 2008; Han *et al.*, 2007; Hopkins *et al.*, 2013). Due to the stability of its reagents, LAMP technology appears to be a suitable diagnostic tool in tropical countries (Thekisoe *et al.*, 2009). As a result, this technology provides hope for a user-friendly and accurate malaria diagnostic tool in most endemic settings and areas with reduced transmission as a result of concerted control interventions.

Other notable molecular techniques applicable in malaria diagnosis include flow cytometry, microarray, and mass spectrometry. Flow cytometry employs hemozoin, a pigment produced when malaria parasites digest hemoglobin, to detect infection by malaria parasites (Fitri *et al.*, 2022;

Tangpukdee *et al.*, 2009). However, with regard to complexities involved in its operation, costly equipment needed, and the risk of false positive outcomes as a result of viral or bacterial infection, the flow cytometry technique is preferred for screening blood samples (Tangpukdee *et al.*, 2009). Microarrays, however, may be considered potential malaria diagnostic tools, are still in early stages of development and improvements (Erdman *et al.*, 2008). Mass spectrometry is an in vitro diagnostic tool for malaria with a detection limit of 10 parasites per microliter of blood (Tangpukdee *et al.*, 2009). Mass spectrometry, like flow cytometry, detects hemozoin to indicate malaria parasite infection (Scholl *et al.*, 2004). It rapidly detects in vitro-cultivated *Plasmodium falciparum* (Demirev *et al.*, 2002).

2.4.4 Other techniques

Serological techniques such as the enzyme-linked immunosorbent assay (ELISA) and the immunofluorescence antibody assay test (IFAT) have primarily been used to detect or estimate the concentration of malaria antibodies produced following parasite infection (Doderer *et al.*, 2007). ELISA can as well be used to detect malaria parasite antigens such as PfHRP-2 in blood (Kifude *et al.*, 2008). Although IFAT has been regarded as the gold standard in the field of serological testing (Candolfi, 2005), the process is considered time consuming and complicated to automate (Doderer *et al.*, 2007). ELISA, on the other hand, is easy to automate but less sensitive than IFAT (Chiodini *et al.*, 1997; Contreras *et al.*, 1999; Silvie *et al.*, 2002). ELISA kits that have recently been developed, on the other hand, have shown sensitivities comparable to IFAT (Doderer *et al.*, 2007; A. D. Kitchen *et al.*, 2004).

Serological techniques involve the detection of antibodies produced in response to asexual blood stage *Plasmodium* species (Duo-Quan *et al.*, 2009; Slater *et al.*, 2022). Merozoite-specific immunoglobulin G (IgG) and immunoglobulin M (IgM) are among the antibodies released in

response to malaria parasite infection (Doderer *et al.*, 2007; C. G. Park *et al.*, 2000). The antibodies can remain in the blood for three to six months after the malaria parasites have been eradicated (Doderer *et al.*, 2007). Due to possible reinfections, semi-immune people living in malaria-endemic areas may have these antibodies in their blood for months or years (Doderer *et al.*, 2007). Relapses caused by *Plasmodium vivax* or *P. ovale* may both induce a secondary immunity resulting in the production of high levels of antibodies (Garraud *et al.*, 2003; Seed *et al.*, 2005). Detection of malaria parasite-specific antibodies is not considered a substitute for microscopy diagnosis, but rather a tool for malaria epidemiological studies or screening blood donors (A. Kitchen *et al.*, 2005; Rodrigues *et al.*, 2003; Tangpukdee *et al.*, 2009). Furthermore, IFAT procedures take time, necessitate highly trained personnel to operate and interpret immunoflorescence microscopy, and require close attention when preparing its reagents (Doderer *et al.*, 2007; Muerhoff *et al.*, 2010).

2.5. Malaria control strategies

Malaria has remained a major public health issue in many sub-Saharan African nations, claiming many lives (Badmos *et al.*, 2021). Pregnant women and young children, have been particularly hard hit by this disease. As a result, there has been a spirited fight involving variety of tools and strategies to save humanity from the disease's devastating effects. The Roll Back Malaria (RBM) initiative was one of the leading global programs developed and implemented in 1998 to reduce malaria burden (Nabarro, 1999). This drive resulted in the development of a number of strong mechanisms aimed at providing accurate and timely disease diagnosis, prompt treatment, vector control, and efficient service delivery at healthcare facilities (Badmos *et al.*, 2021).

Notable tools thought to have the greatest potential to accelerate malaria reduction in Sub-Saharan African settings included the use of insecticidal treated bed nets (ITNs), malaria case management, and application of indoor residual spraying (IRS) (Kleinschmidt *et al.*, 2009; Okumu *et al.*, 2011).

Other interventions such as larval source management (Fillinger *et al.*, 2011), house improvement (Zhao *et al.*, 2016), spatial repellents (Achee *et al.*, 2012), sugar feeding (Beier *et al.*, 2012), and use of genetically modified mosquitoes (B. Greenwood, 2017), have been under trial for vector control and disease management.

2.5.1 Long Lasting Insecticidal Nets (LLINs)

Long Lasting Insecticidal Nets remains the primary tool for malaria prevention in Sub-Saharan Africa (Ng'ang'a *et al.*, 2021). The World Health Organization increased its efforts to ensure that all vulnerable populations are covered and have access to LLINs (WHO, 2008a). The widespread use of LLINs in Africa reduced malaria incidence and mortality by 42% and 66% respectively (WHO, 2018). Besides, extensive use of bed nets was linked to significant reductions in malaria cases between the years 2000 and 2015 (Bhatt *et al.*, 2015). However, the rate of reduction in malaria cases, appears to have slowed since 2015, particularly in sub-Saharan Africa, with cases actually increasing (Talapko *et al.*, 2019; WHO, 2018). For example, there were fewer 204 million fewer cases of malaria in the year 2000 compared to 229 million in 2019 (WHO, 2020). This slow rate of reduction, combined with the likely reversal of malaria cases, jeopardizes the Global Technical Strategy for malaria elimination's long-term objectives (WHO, 2015c).

For many years, LLINs have been treated with three-year-lasting pyrethroid insecticides like permethrin and deltamethrin (WHO, 2011). However, recent studies indicate increasing resistance towards pyrethroid insecticides rendering LLINs less effective (Agossa *et al.*, 2015; Fang *et al.*, 2019; Protopopoff *et al.*, 2018). The stalled reduction in malaria cases has been attributed in part to mosquito pyrethroid insecticide resistance (Minakawa *et al*., 2021). As a result, new and more effective organic compound, such as Piperonyl butoxide (PBO), are being developed and incorporated into LLINs to increase their potency against resistant malaria vectors (WHO-GMP,

2017; WHO, 2019). Crucial considerations, other than pesticide resistance, must be considered to guarantee that LLINs provide comprehensive protection to vulnerable populations. LLINs coverage, ownership, and bed net use are among these characteristics.

Adequate bed net coverage is critical, especially in malaria-endemic areas. The World Health Organization calculates LLIN coverage by taking into account families with one LLIN for every two persons. (WHO, 2017b). When LLINs are used properly, coverage of at least 60% (Killeen *et al.*, 2017), is expected to provide effective protection. For example, in Sub-Saharan Africa, correct and consistent use of LLINs has been related to remarkable decreases in both malaria death and morbidity. (Ng'ang'a *et al.*, 2009). As a result, the African continent currently has greatly expanded LLINs coverage. LLIN ownership grew per household from 3% in 2000 to 54% in 2013. (West *et al*., 2014, WHO, 2013).

Kenya's government, in collaboration with other partners, has increased the distribution of LLINs to safeguard individuals at risk. Approximately 49 million LLINs were distributed throughout the mass distribution periods that began in October 2004 and were repeated in 2006, 2011, and 2014 (MoH, 2016a; Ng'ang'a *et al.*, 2021). A similar mass distribution program was carried out in 2017, with an extra 15.1 million LLINs distributed (MoH, 2019a). This exercise resulted in 83% coverage and 51% universal coverage ownership (1 LLIN against two people in a household).(MoH, 2019a) Aside from a three-year distribution timetable for LLINs, the Kenyan government is still committed to providing bed nets to vulnerable people through antenatal clinics and child welfare clinics for pregnant women and children under the age of one year. (MoH, 2019a).

2.5.2 Indoor Residual Spraying (IRS)

Indoor residual spraying program, which has remained the principal vector control technique in malaria-endemic areas, primarily targets indoor resting mosquitoes (Tangena *et al.*, 2020). The rollout of this initiative resulted in the global elimination and eradication of malaria in a number of nations where malaria posed a significant problem (Tizifa *et al.*, 2018; WHO, 2015c). The IRS program uses five classes of insecticides which includes, pyrethroids, organophosphates, carbamates, organochlorines, and neonicotinoids (Syme *et al.*, 2021; WHO, 2012).

The initiative, which first targeted areas with low or seasonal malaria incidence, was eventually expanded to include areas with high malaria transmission. (Tizifa *et al.*, 2018). This resulted in a substantial expansion in the number of nations dependent on IRS programs. (Tangena *et al.*, 2020). Although the overall number of persons protected by IRS programs in Sub-Saharan Africa climbed from 10 million in 2010 to 124 million in 2013 (WHO, 2015d), the proportion of persons covered by the IRS has been decreasing from 6% in 2010, to 4% in 2013, to 3% in 2017, and finally less than 2% in 2019 (Chaccour *et al.*, 2021; WHO, 2018, 2020). This reduction could be attributed to a variety of issues, including growing IRS operating costs (Chaccour *et al.*, 2021), the evolution of resistance to pyrethroid insecticides, the expensive cost of introducing non-pyrethroid insecticides, and a lack of funds to implement malaria vector control (Chaccour *et al.*, 2021; Coleman *et al.*, 2021; Pluess *et al.*, 2010; WHO, 2012). In Kenya, for instance, increased resistance to pyrethroid insecticides (Chen *et al.*, 2008; Kawada *et al.*, 2011), resulted to the termination of the IRS program in 2012 (Ochomo *et al.*, 2014), but the program was reinstated in February 2018 with organophosphate pesticides along the Lake region where malaria is endemic (PMI, 2018).

Prior to the reintroduction of IRS, the people living around the lake relied solely on long-lasting treated bed nets as malaria vector control. However, the prevalence of malaria remained higher than 20% (Bashir *et al.*, 2019; MoH, 2016b). Furthermore, malaria-related deaths among pregnant women and young children were still prevalent in this region (Bashir *et al.*, 2019; MoH, 2016b). This created a compelling need for improved control measures to reduce transmission rates, leading to the combination of IRS and LLINs. Previous studies using both IRS and LLINs as vector control yielded varying results, with some indicating no significant reductions in malaria rates (Corbel *et al.*, 2012), while others reported significant reduction (West *et al.*, 2014).

2.5.3 Malaria vaccines

Despite tremendous hurdles in developing malaria vaccines, significant progress and breakthroughs have been made (El-Moamly *et al.*, 2023). Malaria vaccine development has numerous challenges, including the parasite's capacity to intelligently elude the human immune system, complex biology, a lack of sterile immunity to malaria, the parasite's DNA, and the parasite's life cycle (Lorenz *et al.*, 2011). To address some of these challenges, scientists attempted to create vaccines containing a variety of parasite antigens in order to elicit a protective immune response (Anders *et al.*, 2010). However, because the small antigens formed less than 1% of the total parasite, adequate protection could not be developed (Hill, 2011). Second, due to antigenic variations (Renia *et al.*, 2016), and a significant number of polymorphisms, single protein vaccinations rarely succeeded (Rappuoli *et al.*, 2011).

Malaria parasites have distinct stages of development, and vaccines currently being tested are stage specific. The figure 2.3 below illustrate a number of vaccines developed to reduce malaria transmission. There are vaccines that target sporozoites, blood stage parasites, and those that target gametocytes to prevent transmission (Duffy *et al.*, 2020; Hill, 2011). Pre-erythrocytic vaccines targeting sporozoites, prevent hepatocyte infection and clear infected liver cells (Duffy *et al.*,

2020). They are considered to be the most effective since they include whole sporozoites as well as circumsporozoite protein (Arora *et al.*, 2021; Hill, 2011). The recent vaccine development focuses on circumsporozoite protein (CSP). For instance, the recently approved RTS,S popularly known as Mosquirix vaccine contains truncated CSP of *Plasmodium falciparum* (Arama *et al.*, 2014; El-Moamly *et al.*, 2023).

RTS,S vaccine was developed and approved by WHO in 2021 for use to reduce malaria transmission in young children (Duffy *et al.*, 2020; El-Moamly *et al.*, 2023). Significant reductions in malaria transmission have been observed following its adoption in areas with moderate to high transmission in Sub-Saharan Africa (El-Moamly *et al.*, 2023). The vaccine is currently being used in three African countries: Kenya, Malawi, and Ghana (El-Moamly *et al.*, 2023). According to a research conducted in Kenya to establish vaccine coverage, the report indicated a 96% and 78% coverage during the first to third dose implementation (Moturi *et al.*, 2023). The vaccine was also reported to provide 39% protection against clinical malaria and 29% protection against severe malaria among children who got the four doses of vaccine (Moturi *et al.*, 2023; Rts, 2015). Homa Bay County is one of the counties in Kenya where the vaccine rollout is taking place, and given the vaccine's proven efficacy, it is predicted to reduce malaria cases in young children

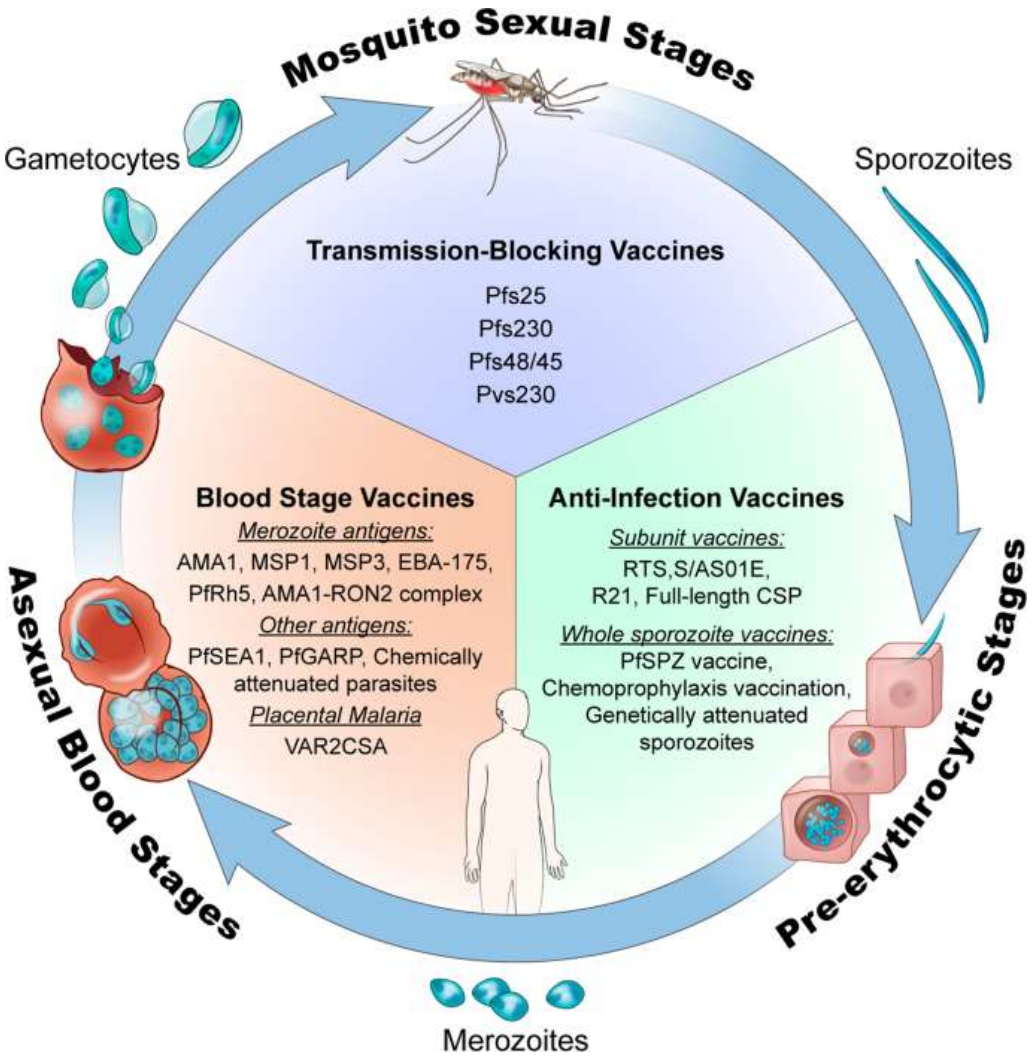

Figure 2.3 *Plasmodium* life cycle phases and vaccines produced for each stage (Duffy *et al.*, 2020)

2.6 Malaria case management

Malaria case management entails early detection of the disease, followed by treatment with effective antimalarial drugs. Easy-to-use diagnostic tools that provide accurate results, as well as the development of more potent antimalarial drugs, are likely to facilitate better malaria management.

Over the last two decades, there has been a renewed focus on improving malaria diagnostic tools to minimize inappropriate use of antimalarial drugs (Bell *et al.*, 2016). Despite significant

advancements in the development of these tools, malaria remains a serious burden in Sub-Saharan Africa. For example, the World Health Organization reported approximately 247 million cases of malaria and 619000 deaths in 2021, with Africa accounting for 95.8% of these deaths (WHO, 2022). A significant challenge confronting the African continent is the continent's high level of poverty and a lack of government support to effectively implement malaria control policies (Badmos *et al.*, 2021). In most remote African settings, poor access to health facilities, inadequately trained personnel, and substandard health services as a result of poorly equipped laboratories are typical (Oleribe *et al.*, 2019). For example, previous research estimated that 40% of Kenyans had to walk long distances to access healthcare services (Noor *et al.*, 2006). These challenges may have far-reaching implications for malaria case management. Furthermore, inaccessible healthcare facilities are likely to raise the cost of seeking treatment due to transportation costs. So, even if the government provides free antimalarial drugs and charges a nominal fee for other services, poor access to hospitals will have a negative impact on treatment seeking.

2.6.1 Malaria treatment

Malaria is a crippling disease caused by single-celled *Plasmodium* parasites (Sato, 2021). *Plasmodium* species that can infect humans are as follow: *Plasmodium falciparum*, *P. malariae*, *P. ovale*, *P. vivax,* and *P. knowlesi* (P. Li *et al.*, 2016; Sato, 2021; B. Singh *et al.*, 2013). Infection with *Plasmodium* species can manifest in a variety of ways, including no symptoms, mild symptoms or severe symptoms (Marteau *et al.*, 2021). *Plasmodium falciparum* can multiply extremely quickly, resulting in severe clinical symptoms and a number of deaths (Andrews *et al.*, 2018; Antinori *et al.*, 2012). Although, infections with *P. vivax , P malariae, P. ovale* and *P. knowlesi* are known to cause mild symptoms (Kotepui *et al.*, 2020b; Marteau *et al.*, 2021; Sato,

2021), they can equally lead to serious illness (D'Abramo *et al.*, 2018; Izri *et al.*, 2019; Kotepui *et al.*, 2020a, 2020b). *Plasmodium vivax* , for example, is less severe but, due to its widespread distribution, is always associated with mass morbidity (Andrews *et al.*, 2018; Antinori *et al.*, 2012). In the absence of treatment, *Plasmodium malariae* infection can rapidly multiply, causing severe illness and, in some cases, death within a few days (Kotepui *et al.*, 2020b; Roucher *et al.*, 2014).

Early treatment with effective antimalarial drugs is the only remaining life-saving intervention and means of reducing malaria burden and mortality (Rathmes *et al.*, 2020). There are five types of medicines used to clear malaria parasites (Ross *et al.*, 2019). They include: endoperoxides (artemisinins and its derivatives), antifolates (sulfadoxine, pyrimethamine, proguanil), 8-aminoquinolines (tafenoquine, primaquine), naphthoquinones (atovaquone), and aryl-amino alcohols (mefloquine, quinine) and 4-aminoquinolines (chloroquine). During the therapeutic phase, a small proportion of undetectable malaria parasites may not be eradicated, resulting in future malaria recurrence (Sato, 2021). *Plasmodium falciparum* and *P. malariae* are two *Plasmodium* species that may undergo recrudescence (Grande *et al.*, 2019; Kugasia *et al.*, 2014; Malvy *et al.*, 2018; Rutledge *et al.*, 2017). *Plasmodium vivax* and *P. ovale*, on the other hand, have been documented to relapse due to dormant cells known as hypnozoites that can remain in liver cells and may cause disease in months or years after the initial infection (Bartoloni *et al.*, 2012). However, no clinical research have demonstrated the potential of the remaining *Plasmodium* species to relapse or recrudescence (Sato, 2021). The only antimalarial medicine class that can target malaria parasites during the liver stage is 8-aminoquinolines (Baird, 2005; B. M. Greenwood *et al.*, 2008). Consequently, the hypnozoites of *Plasmodium vivax* and *P. ovale*, which are responsible for illness relapse, can be targeted and eliminated (Wells *et al.*, 2010).

Malaria disease can be characterized as either uncomplicated or severe based on the gravity of symptoms. Severe malaria can cause symptoms such as respiratory distress, seizures, organ malfunction, and severe anaemia (Dzeing-Ella *et al.*, 2005; Oduro *et al.*, 2007). Disease progression to severe condition may be influenced by health-seeking behaviour, non-malaria co-morbidity, population genetic factors, and malaria case management (Bassat *et al.*, 2008). In contrast, the clinical manifestations of uncomplicated malaria might range from headache to fever to vomiting to aching joints and jaundice (Bartoloni *et al.*, 2012). Specific antimalarial drugs have been recommended for the treatment of either severe or uncomplicated *falciparum* infection. For example, artemisinin-based combination treatments such as artesunate + amodiaquine, artemether + lumefantrine, and artesunate + sulfadoxine-pyrimethamine are some of the suggested treatments for uncomplicated malaria (WHO, 2010). Severe malaria is considered a medical emergency that necessitates immediate administration of effective drugs. Artesunate, which is administered intravenously (IV) or into the muscle (IM), is one of the medications indicated for both children and adults. In the absence of artesunate, quinine can be used. In addition, after successful therapy with artesunate drugs, patients must receive the entire dose of artemisinin-based combination therapy (WHO, 2010).

2.7 Anti-malarial drug resistance

The recommended ACTs include artemether/lumefantrine, artesunate/mefloquine, artesunate/pyronaridine, artesunate /amodiaquine, artesunate/sulfadoxine-pyrimethamine and dihydroartemisinin/piperaquine (WHO, 2010). However, artemisinin-based treatment is already being jeopardized due to an increase in resistant parasites. For example, the widespread emergence of parasites resistant to artemisinin treatment has been reported in a number of Southeast Asian countries (Balikagala *et al.*, 2021). Furthermore, resistance appears to have spread to the African continent, with reports of possible resistance to artemisinin in Southern Uganda (Balikagala *et al.*,

2021). Similarly, the efficacy of chloroquine, Sulphadoxine-Pyrimethamine (SP), amodiaquine, and quinine has been declining, particularly since widespread reports of drug-resistant parasites emerged (Achan *et al.*, 2011; Shujatullah *et al.*, 2012; Trampuz *et al.*, 2003; Wongsrichanalai *et al.*, 2013). The difficulties posed by resistance to current treatments highlight the urgent need for either the development of novel drugs or the implementation of strategies to eliminate factors likely to fuel drug resistance.

2.7.1 Drug resistance drivers

The World Health Organization has consistently insisted that antimalarial treatment ought to be initiated only after parasitological confirmation of suspected cases (WHO, 2015a). This proposal was made to help decrease the misuse of antimalarial medications, which is thought to be one of the factors contributing to drug resistance (Hyde, 2007). Malaria treatment in health institutions based on clinical diagnosis may result to drug misuse (Njama-Meya *et al.*, 2007; Reyburn *et al.*, 2004). Inaccuracy in clinical diagnosis may be caused by malaria symptoms that imbricate with those of other disorders, leading to antimalarial medications being prescribed to non-malaria patients. Similarly, inappropriate drug use, particularly among those who self-treat, has been linked to the emergence of antimalarial drug-resistant parasites (Akilimali *et al.*, 2022). Self-treatment may be prevalent in the most rural and resource-poor regions, where access to health institutions and the provision of high-quality care for patients pose significant challenges. As a result, developing excellent healthcare systems and boosting access to health facilities are crucial to malaria control and management and may encourage proper treatment seeking behavior.

2.8 Anemia

Anemia is a serious health problem that affects roughly one-third of the global population (Harding *et al.*, 2018; Kassebaum *et al.*, 2014). Sub-Saharan Africa bears the heaviest burden of global

anemia cases (Zegeye *et al.*, 2021). Anemia has been observed to impact the majority of people in resource-constrained places who may not be able to purchase balanced healthy meals (Kant *et al.*, 2019; Kassebaum *et al.*, 2014; Milman, 2011). The majority of people mostly affected by anemia are the pregnant women and children under the age of five (Milman, 2011; Stevens *et al.*, 2013). In 2015, the estimated global anemia rates among women and children were 529 million and 273 million, respectively (WHO, 2015b). Anemia is a medical condition that occurs either when hemoglobin concentrations are below normal or when red blood cell sizes are abnormal (Worku *et al.*, 2022). Anemia is caused by excessive blood loss, hereditary abnormalities such as sickle cell, nutritional inadequacies, and parasite infections (Chaparro *et al.*, 2019; Dreyfuss *et al.*, 2000; Jonker *et al.*, 2014). However, in Sub-Saharan Africa, nutritional deficits and infectious illnesses are regarded as the major causes of anemia (Balarajan *et al.*, 2011; Shaw *et al.*, 2011). While iron deficiency is the most common cause of anemia (Ngesa *et al.*, 2014), other micronutrients such as folate, vitamin B12, and hemoglobinopathies can also result in anemia (Q. Li *et al.*, 2019).

Malaria is the parasitic infection most likely to cause anemia (Brooker *et al.*, 2007; White, 2018). However, Human immunodeficiency virus (HIV), heavy hookworms and *Schistosoma mansoni* infections have also been reported to cause anemia (Koukounari *et al.*, 2008; J. L. Smith *et al.*, 2010). *Plasmodium* infections result in the eradication of both healthy and infected red blood cells (RBC), leading to a decrease in hemoglobin and the development of anemia (White, 2018). However, 90% of acute anemia is attributed to the destruction of non-parasitized RBCs (White, 2018). Activated macrophages, for example, may continue to destroy non-parasitized RBCs even after malaria parasites have been completely cleared, resulting in severe anemia in children (Foote *et al.*, 2013). Other notable factors that may result in further RBC destruction following *Plasmodium falciparum* infection include schizont rapture (Wickramasinghe *et al.*, 2000), splenic

filtration (Buffet *et al.*, 2011), and complement-mediated hemolysis (Woodruff *et al.*, 1979). Furthermore, as children grow older, their immune response to malaria parasites improves, and many become asymptomatic, resulting in further RBC destruction and increased anemia (Shankar *et al.*, 2022).

Variable malaria transmission intensities in diverse settings promote the onset and progression to severe anemia. Western Kenya, for example, has historically been a malaria endemic region, with parasite prevalence reported to be high throughout the year (MoH, 2016b). In malaria endemic areas, anemia is a potential public health issue with children being most vulnerable (Kabaghe *et al.*, 2017; White *et al.*, 2014). For example, study conducted in western Kenya reported the occurrence of moderate to severe anemia due to low and high parasite densities (M. R. Desai *et al.*, 2005). Similarly, a study by Foote *et al.* (2013) in western Kenya, high malaria transmission in this region was associated with severe anemia. Anemia prevalence, on the other hand, has been reported to have decreased in low malaria transmission areas following long-term malaria control interventions (Noland *et al.*, 2012). Similarly, other research findings have shown that reduced malaria burden leads to significant reductions in anemia in children (Korenromp *et al.*, 2004). During a malaria control intervention, changes in anemia rates appear to occur more quickly than changes in the prevalence of malaria parasites (Mathanga *et al.*, 2010). As a result, anemia has been proposed as one of the indicators of community malaria burden (Kabaghe *et al.*, 2017; Korenromp *et al.*, 2004).

Anemia is a life-threatening condition that, if left untreated, can lead to an increase in morbidity and mortality, especially among children and women (Black *et al.*, 2013; Scott *et al.*, 2014). Anemia impairs young children's cognitive and behavioral development (Walker *et al.*, 2007), reduced attention, retarded physical growth, lethargy and decreased immune response to infections

(Lozoff, 2007; Ngesa *et al.*, 2014). As a result, anemic students are more likely to perform poorly in school (Melku, Alene, *et al.*, 2018). Multiple studies have documented the negative effects of anemia in expectant women. Premature birth and babies born with weights less than 2500 grams are the two prevalent complications associated with iron deficiency anemia (Kalaivani, 2009). Other possible effects of anemia on women include infection susceptibility, hypertension, stillbirth, and a high level of fatigue (Lee *et al.*, 2006; Zhang *et al.*, 2021). Iron supplementation programs for pregnant women have been implemented in an attempt to alleviate these pressing difficulties and protect the lives of the mother and unborn child (Kalaivani, 2009; Zhang *et al.*, 2021). Furthermore, anemia affects not just children and women, but also adults, who may become less productive, slowing economic growth (Kant *et al.*, 2019).

2.9 Effects of irrigation schemes and dams on malaria burden

Due to climate change, rained agriculture is becoming increasingly unreliable resulting in construction of irrigation schemes to boost food production (Amadou *et al.*, 2006; Kibret *et al.*, 2014; Kibret *et al.*, 2017; Muturi *et al.*, 2008). However, malaria is closely associated with irrigation in Kenya and elsewhere in Africa (Kibret *et al.*, 2014; Kibret *et al.*, 2017; Muriuki *et al.*, 2016). The impacts of irrigation on malaria burden vary from increase to decrease to no effect (Keiser *et al.*, 2005; Kibret *et al.*, 2014; Muriuki *et al.*, 2016; Muturi *et al.*, 2008; Sharma *et al.*, 2008). In areas where irrigation scheme led to increased *Plasmodium* infections, poorly maintained canals, and rice paddy fields have been reported as key driving factors (Haileselassie *et al.*, 2021; Kibret *et al.*, 2014). Rice paddy fields offer conducive breeding fields for Anopheles arabiensis which thrive in open sunlight water habitats (Haileselassie *et al.*, 2021). However, in some studies, rice cultivation led to lower malaria incidence in irrigated areas compared with areas far away from irrigated sites (Sissoko *et al.*, 2004).

Leaking canal pathways create pools of water most suitable for mosquito breeding thereby increasing chances of *Plasmodium* infections (Yohannes *et al.*, 2005). Therefore, proper management of irrigation channels are key towards reduction of malaria transmission in irrigated areas.(Mboera *et al.*, 2010; Sanchez-Ribas *et al.*, 2012). Besides, managing water levels within dams has been advocated as a better approach of reducing malaria transmission in irrigated areas (Kibret *et al.*, 2018). In Homa Bay county, western Kenya, where the current study is situated, the kind of irrigation is a canal system, and the scheme is maintained by community members. Given the region's favorable temperatures for mosquito and parasite development, such schemes are likely to have an impact on malaria prevalence rates (MoH, 2016a)

2.10 Asymptomatic and submicroscopic malaria infection

Asymptomatic malaria is a form of illness in which a person is infected with parasites but does not exhibit clinical symptoms. People who live in malaria-endemic areas develop an immunological response to malaria parasites and are more prone to suffer from asymptomatic malaria (Laishram *et al.*, 2012). The two types of immunity developed include anti-parasitic immunity, which decreases parasite burden, and anti-disease immunity, which permits individuals to exhibit no symptoms while infected with malaria parasites (Laishram *et al.*, 2012). Anti-parasite immunity develops after a particular age and exposure to infective mosquito bites (Laishram *et al.*, 2012; Langhorne *et al.*, 2008).

Asymptomatic *Plasmodium* infections are frequently characterized by undetectable low parasite levels, and hence function as a silent reservoir for continued malaria transmission (Bousema *et al.*, 2014). As a result, asymptomatic infections pose considerable threat to malaria intervention strategies (Abebaw *et al.*, 2022). Previous studies along the lake region of western Kenya indicate increasing rates of asymptomatic *Plasmodium* infections (Idris *et al.*, 2016). Besides,

asymptomatic infections appear to be rising among communities as malaria control programs reduce transmission. Detecting and treating asymptomatic malaria remains critical for malaria management and elimination. In Homa Bay county, malaria control activities are in place with a desire to manage or eliminate malaria. Information regarding the nature of asymptomatic infections in this region will be very important.

Although submicroscopic malaria infections are undetectable by microscopy or RDTs, they are infectious to mosquitos, resulting in continuous transmission (Bousema *et al.*, 2014). Submicroscopic infections not only occur in large numbers in regions with low malaria transmission (Lin *et al.*, 2014), but also in high transmission areas (Ochwedo *et al.*, 2021). Previous studies indicate that more adults are infected with submicroscopic malaria than children (Okell *et al.*, 2012; Whittaker *et al.*, 2021). This is because adults, unlike infants, have gained immunity to malaria parasites as a result of exposure to infective mosquito bites. Accurate detection of submicroscopic infections therefore requires more sensitive molecular techniques such as PCR (Whittaker *et al.*, 2021). Furthermore, as the Ministry of Health expands malaria control in the research area, transmission is likely to decrease, therefore identifying the current proportions of submicroscopic infections will be critical.

2.11 Malaria vectors species, behavior and diversity

Globally, there are 70 Anopheles species that can transmit malaria with 41 of them being primary vectors (Sinka *et al.*, 2010). In Africa, *An. gambiae sensu stricto (s.s)*, *An. funestus*, *An. arabiensis*, *An. merus*, *An. melas*, *An. coluzzii*, and *An. nili* are considered principal malaria vectors (Sinka *et al.*, 2010). *Anopheles gambiae*, *An. funestus* and *An. arabiensis* are the primary malaria vectors in western Kenya, and they significantly contribute to malaria transmission (Okara *et al.*, 2010). Secondary malaria vectors *An. pharoensis* and *An. coustani* have been documented to contribute a

minor percentage (5%) of malaria transmission (Mustapha *et al.*, 2021). *Anopheles arabiensis* forms the majority of Anopheles species in the Rangwe and Rachuonyo North sub-counties of Homa Bay county, where the current study is being conducted (Ondeto *et al.*, 2022).

The three primary malaria vectors in western Kenya prefer different types of aquatic habitats. For example, *Anopheles gambiae* prefer to breed in shallow pools of water that are directly exposed to sunlight (Ondiba *et al.*, 2019). The breeding habitat of *An. gambiae* may vary in size and can be temporary or permanent, manmade or natural, and salty or fresh water (Machault *et al.*, 2009). *Anopheles funestus* prefers to spawn in permanent or semi-permanent water collections with shade, such as swamps with floating algae or on edges of the stream with vegetation (Emidi *et al.*, 2017; Gimnig *et al.*, 2001; Mwangangi *et al.*, 2007). *Anopheles arabiensis*, on the other hand, breeds in clear, shallow, fresh, and sunlight-exposed habitats (Himeidan *et al.*, 2008). While *An. gambiae* favors muddy breeding conditions, *An. arabiensis* loves clear water pools and is most typically seen in rice irrigated areas (Mwangangi *et al.*, 2010).

In terms of resting and feeding habits, *An. gambiae* and *An. funestus* have been observed resting and feeding indoors (Nzioki *et al.*, 2023). These vectors tend to bite in the late hours of the night when people are not protected by treated bed nets (). However, as the campaign against malaria vectors intensifies, many studies have observed a change in biting behavior (Cooke *et al.*, 2015; Gatton *et al.*, 2013; Reddy *et al.*, 2011). For example, *An. gambiae*, which used to feed indoors and late at night, has been reported to feed early at night and outdoors following the application of IRS and the usage of bed nets (Gatton *et al.*, 2013; Reddy *et al.*, 2011). Previously, *An. gambiae* and *An. funestus* preferred to feed solely on human blood; however, several investigations have shown that both Anopheles species can also feed on cattle when a human host is unavailable (Killeen *et al.*, 2001; Lefevre *et al.*, 2009). In a study conducted in Ahero, Kenya, *An. arabiensis*

was observed to feed more outdoor than indoor, and biting was reported to occur late in the evening and near dawn (Nzioki *et al.*, 2023). The implementation of an irrigation scheme and mounted vector control in the study area may alter vector composition and behavior, hence altering malaria transmission.

CHAPTER THREE:

IMPACT OF AGRICULTURAL IRRIGATION ON PREVALENCE OF ANEMIA AND MALARIA IN WESTERN KENYA

3.1 Abstract

Expanding agricultural irrigation efforts to enhance food security and socioeconomic development in sub-Saharan Africa may affect malaria transmission and socioeconomic variables that increase the risk of anemia in local communities. Prevalence of anemia, *Plasmodium falciparum* infection, and indicators of socioeconomic status related to nutrition in communities were compared in Homa Bay County, Kenya where an agricultural irrigation scheme has been implemented to that in nearby communities where there is no agricultural irrigation. Cross-sectional surveys conducted showed that anemia prevalence defined by World Health Organization criteria (Hb < 11 g/dL) was less in communities in irrigated than in the non-irrigated areas during the wet season (38.9% and 51.5%, $\chi^2 = 4.29$, $p = 0.001$) and the dry season 25.2% and 34.1%, $\chi^2 = 7.33$, $p = 0.007$). In contrast, *Plasmodium falciparum* infection prevalence was greater during the wet season in irrigated areas than in non-irrigated areas (15.3% versus 7.8%, $\chi^2 = 8.7$, $p = 0.003$). There was, however, no difference during the dry season (infection prevalence < 1.8%). Indicators of nutritional status pertinent to anemia pathogenesis such as weekly consumption of non-heme and heme containing foods and household income were greater in communities located within the irrigation scheme versus those outside the irrigation scheme ($p < 0.0001$). These data indicate that current agricultural irrigation schemes in malaria endemic communities in this area have reduced the risk of anemia. Future studies should include diagnostic tests of iron deficiency, parasitic worm infections, and genetic hemoglobin disorders to inform public health interventions aimed at reducing community anemia burden.

Key words: Irrigation, Anemia, Malaria, Socioeconomic status, Kenya

3.2 Introduction

Twenty-five to 30% of the global population is estimated to have anemia based on a lower blood hemoglobin (Hb) concentration that is less than that of healthy age and gender-matched residents of high-income countries in non-tropical areas of the world (Kassebaum *et al.*, 2014; Milman, 2011). Women of childbearing age and children in low- and middle`-income countries of Africa are particularly vulnerable to anemia (Chaparro *et al.*, 2019). The underlying causes of anemia include nutritional deficiencies of essential elements needed for Hb synthesis (e.g., iron, vitamins A and B [riboflavin, B12, folate]), malaria and parasitic worm infections (e.g., hookworm and schistosomiais), and genetic disorders of Hb synthesis (e.g., sickle cell) (Brooker *et al.*, 2007; Crawley, 2004; Gutema *et al.*, 2014; Hotez *et al.*, 2008). Severe anemia during childhood, defined as a blood Hb concentration < 7 g/dL, is associated with slow cognitive development and impaired physical growth (Assefa *et al.*, 2014; Phillips *et al.*, 1992; Scott *et al.*, 2014).

Socioeconomic and demographic factors, e.g., household income, education, sex and pregnancy, also play key roles in achieving proper nutrition, and thus, affect the risk of anemia (Galgamuwa *et al.*, 2017). Studies performed in western Kenya during the first 10 years of the 21st century found that poor nutrition was a primary cause of anemia in children (M. R. Desai *et al.*, 2005; M. R. Desai *et al.*, 2004; Foote *et al.*, 2013). Homa Bay County, located near the Lake Victoria basin, has historically experienced perennial malaria transmission and frequent food shortages resulting from prolonged droughts (Development, 2006; Health, 2015). To alleviate this food insecurity, the Kenyan government supported construction of a concrete channel-based irrigation scheme, the Kimira-Oluch Small Holder Farm Improvement Project. Since completion of the irrigation scheme in 2016, a variety of crops (rice, sorghum, millet, beans, and maize) and vegetables (kale, spinach)

have been grown. Increased availability of these crops for local consumption, as well as a source of income resulting from sales to residents of communities outside the irrigation scheme, appear to have improved the nutritional and the socioeconomic status of local communities. The objective of this study was to evaluate the degree of improvement with respect to the prevalence of anemia and *Plasmodium falciparum* infection and socioeconomic status. Accordingly, we compared these variables in residents of communities within the irrigation scheme to nearby communities outside the irrigation scheme.

3.3 Materials and methods

3.3.1 Study area

This study was conducted in Rangwe and Rachuonyo South sub-counties of Homa Bay County in 2018 and 2019, and targeted communities located near canals within irrigated area and non-irrigated areas located 5 to 10 km from irrigation canals (Fig. 3.1). The county is situated in the southern part of former Nyanza Province and lies between latitude 0.15^0S and $0{\cdot}52^0$ S and between longitude 34^0 E and 35^0 E. The study area experiences semi-arid climatic conditions where daily temperatures range between 26^0C and 34^0C during cool months (April and November) and hot months (January to March) respectively. Two annual rainy seasons are associated with increased malaria transmission in Homa Bay County: the "long rains" in April through June and "short rains" in September through November. Annual rainfall ranges from 250mm to 1000mm. The Kenyan government completed the Kimira-Oluch Smallholder Farm Improvement Project, a gravity-fed irrigation system with concrete canal system and dams, in 2016 (Development, 2006). The Tende river is the source of water for Oluch irrigation scheme. The water flows from river Tende along the canals to various dams constructed within the study area. When the canal pathways get blocked, the water flow is usually stopped to allow for clearing of the pathway. Maintenance of the irrigation

scheme is a responsibility of the local farmers within the scheme. However, canal maintenance has been challenging, resulting in leaks and overflow during water release creating pools of water suitable for mosquito breeding. The total area covered by irrigation is approximately 666 ha.

Their major economic activity is subsistence farming. Major crops include: kales, spinach, pumpkin, watermelon, beans, maize, sorghum, rice and variety of fruits. Other activities include fishing, business and livestock keeping. Most of the houses within the study area have mud-walls, open heaves and have iron sheet roof tops. The remaining house types are either permanent or semi-permanent with closed or open heaves and iron sheet roof tops. According Kenya national population census conducted in the year 2019, Homa Bay county has a total population of 1,131,950 people and total area of 3,154.7km^2. There are two private and five public healthcare facilities in the irrigated area, and one private and five public health care facilities in the non-irrigated area. Homa Bay County is a malaria endemic area, where the prevalence of *P. falciparum* blood stage infection has historically been ≥ 20%.(Health, 2015) Annual indoor residual spraying with Actellic insecticide commenced in 2018. The prevalence of malaria infection is thought to have decreased substantially since then (Omondi, Otambo, *et al.*, 2022).

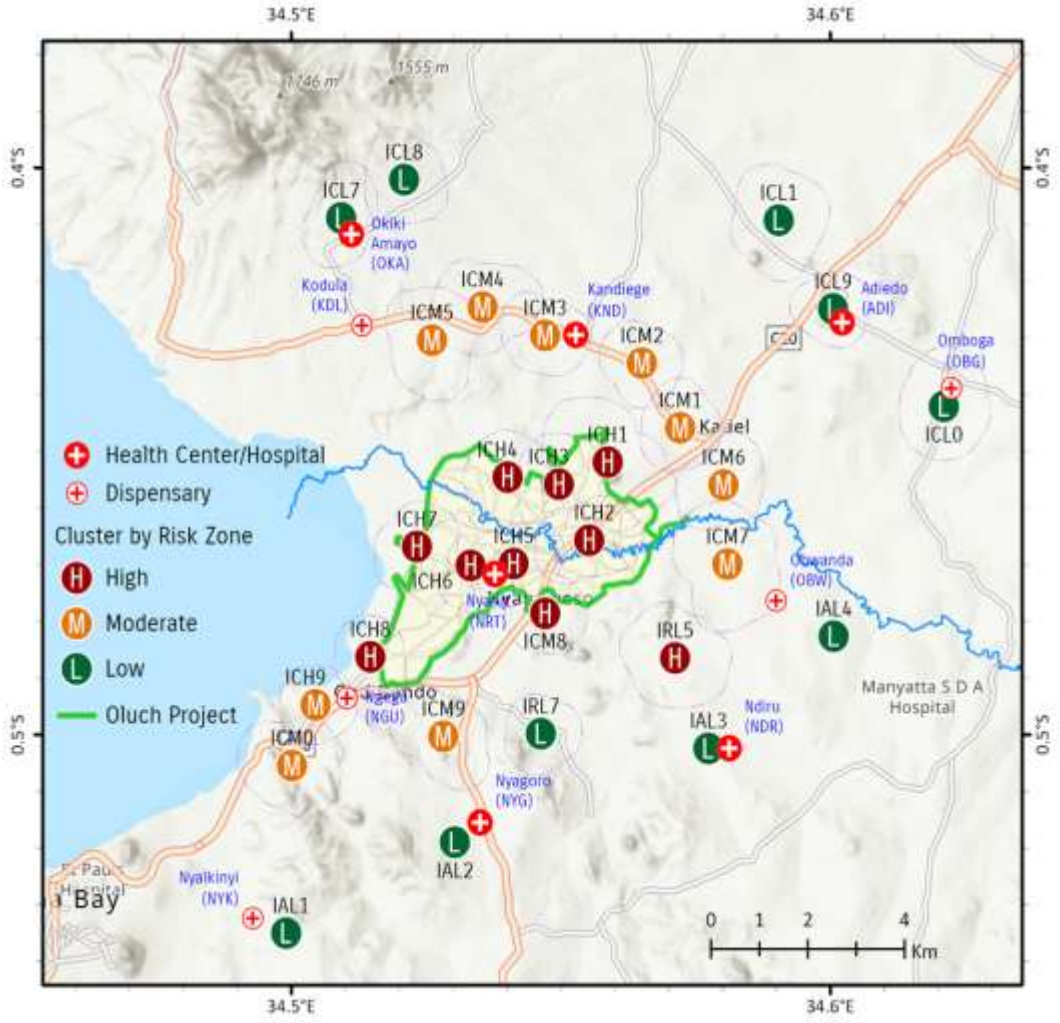

Figure 3.1 Map of study area (part of Rangwe and Rachuonyo South sub-counties) in Homa Bay county shaded red in the map of Kenya

3.3.2 Selection of study communities

As described by Galway *et al.*, a two-stage cluster-sampling method was adopted, with clusters (defined as collection of households with a single geographic unit) and households serving as the primary and secondary sampling units, respectively (Galway *et al.*, 2012). The size (radius) of each cluster ranged from 250-500m with a population size of ~200-250 people. Briefly, 20 clusters were selected based on population size. Ten clusters were located in irrigated areas and ten in non-irrigated areas. Six clusters were selected at random from both regions. After a cluster was

identified, the total number of households within each cluster was established and assigned unique numbers. Based on the required sample size, 20 households per cluster were randomly selected from the previously marked households. Every household member (i.e., a permanent resident age ≥ 6 months), was enrolled in the study after obtaining signed informed consent or an assent form (Appendix 1) from children 5 to < 18 years. Pregnant women were excluded from the study. However, pregnant women were tested for anemia and malaria infection and referred to the local healthcare facility for further assessment and treatment. The total number of participants who met the inclusion criteria was 836. The sample size was calculated using the formula: $n = Z^2P(1-P)/D^2$ where **D** represents precision (0.05), **P** = proportion (0.27), **Z** = confidence interval (1.96), and **n** is the required minimum sample size. Since anemia determination was part of a major study (*Plasmodium* infection), an estimated malaria prevalence (P) of 27% was assumed in sample size calculation. The adopted cluster sampling procedure, hence, the sample size, was multiplied by a design effect of two.(W, 1977) The required sample size was 606 people. To avoid attrition during surveys, all eligible participants (N = 836) were enrolled. There were 712 and 826 participants in the 2018 wet season and 2019 dry season surveys respectively.

3.3.3 Study design and laboratory methods

Cross-sectional surveys of community residents aged 6 months and old and above in the irrigated area and non-irrigated area were conducted during the June 2018 wet season and the February 2019 dry season.

3.3.4 Sample collection and examination for Hemoglobin level

Blood samples were collected by fingerpick to determine Hb levels as illustrated in appendix 6. To measure Hb concentration, fresh blood was placed in the optical window of a micro-cuvette HemoCue HB 201+ analyzer (Angelholm, Sweden). Based on WHO-recommended

criteria,(Organization, 2008) Hb concentration of each study participants was classified as having severe , moderate, mild, or no anemia, after adjusting for age and gender, as shown in Table 3.1.

Table 3.1 Hemoglobin values for classification of anemia

Study participants	**Hemoglobin concentration (g/dL)**			
	Severe	Moderate	Mild	Non-anemic
Children 6 - 59 months	< 7	7 – 9.9	10 – 10.9	≥ 11
Children 5 - 11 yrs	< 8	8 – 10.9	11 – 11.4	≥ 11.5
Children 12 - 14 yrs	< 8	8 – 10.9	11 – 11.9	≥ 12
Non-pregnant women ≥ 15 yrs	< 8	8 – 10.9	11 – 11.9	≥ 12
Men ≥ 15 yrs	< 8	8 – 10.9	11 – 12.9	≥ 13

3.3.5 Sample collection and microscopic examination of malaria parasites

Blood sample was obtained by pricking the finger as described in appendix 6. Thick and thin smears were prepared in the field, air-dried, and then transported to the laboratory. Thick and thin blood smear slides were stained with 10% Giemsa for 15 minutes, washed, air-dried, and examined by microscopy. A minimum of microscopic fields containing 200 leukocytes were examined at X100 magnification. Two independent microscopists read blood smears for quality control. A third reading was performed by a different microscopist if there was a discrepancy. Parasite density was determined by counting the number of parasite-infected red blood cells in microscopic fields that included 200 leukocytes and multiplying by 40 with the assumption that there were 8,000 leukocytes per µL blood for all study participants.

3.3.6 Data collection for household income and iron-rich foods intake

With respect to socioeconomic status and intake of nutrients pertinent to Hb production, the head of each household was interviewed using a pretested questionnaire (Appendix 2) to determine the source of income, annual household income, and consumption of heme and non-heme containing

foods and vitamins A and B in the previous week. Food intake was quantified according to one of four categories: no intake, intake 1 to3 days, intake 4 to 6 days, and daily intake for the past week. Annual household income was categorized as severe poverty (annual income less than U.S. dollars [USD] 365), moderate poverty (income USD 365 - 1095), and non-poor (income > USD 1095).

3.3.4 Statistical analysis

Data were entered into Excel spreadsheets and analyzed using GraphPad Prism version 8 and SPSS version 20 (SPSS Inc., Chicago, IL). The χ^2 or Fisher's exact test were used to analyze categorical variables. Means (Hb concentration, malaria parasite densities) between groups residing in irrigated and non-irrigated areas were tested for significance using t-tests. Analysis of variance (ANOVA) was used to compare the means of three or more groups. A post-hoc test was performed when the ANOVA revealed a significant p-value. Data sets with non-normal distributions were logarithm-transformed. A p-value < 0.05 was considered significant. To determine the independent association of anemia with various risk factors, adjusted odds ratio (aORs) and 95% confidence intervals were calculated using multivariate logistic regression.

3.3.5 Ethical considerations

Ethical approval (Appendix 3) for the study was obtained from the University of California, Irvine Institutional Review Board (HS no 2017-3512) and Maseno University Ethics and Research Committee in Kenya (MSU/DRPI/MUERC/00456/17). Permission to conduct the study was obtained from the Ministry of Health Homa Bay county government (Appendix 4). The candidate ethic certificate (protecting human research participants) (Appendix 5), was awarded before the onset of cross-sectional surveys.

3.4 Results

There were no significant differences in age or gender distributions of the study populations in the irrigated and non-irrigated area during the 2018 wet season or 2019 dry season (Table 3.2). The median age of participants in both the irrigated and non-irrigated area was 21 years (interquartile range, 44-7 years).

Table 3.2 Demographic characteristics of study participants by sex and age groups in irrigated and non-irrigated areas.

Survey period	Sex	Age group (yrs)	Irrigated n (%)	Non-irrigated n (%)
June 2018	Females	< 5	27 (11.7)	24 (12.4)
		5-11	63 (27.4)	42 (21.6)
		≥ 12	140 (60.9)	128 (66)
	Males	< 5	26 (17.3)	29 (21)
		5-11	49 (32.7)	46 (33.3)
		≥ 12	75 (50)	63 (45.7)
		Total	380	332
Feb 2019	Females	< 5	40 (15.9)	34 (13.3)
		5-11	32 (12.7)	30 (11.7)
		≥ 12	180 (71.4)	192 (75)
	Males	< 5	52 (30.2)	51 (34.9)
		5-11	37 (21.5)	25 (17.1)
		≥ 12	83 (48.3)	70 (47.9)
		Total	424	402

3.4.1 Prevalence of anemia and *Plasmodium* infection

Anemia prevalence was 44.8% (319 of 712) during the wet season (June 2018) and 29.5% (244 of 826) during the dry season (February 2019) (χ^2= 17.72, degrees of freedom [df] = 1, p< 0.0001). Table 3.3 presents data showing that the overall prevalence of anemia and *Plasmodium* infection during the wet season differed in residents of households located in the irrigated and non-irrigated areas. Anemia prevalence was significantly less in the irrigated area than in the non-irrigated area (38.9% versus. 51.5%, p < 0.05). A similar trend was observed when age was stratified to 5 to 11 and ≥ 12 years (but not for children under 5 years). In contrast, *Plasmodium* infection prevalence

was significantly higher in the irrigated than in the non-irrigated area (15.3% vs 7.8%, $p < 0.05$). The geometric mean density of *Plasmodium* infection was similar in the irrigated versus. non-irrigated study areas (381 versus. 309 parasites/μL, respectively). Infection prevalence for the 5 to 11 year and ≥ 12-year age groups, but not for children under 5 years, was greater in the irrigated areas than the non-irrigated areas. Given the relative lack of naturally acquired immunity to blood-stage infection in young children in this area of western Kenya,(Weber *et al.*, 2017) we speculate that our study was underpowered to make meaningful conclusions with respect to the impact of residency in irrigated versus. non-irrigated areas. As in the wet season, anemia prevalence in the dry season was significantly less in study participants residing in the irrigated area than in the non-irrigated area (25.2% versus. 34.1%, $p < 0.05$). This difference was evident in the two older age groups, but as in the survey conducted in the wet season, not in children under 5 years.

Prevalence of anemia was also compared according to gender and severity in the wet and dry seasons. With regards to the former, females ≥ 12 years were more commonly anemic than males in both the wet and dry seasons. Females <12 years, for example, were 38.5% anemic, whereas the males of the same age group were 50% anemic during the wet season (p value = 0.06). In contrast, female ≥ 12 years were significantly anemic (56.9%) compared with males (24.6%, p value < 0.0001). During the dry season, the proportion of anemic females and males < 12 years was 29.4% and 32.7% respectively (p value = 0.6). However, females ≥ 12 years were more anemic (31.7%) compared with males of the same age group (20.9%, p value = 0.01).

In terms of anemia severity, severe, moderate and mild anemia accounted for 3.5% (25 of 712), 18.8% (134 of 712) and 22.5% (160 of 712) of the total for irrigated and non-irrigated areas combined during the wet season ($\chi^2 = 113.4$, df = 2, p value < 0.0001). Similarly, during the dry

season, the prevalence of severe, moderate, and mild anemia was 3.1% (26 of 826), 12.3% (102 of 826) and 14.0% (116 of 826) respectively ($\chi^2 = 63.97$, df = 2, p value = < 0.0001).

Table 3.3 Prevalence of anemia and *Plasmodium* infection in irrigation and no irrigation communities

Variables	**Irrigation n/N (%)**	**No irrigation n/N (%)**
Wet season (June 2018)		
Overall anemia prevalence	148/380 (38.9)*	171/332 (51.5)
Age, years		
< 5	28/53 (52.8)	34/53 (64.2)
5-11	31/112 (27.7)*	42/88 (47.7)
≥ 12	88/215 (40.9)*	98/191 (50.3)
Overall *Plasmodium* infection prevalence	58/380 (15.3)*	26/332 (7.8)
Age, years		
< 5	5/53 (9.4)	5/53 (9.4)
5-11	25/112 (22.3)*	9/88 (10.2)
≥ 12	28/215 (13)*	12/191 (6.3)
Dry season (February 2019)		
Overall anemia prevalence	107/424 (25.2)*	137/402 (34.1)
Age, years		
<5	40/92 (43.5)	39/85 (45.9)
5-11	3/69 (4.3)*	12/55 (21.8)
≥12	64/263 (24.3)*	86/262 (32.8)
Overall *Plasmodium* infection prevalence	7/424 (1.7)	4/402 (1)
Age, years		
<5	1/92 (1.1)	1/85 (1.2)
5-11	1/69 (1.45)	1/55 (1.82)
≥12	5/263 (1.9)	2/262 (0.8)

*Number in parentheses is percent with p-value < 0.05 in irrigated versus non-irrigated areas

3.4.2 Mean hemoglobin concentrations

Children younger than five years had significantly lower Hb concentrations than other age groups in both the irrigated and non-irrigated areas during the wet season. For example, the mean Hb concentration for participants aged < 5, 5 to 11 and ≥ 12 years old was 10.4, 11.8 and 12.4 g/dL, respectively (ANOVA: F = 47.69, df = 2, n = 709, p value < 0.0001). Similarly, during the dry season, the mean Hb concentration among children < 5, 5 to 11 and ≥ 12 years old was 10.9, 12.9 and 12.9 g/dL, respectively (ANOVA: F = 67.9, df = 2, n = 823, p value = 0.002).

During wet season, the mean Hb concentration among study participants living in non-irrigated area was less (mean ± SD, 11.6 ± 2.098 g/dL) than in irrigated areas (12.2 ± 1.832 g/dL p value = 0.0002). The same trend was observed during the dry season. The mean Hb concentration of non-irrigated area residents (12.3 ± 2.18 g/dL) was significantly less than that of irrigated area participants (12.7 ± 2.004 g/dL, p value = 0.006)

Females had significantly lower Hb concentrations (11.7 ± 1.860 g/dL) than males (12.2 ± 2.115 g/dL) during the wet season (p value = 0.002). Similarly, during the dry season, Hb concentration in females (12.3 ± 2.084 g/dL) was significantly less than in males (12.8 ± 2.279 g/dL, p value = 0.0005).

3.4.3 Household income

Annual household income differed significantly between irrigated and non-irrigated areas (Table 3.4). The proportion of those earning less than USD 365 per year was significantly greater in non-irrigated compared with irrigated areas (χ^2 = 6.31, df = 1, p value = 0.01). The proportion of

households earning more than USD 1,095 per year was significantly greater in irrigated than in non-irrigated areas ($\chi^2 = 19.7$, df = 1, p value < 0.0001).

3.4.4 Nutrition and food intake

The majority of participants (74.7%) from irrigated areas consumed non-heme iron foods on a daily basis compared to 54.2% from non-irrigated area (Table 3.4). Similarly, a greater proportion of residents in irrigated area consumed heme iron foods seven days per week versus. residents of non-irrigated areas ($\chi^2 = 4.9$, df = 1, p = 0.03). The results further indicate that a significant proportion of study participants from non-irrigated area did not consume heme iron foods when compared with those from irrigated areas ($\chi^2 = 10.73$, df = 1, p value = 0.001). Although there was a significant difference in vitamin consumption between irrigated and non-irrigated areas, it is clear that few people in both areas consumed vitamin B and C. (Table 3.4)

Table 3.4 Annual household income and food intake per week

Variable	Irrigated (n = 91) n (%)	Non-irrigated (n = 120) n (%)	χ^2, df	p-value
Household income per year				
< USD 365	8 (8.8)	26 (21.7)		
USD 365- 1095	47 (51.6)	78 (65)	21.3, 2	< 0.0001
>USD 1095	36 (39.6)	16(13.3)		
Iron-rich foods (plant foods) in a week				
Daily	68 (74.7)	65 (54.2)		
4-6 times	9 (9.9)	16 (13.3)	150.4, 2	< 0.0001
1-3 times	14 (15.4)	39 (32.5)		
Iron-rich foods (animal products) in a week				
Daily	10 (11)	4 (3.3)		
4-6 times	14 (15.4)	11 (9.2)	260.6, 3	<0.0001
1-3 times	61 (67)	77 (64.2)		
No intake	6 (6.6)	28 (15)		
Vitamin B rich-fruits intake in a week				
Daily	9 (9.9)	7 (5.8)		
4-6 times	14 (15.4)	14 (11.7)	238.5, 3	<0.0001
1-3 times	56 (61.5)	80 (66.7)		
No intake	12 (13.2)	19 (15.8)		
Vitamin C rich-fruits intake in a week				
Daily	7 (7.7)	8 (6.7)		
4-6 times	9 (9.9)	11 (9.2)	132.7, 3	< 0.0001
1-3 times	36 (39.6)	59 (49.2)		
No intake	39 (42.9)	42 (35)		

3.4.5 Factors associated with anemia

Anemia risk factors were determined during the dry season (second survey). Prior to the start of the study, it was hypothesized that *Plasmodium* infection would be associated with anemia. During the wet season, however, there was no link between anemia and *Plasmodium* infection. This prompted us to consider other possible risk factors which were evaluated during the dry season as indicated in table 3.5.

Children younger than 5 years were 2.1 times (aOR, 2.1; 95% CI, 1.13 – 4.08) more likely to be anemic than those ≥5years. Study participants with a family annual income of less than USD 365 and USD 365 to 1,095 were 9.7 times (aOR, 9.7; 95% CI, 3.66 to 26.0) and 4.4 times (aOR, 4.4; 95% CI, 2.1 to 9.1) more likely to be anemic, respectively, than participants with a family annual income of more than USD 1,095.

Study participants who consumed non-heme iron foods one to three times per week were 2.8 times (aOR, 2.8; 95% CI, 1.57 – 4.84) more likely to be anemic than those who ate non-heme iron foods four to six/week times or on a daily basis. Participants who did not eat heme iron foods during the week were 7.1 times (aOR, 7.1; 95% CI, 1.98 – 25.4) more likely to be anemic than those who consumed it once a week, one to three times per week, four to six times per week and on a daily basis. Study participants who did not consume vitamin C were 8.7 times (aOR, 8.7; 95% CI, 2.41 – 31.27) more likely to be anemic than those who did one to three, four to six times per week and daily.

Participants who did not consume vitamin B were 4.1 times (aOR, 4.2; 95% CI, 1.43 – 12.1) were more likely to be anemic than those who consumed it for one to three, or four to six times daily. There was no association between *Plasmodium* infection and anemia (aOR,1.4, 95% CI, 0.28 – 4.45). Similarly, neither gender nor place of residence was associated with anemia (aOR, 1.4, 95% CI, 0.98 – 1.92).and (aOR, 1.2, 95% CI, 0.87 – 1.79) respectively (Table 3.5).

Table 3. 5 Factors associated with anemia among study participants during the dry season, (n = 826)

Variable	Anemia		Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
	Yes, n (%)	No, n (%)				
Region						
Non-irrigated	137 (34.1)	265 (65.9)	1.5 (1.13 – 2.07)	0.005	1.2 (0.87 – 1.79)	0.23
Irrigated	107 (25.2)	317 (74.8)	1.00		1.00	
Age (yrs)						
< 5	73 (42.7)	98 (57.3)	2.0 (1.42 – 2.87)	< 0.0001	2.1 (1.13 – 4.08)	0.02
5-11	15 (12.1)	109 (87.9)	0.34 (0.19 - 0.61)	< 0.0001	0.35 (0.14 - 0.89)	0.03
≥ 12	156 (29.4)	375 (70.6)	1.00		1.00	
Sex						
Female	158 (31.1)	350 (68.9)	1.22 (0.89 – 1.66)	0.21	1.4 (0.98 – 1.92)	0.06
Male	86 (27)	232 (73)	1.00		1.00	
Annual Income (USD)						
<. 365	77 (63.6)	44 (36.4)	22.1 (10.3 – 47.5)	< 0.0001	9.7 (3.66 – 26.0)	< 0.0001
365-1095	164 (36.4)	287 (63.6)	6.0 (3.2 – 11.1)	< 0.0001	4.4 (2.1 – 9.1)	< 0.0001
> 1095	3 (1.2)	251 (98.8)	1.00		1.00	
Iron-rich foods intake (Plants) per week						
1-3 times	62 (35.2)	114 (64.8)	3.7 (2.36 – 5.70)	< 0.0001	2.8 (1.57 – 4.84)	< 0.0001
4-6 times	42 (36.8)	72 (63.2)	1.8 (1.02 – 3.24)	0.4	0.6 (0.27 – 1.33)	0.22
Daily	140 (26.1)	396 (73.9)	1.00		1.00	
Iron-rich foods intake (Animals) per week						
No intake	77 (58.8)	54 (41.2)	14.8 (5.14 – 42.5)	< 0.0001	7.1 (1.98 - 25.4)	0.003
1-3 times	148 (29.7)	350 (70.3)	3.0 (1.14 – 8.12)	0.03	2.3 (0.76 – 7.09)	0.1
4-6 times	10 (10)	90 (90)	1.7 (0.54 – 5.27)	0.4	2.5 (0.7 – 9.47)	0.2
daily	9 (9.3)	88 (90.7)	1.00		1.00	
***Plasmodium* infection**						
Infected	4 (36.4)	7 (63.4)	1.4 (0.4- 4.72)	0.6	1.13 (0.28 – 4.45)	0.9

Not infected	240 (29.4)	575(70.6)	1.00		1.00	
Vitamin C intake per week						
No intake	213 (67.8)	101 (32.2)	10.2 (4.0 – 25.8)	< 0.0001	8.7 (2.41 – 31.27)	0.001
1-3 times	86 (23.1)	287 (76.9)	1.4(0.57 – 3.7)	0.4	0.98 (0.28 – 3.39)	0.97
4-6 times	17 (21.5)	62 (78.5)	1.3 (0.4- 4.1)	0.6	0.89 (0.18 – 4.47)	0.9
Daily	10 (16.7)	50 (83.3)	1		1	
Vitamin B intake per week						
No intake	84 (68.9)	38 (31.1)	8.1 (3.18 – 20.47)	< 0.0001	4.2 (1.43 – 12.1)	0.009
1-3 times	192 (36.2)	338 (63.8)	2.06 (0.91– 4.66)	0.08	1.6 (0.65 – 3.90)	0.3
4-6 times	36 (32.7)	74 (67.3)	1.77 (0.69 – 4.54)	0.2	1.9 (0.68 – 5.43)	0.2
Daily	14 (21.9)	50 (78.1)	1		1	

OR= odds ratio, CI= confidence interval, 1= reference

3.5 Discussion

This study was undertaken to compare the rates of anemia, *Plasmodium* species infection, nutritional intake, and household income in the study area. Anemia is a major health concern in malaria-endemic regions, causing significant morbidity and mortality (Obonyo *et al.*, 2007). Surveys assessing hb levels in populations are critical to developing appropriate interventions that may alleviate severity of anemia. During the wet season, the prevalence of anemia was 44.8%. This rate of anemia is regarded as a severe public health problem per WHO standards (WHO, 2008b). These results are consistent with other study findings from malaria-endemic zones in Kenya where anemia has also been reported to pose a considerable challenge to public health (M. R. Desai *et al.*, 2005; Foote *et al.*, 2013; Newton *et al.*, 1997; Zucker *et al.*, 1994). All of these studies pointed to malaria as the primary cause of hb deficiency (M. R. Desai *et al.*, 2005; Foote *et al.*, 2013; Newton *et al.*, 1997). However, in this study, there was no association between *Plasmodium* infections and anemia during the two cross-sectional surveys.

The lack of association could be attributed to a significant reduction in parasite density and prevalence. During the study period, an indoor residual spraying (IRS) program using Actellic 300 CS insecticide was implemented in February 2018 and repeated in February 2019. The indoor residual spray program significantly reduced both the *Plasmodium* infections and parasite density (Omondi, Otambo, *et al.*, 2022).These findings are consistent with previous research that found no significant association between *Plasmodium* infection and anemia resulting from low malaria prevalence and parasitemias (Cornet *et al.*, 1998; Gutema *et al.*, 2014). The *Plasmodium* infection rate in irrigated areas was higher than in non-irrigated areas. Previous studies in Tanzania, (Woldie *et al.*, 2015) Ethiopia, (Kibret *et al.*, 2014) and Mali, (Sogoba *et al.*, 2007) reported increased malaria within irrigated compared with non-irrigated areas.

This study found that males were more likely than females to be infected with *Plasmodium.* Although some studies reported similar findings (Rumisha *et al.*, 2019), others indicated that females were more infected by malaria parasites than males (Nzobo *et al.*, 2015b). In this study area, some males were engaged in night fishing activities, whereas others were engaged in rice farming, which typically begins very early and ends at dusk. These activities may have exposed males to infectious mosquito bites, resulting in an increase in *Plasmodium* infections. Children under the age of five had the lowest *Plasmodium* infection rate. This finding is in agreement with previous studies that reported an increase in malaria infections with increasing age (Sultana *et al.*, 2017). It was also important to note that, although the *Plasmodium* infection rate among children under the age of five years was similar in irrigated and non-irrigated areas, the malaria infection rate among children >5 years, was significantly higher in irrigated areas. Older children have been reported to use bed nets infrequently (Gitonga *et al.*, 2012). Inadequate use of bed-nets will almost certainly lead to an increase in infections, particularly in irrigated areas. Furthermore, this infection

pattern was only observed during the wet season, when rice farming is at its peak, implying that older children may be involved in rice farming. Despite an increase in *Plasmodium* infection rates in irrigated areas, anemia cases were significantly less in irrigated areas than in non-irrigated areas. This finding is similar to a study conducted in Tanzania (Rumisha *et al.*, 2019), which reported lower anemia rates in areas with higher *Plasmodium* infection rates.

The variation in anemia rates between the two areas may have been influenced by household income and diet, which was better in irrigated areas than in non-irrigated areas. Low-income households were 4.4 to 9.7 times more likely to be anemic. This is consistent with other studies that reported increased anemia rates in low-income populations (Gebreweld *et al.*, 2019). A higher income influences the affordability and the frequency with which that food can be consumed. Iron intake, both heme and non-heme, was also associated to the prevalence of anemia. Those who could not afford to eat foods rich in heme iron, for example, were 7.1 times more likely to be anemic. Similarly, those who could only consume foods rich in non-heme one to three times per week were 2.8 times more likely to be anemic than those who consumed it on a daily basis. A study conducted in northeast Ethiopia also reported that children with poor dietary intake were more likely to be anemic compared with those who ate a healthy diet (Woldie *et al.*, 2015). Although there are many underlying factors that may lead to anemia, poor diet and low household income were some of the possible causes of anemia in the study area.

This study reported a general poor intake of vitamin B and C in both irrigated and non-irrigated areas. Vitamin C is an essential nutrient that aids in absorption of non-heme iron (Monsen, 1988). In both surveys, moderate and mild anemia, which most often occurs silently within populations, were significantly greater compared with severe cases. This finding is in agreement with other studies (Kuziga *et al.*, 2017). It is also worth noting that the prevalence of anemia declined

significantly during the dry season survey. Reduced malaria cases during the dry season may have contributed to the observed reduction in anemia rates.

In both surveys, the prevalence of anemia varied significantly by age group. The majority of anemia cases were observed in children younger than 5 years. It is noteworthy that this age group had the lowest prevalence of malaria infections. Previous studies also reported the same (Ewusie *et al.*, 2014; Gebreweld *et al.*, 2019; Kuziga *et al.*, 2017). Young children, in general, experience rapid physical and mental development, which requires an adequate supply of micronutrients such as iron, vitamin B_{12}, and folic acids for optimal growth (Armitage *et al.*, 2019). Anemia in actively growing children, may be caused by the lack of or insufficient supply of these nutrients. Anemia has a negative and long-term impact on children's mental growth (Hurtado *et al.*, 1999), it retards normal body development (Soliman *et al.*, 2014), and affects their behavior (Lozoff *et al.*, 2007). Furthermore, our results show a progressive decline in anemia severity with age, with adults having higher rates of mild anemia than younger age groups. This is consistent with other research findings, which show that as one gets older, the severity of anemia decreases (Bouyou-Akotet *et al.*, 2009; Cornet *et al.*, 1998; M. R. Desai *et al.*, 2005; Premji *et al.*, 1995). It has been suggested that as the immune system matures with the increase in age, increased synthesis of hemoglobin is enhanced (Baird, 1998; Kurtis *et al.*, 2001). This can explain in part the declining trend of anemia towards adulthood. Previous research suggests that frequent exposure to *Plasmodium* infections in adults may boost the immune system against malaria, resulting in a reduction in anemia (Marsh *et al.*, 1997).

Males had higher anemia rates than females among participants <12 years. This is consistent with previous research (Melku, Takele, *et al.*, 2018). According to one study, males have greater levels of testosterone before puberty, which stimulates rapid growth and thus an increased demand for

iron than females (Melku, Takele, *et al.,* 2018). However, from the age of 12 years and above, anemia was found to be more prevalent in females than in males. This is also in agreement with other previous findings (Zuffo *et al.,* 2016). The onset of menstruation, coupled with rapid growth in adolescent females, are considered major causes of anemia in this age group (Leenstra *et al.*, 2004; Milman, 2011). There is greater demand for iron lost during menarche, as well as a faster growth rate. According to previous studies, females from low-income families are more likely to develop anemia due to unhealthy diets (Gebreyesus *et al.*, 2019).

One of the study's limitations was its inability to investigate the morphological appearance of red blood cells. This made it impossible to distinguish between anemia caused by *Plasmodium* and other forms of anemia such as iron deficiency anemia, sickle cell anemia, and anemia caused by other parasitic infections. Due to the cross-sectional nature of the study, determining the causal relationships between anemia and independent variables was also difficult.

3.6 Conclusion

Anemia is a severe public health problem, particularly in non-irrigated areas. Anemia was most prevalent in children younger than 5 years and in females 12 years older. Anemia was associated significantly with age, household income, iron-rich food consumption and vitamin consumption. As a result, poverty alleviation and proper dietary intake are critical to reducing the anemia burden in this region. In order to clearly guide policy makers on how to mount expanded and targeted control intervention measures, detailed studies revealing the causal links are also required. It is noteworthy that the type of irrigation practiced in this study contributed to better nutrition that mitigated against anemia in the irrigated area. Intensive vector control may have reduced the impact of malaria infections on anemia in both the irrigated and non-irrigated areas.

CHAPTER FOUR:

ASYMPTOMATIC AND SUBMICROSCOPIC *PLASMODIUM* INFECTIONS IN AN AREA BEFORE AND DURING INTEGRATED VECTOR CONTROL IN HOMA BAY, WESTERN KENYA

4.1 Abstract

Long-lasting insecticidal nets (LLINs) have been the primary vector control strategy until indoor residual spraying (IRS) was added in Homa Bay and Migori Counties in western Kenya. The objective of this study was to evaluate the impact of LLINs integrated with IRS on the prevalence of asymptomatic and submicroscopic *Plasmodium* infections in Homa Bay County. A two-stage cluster sampling procedure was employed to enroll study participants aged ≥6 months old. Four consecutive community cross-sectional surveys for *Plasmodium* infection were conducted in residents of Homa Bay county, Kenya. Prior to the start of the study, all study households received LLINs, which were distributed between June 2017 and March 2018. The first (February 2018) and second (June 2018) surveys were conducted before and after the first round of IRS (Feb-Mar 2018), while the third (February 2019) and fourth (June 2019) surveys were conducted before and after the second application of IRS (February-March 2019). Finger-prick blood samples were obtained to prepare thick and thin smears for microscopic determination and qPCR diagnosis of *Plasmodium* genus. *Plasmodium* spp. infection prevalence by microscopy was 18.5% (113/610) before IRS, 14.2% (105/737) and 3.3% (24/720) after the first round of IRS and 1.3% (11/849) after the second round of IRS ($p < 0.0001$). Submicroscopic (blood smear negative, qPCR positive) parasitaemia reduced from 18.9% (115/610) before IRS to 5.4% (46/849) after IRS ($p < 0.0001$). However, the proportion of PCR positive infections that were submicroscopic increased from 50.4% (115/228) to 80.7% (46/57) over the study period ($p < 0.0001$). Similarly, while the absolute number and proportions of microscopy positives which were asymptomatic decreased from 12%

(73/610) to 1.2% (9/849) ($p < 0.0001$), the relative proportion increased. Geometric mean density of *P. falciparum* parasitaemia decreased over the 2-year study period ($p < 0.0001$). These data suggest that two annual rounds of IRS integrated with LLINs significantly reduced the prevalence of *Plasmodium* parasitaemia, while the proportion of asymptomatic and submicroscopic infections increased. To reduce cryptic *P. falciparum* transmission and improve malaria control, strategies aimed at reducing the number of asymptomatic and submicroscopic infections should be considered.

Keywords *Plasmodium falciparum***,** asymptomatic malaria, submicroscopic infection, vector control, Kenya

4.2 Introduction

Despite increased malaria control interventions, malaria morbidity and mortality remain high, making the disease a major public health concern in sub-Saharan Africa (Ochwedo *et al.*, 2021; Otambo, Omondi, *et al.*, 2022; WHO, 2020). Out of the 229 million malaria cases in 2019, 94% of them were from sub-Saharan Africa. In Kenya, malaria remains a major public health challenge, with approximately 70% of the population at risk, resulting in about 13-15% of outpatient consultations (MoH, 2021). Malaria transmission is perennial with parasite prevalence consistently above 20% in the Lake Victoria Basin region (Bashir *et al.*, 2019; MoH, 2016b; Otambo, Olumeh, *et al.*, 2022). Morbidity and mortality mostly in young children and pregnant women remains prevalent along the lake region (Bashir *et al.*, 2019; MoH, 2016b). *Plasmodium falciparum* is the most abundant species (92%) accompanied by *Plasmodium malariae* (6%) and *Plasmodium ovale* at 2% (MoH, 2016a).

The fight against malaria has been scaled up along the Lake Victoria Basin region, where the infection rates remain high. For instance, mass distribution of LLINs occurs every three years (MoH, 2019b). Besides, indoor residual spraying (IRS) was initiated in 2008 (PMI, 2018), but due to reports of emerging resistance to pyrethroid insecticides by malaria vectors, IRS was suspended in endemic parts of Kenya in 2012 (Bashir *et al.*, 2019; MoH, 2016b; Ochomo *et al.*, 2014). Since then, Kenya has relied on long-lasting insecticidal nets as the primary vector control until February 2018 when IRS was re-introduced using Actellic 300 CS, an organophosphate insecticide (PMI, 2018). These control strategies toward malaria in Kenya have been intensified to minimize malaria burden in most affected regions. However, this may be undermined by the residual proportions of both asymptomatic and submicroscopic *Plasmodium* infections in low transmission areas (Almeida *et al.*, 2018; Bousema *et al.*, 2014). These infections act as major reservoirs of malaria parasites, thus will sustain transmission. Therefore, formulation of sustainable control strategies to mitigate challenges fueling transmission is very critical and should be regarded as a priority investment.

While the impact of long-lasting insecticidal nets on malaria has been well documented in western Kenya, little is known on the impacts of the addition of IRS on parasite profiles at the population level. Therefore, a study targeting the entire community to identify the prevalence of malaria parasites, asymptomatic and submicroscopic infections was undertaken before the application of indoor residual spraying (IRS, Actellic 300 CS) and during IRS intervention period. Highly sensitive molecular tools were used to detect submicroscopic parasites and improve species identification. Results of this study will guide policy makers in the health, and other concerned stakeholders with regard to enhanced malaria control and management.

4.3 Materials and methods

4.3.1 Sample size calculation formula

The formula proposed by Naing *et al.* (2006) was used to determine sample size. Briefly, assuming malaria prevalence (p) of 27% (MoH, 2016b), precision (d) of 0.05 and level of confidence (z) as 1.96, the minimum number of study participants needed was 303. This was arrived at as follows:

$$N = \frac{Z^2 P(1-P)}{d^2} \rightarrow N = \frac{1.96^2 \times 0.27(0.73)}{0.05^2} = 303 \rightarrow 303 \times 2(\text{design effect}) = 606$$

Due to the cluster sampling procedure, the sample size was multiplied by a design effect as proposed by Cochran (1997). The design effect in most cluster surveys has been reported to be 2 (Macfarlane, 1997) Hence, the minimum number of study participants required was 606. To prevent survey attrition, we decided to recruit 4 participants per family because the average number per household was 4.4, resulting in 80 individuals per cluster and 960 for the entire study region. However, only 610, 737, 720, and 849 participants were tested during the first, second, third and fourth surveys, respectively, due to participants' unavailability during surveys.

4.3.2 Cross-sectional surveys

Four community cross-sectional surveys were conducted from February 2018 to June 2019. The first survey was in February 2018 before the first IRS, and the second in June 2018. A similar schedule was executed in 2019 before and after the second IRS. Selection for study participation was based on a two-stage procedure similar to that described by Galway *et al.* (2012). The first stage involved identification of clusters (essentially villages) as primary sampling units. For example, with the help of chiefs and village heads the study team identified the total number of clusters (30). Based on the number of participants required, 12 clusters were randomly selected from the previously identified 30 clusters. In the second stage, the total number of households in each cluster was determined and assigned unique identification numbers. Twenty households from

each cluster were then randomly selected provided the household head accepted to participate. The study team recruited 80 permanent dwellers from each cluster. In every household, 4 participants, aged 6 months and above, who were permanent dwellers and who were willing to participate and sign the informed consent or assent forms (for participants <18 years) (appendix 1), were recruited. In case there were more than 4 eligible participants in a household, the 4 participants were randomly selected. However, in households with less than 4 eligible participants, the next household was selected. A questionnaire (appendix 2) was used to collect information such as gender, age and body temperature. An infrared thermometer was aimed at the forehead to capture the body temperature. Individuals who had a fever in the previous 48 hours were also recorded in the questionnaire. During the survey period, any study participant who had a fever or tested positive for *Plasmodium* infection was referred to the nearest health center for further examination and treatment.

4.3.3 Sample collection and microscopic examination

Finger-prick blood samples were obtained from study participants to screen for *Plasmodium* infections by microscopy as described in appendix 6. Thick and thin blood smears were prepared and stained with 10% Giemsa for 15 minutes and examined using a microscope. A total of 200 microscopic fields containing leukocytes in the thick smear were examined before declaring a slide negative. For quality control, each slide was examined by two independent microscopists and, in the case of a discrepancy, a third reader was involved. To estimate the *P. falciparum* density, the number of parasites observed in microscopic fields containing based on the 200 leucocytes on a thick smear. This number was then multiplied by 40 assuming 8000 leukocytes/μL blood (McKenzie *et al.*, 2005). The parasite density was further classified as either low (< 1,000 parasites/μL), moderate (1,000 – 4,999 parasites/μL), high (5,000 – 99,999 parasites/μL) or

hyperparasitaemia (≥100,000 parasites/μL) (Chipwaza *et al.*, 2020).Gametocyte density was also estimated by counting gametocytes against 500 leukocytes in thick smear (Subussa *et al.*, 2021). This was then converted to gametocytes/ μl of blood by multiplying with the standard count of 8,000 white blood cells /μl.

4.3.4 DNA extraction and qPCR detection of parasite infections

Dry blood spots were collected from study participants as described by Wampfler *et al.* (2013). Briefly, 50 μL of finger-prick blood was spotted on Whatman® 3MM filter paper (3 spots) in the field. Filter paper was air dried and kept at 4^0C in sealed plastic bags with a desiccant and transported to the laboratory. DNA was extracted from each filter paper using Chelex resin (chelex -100) as described previously (Wooden *et al.*, 1993) with minor modifications (Onyango *et al.*, 2021). For instance, discs measuring 3 mm were cut from the prepared dry blood spot. These discs were soaked in 10% Saponin and Phosphate Buffer Saline (PBS) and then left to incubate overnight. The mixture was washed twice in 1x PBS and then boiled in 20% (wt/vol) chelex suspension (styrene-divinylbenzene co-polymer containing iminodiacetic acid groups). The study focused on examination of the three *Plasmodium* species (*P. falciparum, P. malariae*, and *P. ovale*) since they had been previously reported as the most possible species to exist in study area (MoH, 2016a). Malaria parasite detection was performed on all extracted DNA (all blood smear positives and negatives) using multiplex real-time PCR (qPCR) targeting 18S rRNA gene as described elsewhere (Shokoples *et al.*, 2009; Veron *et al.*, 2009) with modifications. Briefly, the probes and primers of three species (*P. falciparum, P. malariae*, and *P. ovale*) were used for multiplex qPCR. The qPCR was run on the Applied Biosystems QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific Inc. USA) in a final volume containing 6μl of PerfeCTa® qPCR ToughMix™, Low ROX™ Master mix (2X), 0.4μl of the species specific forward and reverse primers (10μM),

0.5µl of the species-specific probe, 0.1µl of double-distilled water and 2µl of sample DNA. The thermo profile was set as follows; 50°C for 2 min, (95°C for 2 min, 95°C for 3 sec and 58°C for 30 sec) for 45 cycles.

4.3.5 Data analysis

An asymptomatic infection was defined as a *Plasmodium* positive test result with the body temperature below 37.5ºC during the time of blood sample collection or in the last 48hrs. Blood samples testing negative by microscopy but positive by qPCR were considered to be submicroscopic. All data collected was entered in Excel spreadsheet, cleaned and malaria prevalence from the study area analyzed using GraphPad Prism version 8. Differences in prevalence among 4 cross-sectional surveys and age sets were determined using inferential statistics (Pearson χ^2). Parasite densities were logarithm-transformed (log (x+1) and mean difference among age groups or survey periods determined using analysis of variance (ANOVA).

4.4 Results

4.4.1 Demographic characteristics of participants

The study team approached 600 households in the study area during the recruitment stage. However, 42 household heads refused to participate, and 70 household stated that they did not have LLINs and were, therefore, excluded. A total of 240 households were chosen at random from the remaining 498 households to participate in the study. These 240 households had a total population of 1060 people. However, 24 of them refused to participate, 40 children under the age of 6 months were excluded, and 36 people from households with more than 4 eligible participants were also excluded. Finally, the total number of participants who met the inclusion criteria was 960 as indicated in Fig. 4.1. Due to unavailability of participants during surveys, the participants who were tested for *Plasmodium* species infections during the first, second, third, and fourth

survey, were 610, 737, 720, and 849 study participants respectively. Age group distribution during the survey period are shown in Table 4.1.

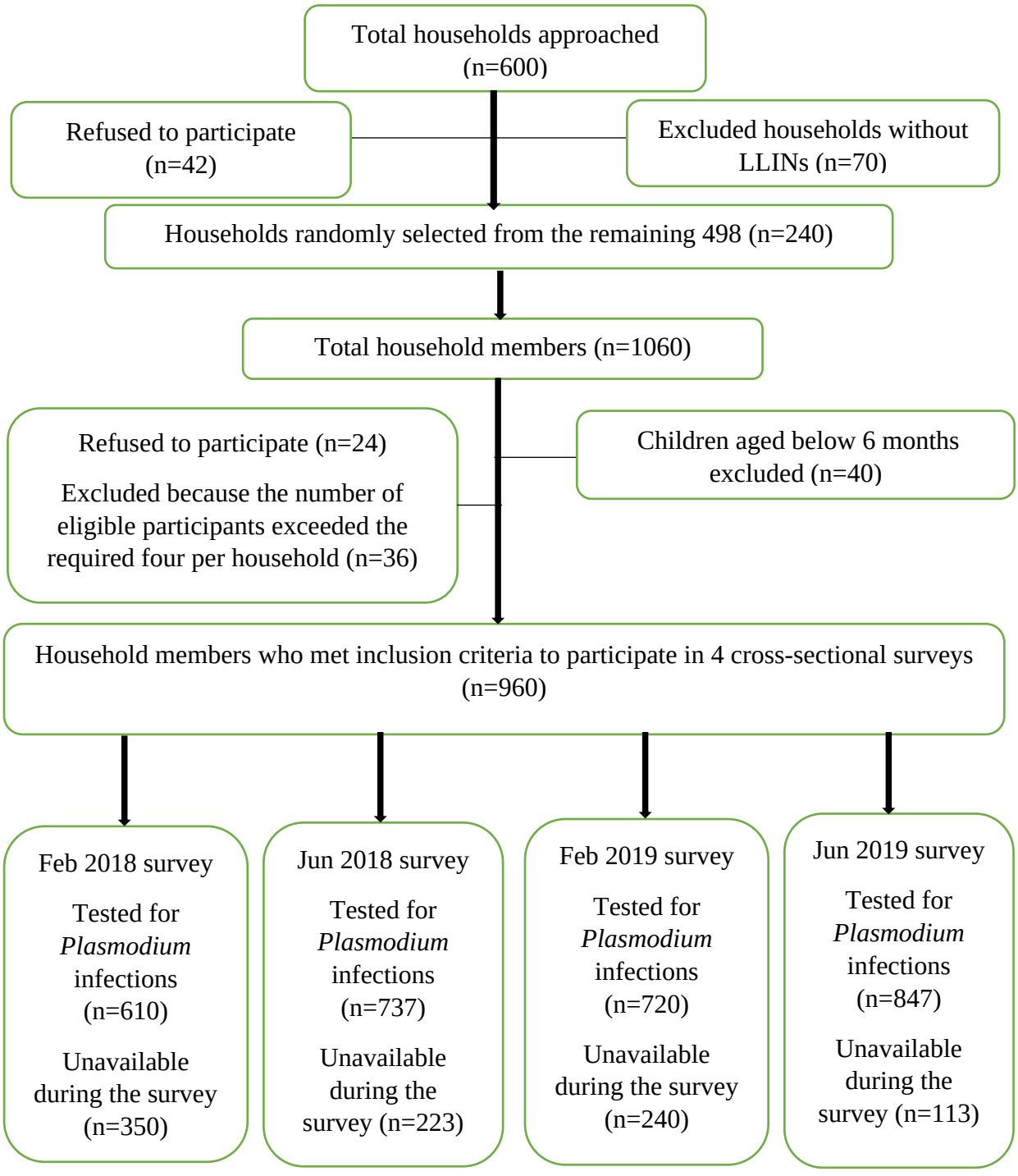

Figure 4.1 The trial diagram showing the study participants' recruitment criteria

Table 4.1 Demographic characteristics of the study participants, parasite density, asymptomatic and submicroscopic Plasmodium infections during the 4 cross-sectional surveys

Variable	**Survey period**			
	Feb 2018 (dry season)	June 2018 (wet season)	Feb 2019 (dry season)	June 2019 (wet season)
Age group (yrs.) n (%)				
<5	96 (15.7)	139 (18.9)	163 (22.6)	144 (17.0)
5-14	210 (34.4)	285 (38.7)	250 (34.7)	260 (30.6)
≥15	304 (49.8)	313 (42.5)	307 (42.6)	445 (52.4)
Microscopic malaria parasite prevalence % (n)	18.5 (113/610)	14.2 (105/737)	3.3 (24/720)	1.3 (11/849)
Malaria parasite prevalence by qPCR % (n)	37.4 (228/610)	25.1 (185/737)	10.4 (75/720)	6.7 (57/849)
Geometric mean parasite density (parasites/µl of blood)	2229	353	578	184
Plasmodium falciparum parasite density category % (N)	100% (113)	100% (102)	100% (24)	100% (11)
Low % (n)	33.6 (38)	77.8 (77)	58.3 (14)	90.9 (10)
Moderate % (n)	36.3 (41)	19.2 (19)	41.7 (10)	9.1 (1)
High % (n)	27.4 (31)	3.0 (3)	0.0 (0)	0.0 (0)
Hyper % (n)	2.7 (3)	(0)	0.0 (0)	0.0 (0)

Low = < 1,000 parasites/µL, moderate = 1000 – 4,999 parasites/µL, high = 5,000 – 99,999 parasites/µL, hyperparasitaemia = ≥ 100,000 parasites/µL

4.4.2 Parasite prevalence

During the first survey (dry season), conducted in February 2018, the parasite prevalence by microscopy and qPCR was 18.5% (113/610) and 37.4% (228/610), respectively. A significant reduction in prevalence was recorded during the second cross-sectional survey (wet season) by both microscopy and qPCR. For instance, parasite prevalence reduced to 14.2% (105/737) (χ^2=

5.117, df = 1, p = 0.02) by microscopy and 25.1% (185/737) (χ^2 =23.08, df = 1, p < 0.0001) by qPCR. Further decline in parasite prevalence was reported in the third survey with microscopy and qPCR recording 3.3% (24/720) and 10.4% (75/720) respectively. During the last survey, malaria parasite prevalence was finally reduced to 1.3% (11/849) and 6.7% (57/849) by microscopy and qPCR respectively. Overall, the reduction in parasite prevalence during the 4 cross-sectional surveys was significant by microscopy (χ^2= 186.9, df = 3, p < 0.0001) and by qPCR (χ^2= 266.2, df = 3, p < 0.0001) as indicated in Table 4.1.

Parasite prevalence as determined by both microscopy and qPCR, varied significantly among age groups during the first, second and third survey. For instance, during the first survey, *Plasmodium* infection rate among participants aged <5, 5-14 and ≥15 years old by microscopy was 24% (23/96), 28.1% (59/210), and 10.2% (31/304), respectively (χ^2 = 28.6, df = 2, p < 0.0001). During the second survey, parasite prevalence among children aged below 5, 5-14 and those aged ≥ 15 years was 15.8% (22/139), 20.7% (59/285), and 7.7% (24/313), respectively (χ^2 = 25.1, df = 2, p < 0.0001). Similarly, during the third survey, *Plasmodium* infection rate among <5, 5-14, and ≥15 years old was 2.4% (4/163), 6% (15/250) and 1.6% (5/307), respectively (χ^2 = 8.6, df = 2, p = 0.01). However, during the fourth survey, the parasite prevalence among <5, 5-14, and ≥15 years old was 1.4% (2/144), 2.3% (6/260), and 0.7% (3/445), respectively (χ^2 = 1.9, df = 2, p = 0.39). (Fig. 4.2). A similar pattern of variation in parasite prevalence among age groups was recorded by qPCR during the 4 cross-sectional surveys as shown in Fig. 4.2 A.

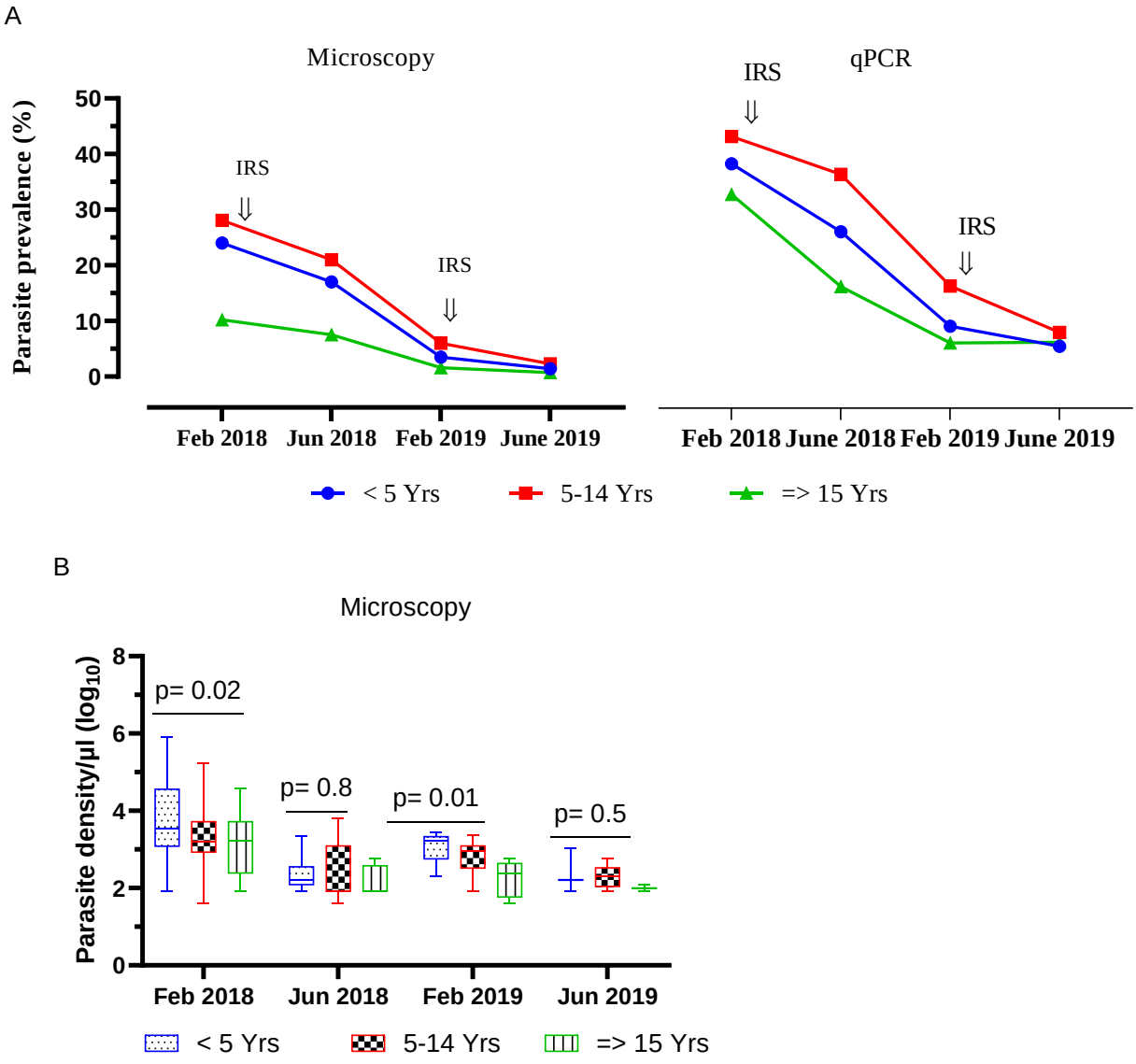

Figure 4. 2 Graph 4.2A shows the prevalence of *Plasmodium* infection by microscopy and qCR among age groups during the 4 surveys. Graph 4.2B indicates the parasite density among age groups by microscopy

4.4.3 *Plasmodium falciparum* parasite density and category

The 4 cross-sectional surveys indicated significant reductions in parasite burden among the study population. The overall geometric mean parasite densities (GMPD) during first, second, third and

fourth survey were 2229 infected red blood cells (irb)/µl, 353 irb/µl, 578 irb/µl and 184 irb/µl, respectively (ANOVA, $F = 28.95$, $df = 3, 243$, $p < 0.0001$) (Table 4.1). Geometric mean parasite density varied significantly among age groups during the first (ANOVA, $F = 4.142$, $df = 2,110$, $p = 0.018$) and the third survey (ANOVA, $F = 5.463$, $df = 2, 21$, $p = 0.012$ (Fig. 3B). During the first and third surveys, children aged below 5 years old, had highest mean parasite densities compared to other age groups. However, during the second and fourth surveys, geometric mean parasite density did not vary significantly among age groups (ANOVA, $F = 1.361$, $df = 2, 96$, $p = 0.26$) and (ANOVA, $F = 0.67$, $df = 2, 8$, $p = 0.54$), respectively. The parasite density during each survey was categorized into low, moderate, high and hyperparasitemia. The findings indicated elimination of high and hyperparasitemia during the 4 cross-sectional surveys. For example, the study reported zero prevalence rates of hyperparasitemia during the second, third and the fourth survey. Equally, during survey 3 and 4, there were no cases of high parasite density. With the onset of indoor residual spray program, most infections were due to low parasite densities as illustrated in Table 4.1.

4.4.4 *Plasmodium* species distribution and *P. falciparum* gametocyte prevalence

Three malaria species were found in the study population with *P. falciparum* being most prevalent, followed by *P. malariae* and *P. ovale* as the least prevalent, as shown in Table 4.1. Both *P. malariae* and *P. ovale* occurred at low parasite densities hence mostly detected by PCR. Considering the prevalence of mono-infections during the 4 cross-sectional surveys, *Plasmodium falciparum* accounted for 94.3% (215/228), 81.6% (151/185), 68% (51/75) and 87.7% (50/57) of all infections during the first, second, third and fourth survey respectively. Similarly, the proportion of *P. malariae* during survey 1, 2, 3 and 4 was 0.9% (2/228), 8.6% (16/185), 10.7% (8/75) and 1.8% (1/57) respectively. The infections due to *P. ovale* only occurred during the first

(0.4% (1/228), second (1.6% (3/185), and fourth survey (5.3% (3/57). Mixed species infections due to *P. falciparum* and *P. malariae* mostly occurred throughout the study period. Mixed infections involving *P. ovale* were rare and were reported during the first and second surveys, as illustrated in table 4.2 below. Results further indicated lowest levels of gametocyte prevalence during survey periods. The declining trend of gametocyte prevalence was 1.3% (8/610), 0.5% (4/737), 0.14% (1/720) and 0.12% (1/849) during the first, second, third and the fourth survey respectively ($\chi^2 = 12.97$, df = 3, p = 0.005). The geometric mean gametocyte density was: 66.6, 35.9, 48 and 16 gametocytes/ µl of blood during survey 1, 2, 3 and 4 respectively (ANOVA, F = 1.24, df = 3, 10, p = 0.35).

Table 4.2 *Plasmodium* species composition during the 4 cross-sectional surveys as detected by PCR

Survey period	***Plasmodium* species composition**							
	Pf	*Pf+Pm*	*Pf+Pm+Po*	*Pf+Po*	*Pm*	*Pm+Po*	*Po*	**Total**
Feb 2018	215	7	2	1	2	0	1	228
Jun 2018	151	12	0	0	16	3	3	185
Feb 2019	52	15	0	0	8	0	0	75
Jun 2019	50	3	0	0	1	0	3	57

4.4.5 Submicroscopic, asymptomatic and symptomatic *Plasmodium* infections

The absolute numbers of submicroscopic (qPCR positive alone) infections decreased during the four cross-sectional studies. For instance, the proportion of submicroscopic infections was 18.9% (115/610), 10.9% (80/737), 7.1% (51/720), and 5.4% (46/849) ($\chi^2 = 80.2$, df = 3, $p < 0.0001$) for the first, second, third and fourth surveys, respectively (Fig. 4.3). However, the relative proportion of submicroscopic infections increased during the four cross-sectional surveys. For example, the

proportion of PCR positive infections that were submicroscopic increased from 50.4% (115/228) to 80.7% (46/57) over the study period (χ^2= 31.98, df = 3, $p < 0.0001$).

The rate of symptomatic infections as determined by microscopy decreased during the survey period. For instance, the rate of microscopy positive that were symptomatic during the first, second, third, and fourth surveys, was 6.6% (40/610), 4.6% (44/737), 0.8% (6/720), and 0.2% (2/849), respectively (χ^2 = 78.6, df = 3, $p < 0.0001$) (Fig. 4.3). Similarly, the absolute numbers of microscopy positive that were asymptomatic during surveys 1,2,3 and 4, decreased from 12% (73/610) to 1.2% (9/849) (χ^2 = 110.4, df = 3, $p < 0.0001$). However, the relative proportions of microscopy positive that were asymptomatic increased from 64.6% (73/113) to 83.3% (9/11) (χ^2 = 1.349, df = 3, p = 0.7). The detection of *Plasmodium* species infection prevalence by qPCR was significantly higher than that of microscopy as illustrated in Fig. 4.4.

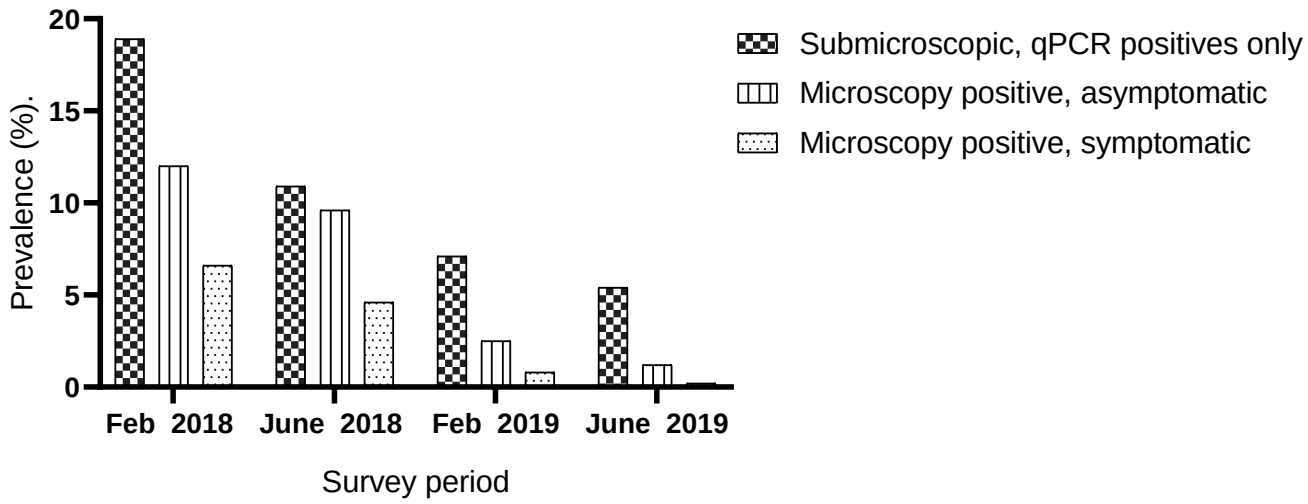

Figure 4.3 The graph indicates the proportions of submicroscopic, asymptomatic, and symptomatic infections during the 4 cross-sectional surveys

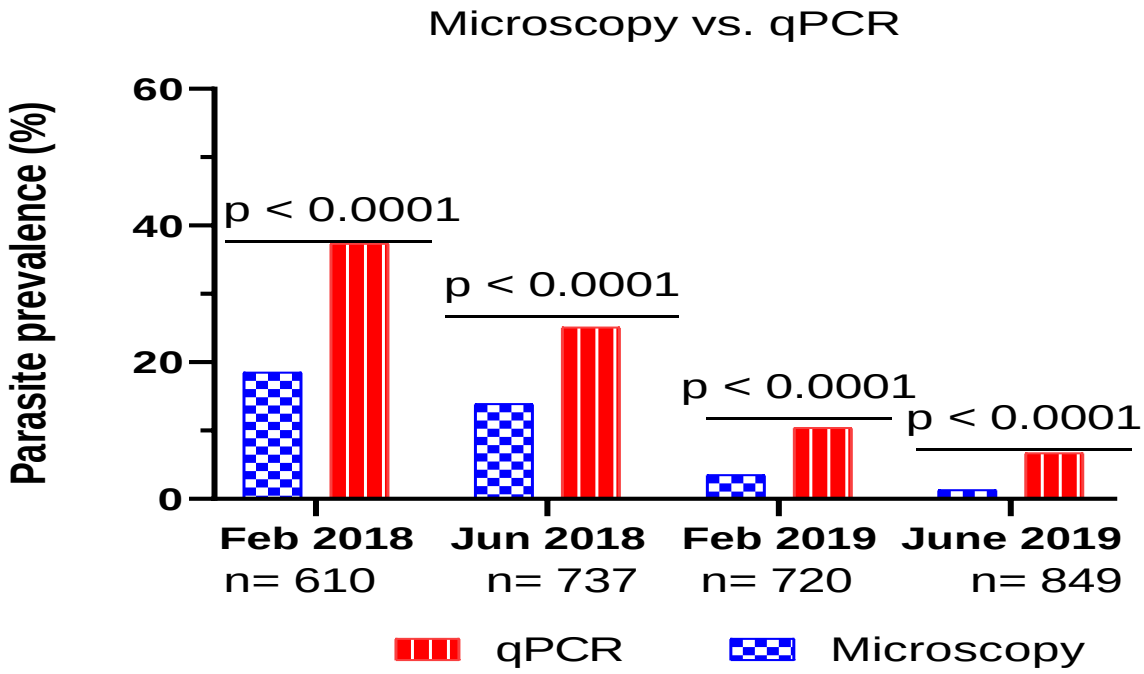

Figure 4.4 The graph shows the detection of malaria parasites by microscopy and qPCR during the 4 cross-sectional surveys.

4.5 Discussion

This study was undertaken to document the impact of IRS and LLINs on parasitological profiles of malaria parasites in a human population in an endemic site in western Kenya. Accurate determination of *Plasmodium* infection rates is key towards monitoring the progress of malaria intervention programmes initiated within endemic regions (Okell *et al.*, 2012). To accurately document malaria prevalence rates within populations, more sensitive detection tools are essential. This will help detect infections including those with very low parasite densities which mostly go undetected by microscopy (Wu *et al.*, 2015). The present study investigated the prevalence rates of malaria parasites, asymptomatic and submicroscopic *Plasmodium* infections before the introduction of IRS and during two rounds of IRS in an area where LLINs were widely used. Four cross-sectional surveys were conducted and findings indicated a sustained reduction in *Plasmodium* infection rates especially after the rollout of IRS program. Just before the onset of IRS, the *Plasmodium* prevalence rate by both microscopy and qPCR was at 18.5% and 37.4%, respectively. However, during the last survey, the prevalence of malaria parasites had declined to 1.3% by microscopy and 6.7% by qPCR.

The geometric mean parasite density significantly declined from 2229 parasites/µl of blood before intervention to 184 parasites/µl of blood after 2 rounds of IRS. The most plausible explanation to such a drastic reduction in parasite prevalence and parasite density could be due to very low parasite transmission after the application of IRS. For instance, in a neighboring Migori county, where a similar intervention was initiated, malaria transmission was reported to have declined significantly (Abong'o *et al.*, 2020). Furthermore, in Uganda (Tukei *et al.*, 2017), Mali (Wagman *et al.*, 2020) and Tanzania (Mashauri *et al.*, 2013; Mashauri *et al.*, 2017) where IRS was initiated

as a vector control strategy, the *Plasmodium* infection rate and parasite density decreased significantly.

Parasite prevalence determined by both microscopy and qPCR were reported to be highest among the age group 5-14 years old. This is in agreement with other previous studies(Idris *et al.*, 2016; Omondi *et al.*, 2017; Sultana *et al.*, 2017). A likely explanation to this trend has been highlighted in past studies (M. Desai *et al.*, 2014; Gitonga *et al.*, 2012; Iwashita *et al.*, 2010). For instance, majority in this age group are not under direct care of parents as compared to under 5 years old. They tend to be independent and most often may not sleep under bed nets or nets poorly placed, leaving them vulnerable to infectious mosquito bites. Poor health-seeking pattern has also been reported as a possible challenge in this age group, leading to more parasite infections (Bigogo *et al.*, 2010), possibly because they have mild clinical symptoms. Unlike parasite prevalence, parasite density was highest in children under the age of 5, but decreased with age. Antiparasitic immunity develops with age, providing protection against high malaria parasite density (Doolan *et al.*, 2009; Moormann, 2009). Due to repeated *Plasmodium* infections, older children, for example, develop an improved immune response that significantly suppresses the multiplication of asexual parasites.

The prevalence of asymptomatic and symptomatic cases decreased during the study period. This could be due to sustained malaria vector control in the study area through the use of IRS and LLINs. It was important to note, however, that the relative proportions of microscopy positives that were asymptomatic increased with each subsequent survey. This observed trend could be explained by a significant decrease in parasite prevalence and density. For example, the study revealed a complete elimination of hyperparasitaemia and high parasite density infections during the third and fourth survey. Likewise, there was a significant decrease in infections with moderate parasitaemia. As a result, the few existing *Plasmodium* infections with low parasite densities may

be easily tolerated, increasing the number of asymptomatic cases. Similarly, the current study area has historically been a malaria endemic region. Areas undergoing perennial *Plasmodium* infections endure elevated rates of asymptomatic infections (Badiane *et al.*, 2021; Imwong *et al.*, 2016). This is due to increased tolerance towards parasite density (Ademolue *et al.*, 2017). Frequent exposure to *Plasmodium* infections may trigger strong immune modulation resulting in reduced clinical symptoms (A. Kamau *et al.*, 2020; McQueen *et al.*, 2013; White, 2017). However, rising asymptomatic rates are likely to negate the gains and jeopardize vector control efforts in the study area.

The absolute number of submicroscopic infections decreased with decrease in *Plasmodium* infection rate. However, the relative proportion of submicroscopic infections increased during study period. As the parasite densities decrease, submicroscopic infections may increase due to microscopy's low detection limit (Bousema *et al.*, 2014; Okell *et al.*, 2012). This is comparable to previous studies, which reported high submicroscopic infections in low-transmission settings (Harris *et al.*, 2010). Submicroscopic infections are of public health interest as they pose serious health challenge to pregnant women (Arango *et al.*, 2013) as well as causing mild anemia (Ladeia-Andrade *et al.*, 2009). The prevalence and density of gametocytes were extremely low and steadily declined with subsequent surveys. Although infectious gametocyte carriage is modulated by a variety of factors including immune response, treatment, and transmission intensity(Bousema *et al.*, 2011a), the current study's reduced levels of gametocytes could be attributed to a sustained vector control program within the study area.

Plasmodium species detected in the current study varied with *P. falciparum* accounting for the highest proportions. This is consistent with other observations along the lake region, western Kenya (Idris *et al.*, 2016; Olanga *et al.*, 2015). The study further reported existence of *P. malariae*

and *P. ovale*. While the number of *P. falciparum* infection cases reduced, the proportion of *P. malariae* cases remained relatively stable over the course of four surveys. Most importantly, these *P. malariae* infections occurred at very minimal parasite densities, hence the majority of cases were diagnosed using qPCR. This is similar to a previous study which reported subpatent *P. malariae* with low parasite densities (Mueller *et al.*, 2007). Low parasite density in *P. malariae* infections may be a challenge to microscopy based diagnosis leading to misdiagnosis or underreporting of malaria cases (Mueller *et al.*, 2007) Accurate detection and treatment of *P. malariae* infections is paramount to minimize cases of nephrotic syndrome, which are associated with excessive mortality (Eiam-Ong, 2003).

Detection rate of *Plasmodium* species by qPCR was significantly higher compared to that of microscopy. This is consistent with previous studies which compared the two diagnostic tools (Kang *et al.*, 2017; Wang *et al.*, 2014). Molecular based detection tools are known to be very sensitive hence able to identify cases with lowest parasite densities (Jones *et al.*, 2012; Kuamsab *et al.*, 2012). Highly sensitive detection tools may be useful especially in settings where submicroscopic infections or co-infections with low parasite density *P. malariae* are common.

The study was limited to two sub-counties; hence the findings cannot not be generalized to the entire county. Besides, the study did not include the *Plasmodium* infection rate prior to net interventions. Finally, due to the study's cross-sectional design, it was difficult to directly link the lower *Plasmodium* infections to LLINs and IRS interventions.

4.6 Conclusion

The study findings report significant reduction in *Plasmodium* infection rates and parasite densities following the introduction of the indoor residual spray programme. The reports also show that,

while the absolute number of asymptomatic and submicroscopic infections decreased in the study area, the relative proportion of both asymptomatic and submicroscopic infections increased. This is likely to negate the progress achieved towards vector control. The study also demonstrated a large reduction in clinical malaria, which is an important public health benefit.

CHAPTER FIVE:

MALARIA DIAGNOSIS IN HEALTHCARE FACILITIES AND TREATMENT-SEEKING BEHAVIOR IN MALARIA ENDEMIC SETTINGS IN WESTERN KENYA

5.1 Abstract

Accurate malaria diagnosis and timely treatment are requirements for effective management of the disease. However, treatment efficacy may be significantly reduced in resource-constrained healthcare facilities with poorly equipped laboratories and frequent drug and rapid diagnostic test kit (RDT) stock-outs. Furthermore, patient may avoid seeking treatment from such facilities. The study's goal was to determine treatment-seeking behavior, malaria diagnosis and treatment quality, and likely treatment-seeking determinants in the local population. Passive case detection, which targeted all patients with suspected malaria cases, was conducted in ten public healthcare facilities (7 dispensaries, 2 health centers and 1 sub-county hospital) over a three-month period. Monthly malaria cases, methods of diagnosis and antimalarial drug availability were assessed. A household-based survey was also carried out. Structured questionnaires were used to collect knowledge, attitude and practice (KAP) data from household heads. Malaria knowledge, treatment seeking behavior, and predictors of malaria treatment-seeking were all determined. Three of the seven dispensaries lacked a laboratory to conduct microscopy- diagnosis. These three dispensaries also experienced frequent RDT stock-outs, which resulted in depending on clinical signs as diagnosis for malaria. The majority of local residents with fever (50.3%) purchased antimalarial drugs from a chemist. About 37% of fever patients sought treatment at healthcare facility while the remaining 12.7% did not treat their fevers. In irrigated areas, 45.5% (46/64) of fever patients sought treatment at healthcare facilities, compared to 25% (18/64) in non-irrigated areas ($p = 0.009$). Most children aged below 5 who had fever (77.7%) were taken to healthcare facility for treatment compared to

31.4% of children aged 5 – 14 years or 20.9% of adults (0.0001). Predictors of treatment seeking included access to healthcare facility (OR = 16.23, 95% CI: 2.74-96.12), and ability to pay hospital bills (OR = 10.6, 95% CI: 1.97- 57). Other factors that influenced health-seeking behavior included the severity of symptoms, the age of the patient and knowledge of malaria symptoms.

5.2 Introduction

Malaria is still a major public health challenge in sub-Saharan Africa, with young children suffering the highest morbidity and mortality rates (Ajakaye *et al.*, 2020; A. Kamau *et al.*, 2020; WHO, 2020). The debilitating nature of the disease is exacerbated by the overlap of malaria symptoms with those of other diseases such as upper respiratory infections and typhoid (Chandramohan *et al.*, 2002; Kallander *et al.*, 2004; Rafael *et al.*, 2006). As a result, accurate diagnosis combined with timely medication remains the primary life-saving intervention and means of reducing transmission (Ajakaye *et al.*, 2020; WHO, 2000) Previously, the World Health Organization recommended treating any febrile child with antimalarial drugs in the absence of microscopy or malaria diagnostic test kits (WHO,2006). However, this was later revised to emphasize parasite-based diagnosis in all ages (WHO, 2010). The widespread adoption of malaria diagnostic test kits (RDT) in healthcare facilities, as well as a decrease in febrile illness caused by malaria, prompted the revision (D'Acremont *et al.*, 2010; WHO, 2010). In addition, the emergence of drug-resistant malaria parasites due to drug overuse, possibly led to the implementation of the 'test and treat' strategy (Hyde, 2007; Parija *et al.*, 2011). Malaria RDT kits were relatively easy to use in malaria diagnosis (Moody, 2002). This made parasitological diagnosis of malaria possible, especially in resource-poor healthcare facilities where microscopy diagnosis was a challenge (Cunningham *et al.*, 2019).

In an effort to streamline with WHO recommendation, the Kenyan ministry of health endorsed the test and treat policy in all patients presenting with fever (MoH, 2016d). This ensures that only malaria-confirmed cases receive antimalarial treatment, as well as proper management of fever patients suffering from non-malarial illnesses (MoH, 2016d). Most importantly, it is prudent to minimize indiscriminate antimalarial prescription (Yukich *et al.*, 2010; Zikusooka *et al.*, 2008). However, treatment of suspected malaria based on clinical diagnosis is increasingly becoming common in most resource-poor settings (Amexo *et al.*, 2004; Nankabirwa *et al.*, 2009; Reyburn *et al.*, 2004). This situation has been aggravated by a number of factors, including ill-equipped healthcare facilities (Kyabayinze *et al.*, 2012) and self-treatment among populations (Akilimali *et al.*, 2022).

Significant reductions in parasite prevalence have been reported in Homa Bay county, where malaria infections were previously above 20% (Bashir *et al.*, 2019; MoH, 2016b; Omondi, Otambo, *et al.*, 2022). The current control efforts using indoor residual spraying (IRS with an organophosphate insecticide) and long-lasting insecticide treated bed nets (LLINs) had significantly reduced malaria prevalence in the area. Accurate diagnosis and proper treatment-seeking practice are critical for maintaining effective treatment and possibly reaching malaria-free Kenya, as envisioned in Kenya's malaria strategy 2019-2023 (MoH, 2021). However, studies investigating malaria diagnosis practice at the healthcare facilities and treatment-seeking around this region are few. The study therefore seeks to investigate the challenges faced by healthcare facilities with regard to diagnosis and treatment as well as treatment-seeking behavior among the local population. The findings of the study will further guide the ministry of health and other stakeholders determine the best way to improve the situation.

5.3 Materials and methods

5.3.1 Study design and data collection

This was a cross-sectional household and healthcare facility-based survey. Healthcare facility survey was conducted between October and December 2019, during the short rainy season. Healthcare facilities with a minimum catchment population of 3000 people were selected. As a result, ten government healthcare facilities were identified and included in the study due to low cost of services. Children under the age of five, for example, are eligible for free malaria testing and treatment. However, for older children and adults, a malaria laboratory test costs KSH. 100, but antimalarial drugs are free. Table 5.1 further describes the total number of villages, households and population per healthcare facility. According to the Kenya national guidelines for diagnosis, treatment, and control, all patients in all age groups with suspected malaria undergo a parasite-based diagnosis before treatment (MoH, 2016d). Microscopy and malaria rapid diagnostic test kits (RDT) were the recommended malaria diagnostic tools particularly in level 2 and 3 health facilities (MoH, 2016d).

The study therefore aimed to determine the average monthly clinical malaria cases and to assess the available diagnostic techniques for suspected malaria cases. The study targeted all patients with suspected malaria attending the ten government-based healthcare facilities and agreed to signed informed consent or assent form (for minors aged <18 years old) (Appendix 1). The patient had to be a resident of the study area in order to be included in the study. Clinical officers in charge of each healthcare facility provided information on Artemether Lumefantrine (AL) availability. AL is the recommended first-line drug for the treatment of uncomplicated *Plasmodium* infections in Kenya (MoH, 2016d).

Table 5.1. List of the 10 healthcare facilities within the study area

Name	**level**	**Catchment population**			**Type of malaria diagnosis offered**
		No. of villages	No. of households	Total population	
Nyarut	Dispensary	18	792	3,774	RDT, clinical
Kodula	Dispensary	12	930	6,797	RDT, clinical
Omboga	Dispensary	25	2165	9,937	RDT, clinical
Ngegu	Dispensary	20	1614	7,760	RDT, microscopy
Nyagoro	Health center	34	3203	15,583	RDT, microscopy
Nyalkinyi	Health center	20	2147	10,730	RDT, microscopy
Okiki Amayo	Dispensary	10	477	3,142	RDT, microscopy
Adiedo	Dispensary	29	1727	9,234	RDT, microscopy
Obwanda	Dispensary	16	927	4,864	RDT, microscopy
Kandiege	Sub-county hospital	51	2809	15,338	RDT, microscopy

Dispensary- Level 2, Health center-3, Sub-county hospital-level 4

5.3.2 Household survey

Household-based survey was conducted in the month of July 2020, at the end of long rains, when malaria transmission is typically at its peak. Households were the study units in the selected villages. A total of 240 households were considered sufficient to answer study objectives and were randomly selected using a two-stage cluster sampling design as previously described (Galway *et al.*, 2012). First, the ten health facilities served a total of 208 villages (clusters) in both irrigated and non-irrigated areas. There were 73 clusters in irrigated area and 135 clusters in non-irrigated areas. Based on the number of households in each cluster, 12 clusters (6 from each region) were randomly selected. The second stage involved household selection. All households in the 12 identified clusters were assigned unique numbers by the study team. Thereafter, 20 households in each of the 12 clusters, were chosen at random.

Trained interviewers administered structured questionnaires (appendix 2) to the household heads. All 240 household heads responded to questions regarding knowledge, attitude and practice towards malaria. However, to determine the treatment seeking behavior, only households that had

reported cases of fever (increase in body temperature) accompanied by headache or joint pains or both in the two weeks preceding data collection, were interviewed. Information on the age, gender, severity of fever, treatment-seeking behavior, bed net ownership, and use was collected.

The integrity of nets was physically examined. For instance, the nets were examined for the presence of holes. If the nets had holes, the sizes and the number of those holes were noted.

Health-seeking behavior was the primary outcome of the study. The operational definition of treatment-seeking behavior was considered to be seeking treatment at health facility or self-medication using antimalarial drugs bought from a chemist. The proportion of participants who did not treat their fever was also recorded. Availability of antimalarial drugs in healthcare facilities for fever patients seeking treatment was enquired, or if fever patients were referred to a chemist. A chemist classified as store that specialized in selling for drugs. Most chemists in the study area were not run by a pharmacist. The cost of AL at the chemist is KSH. 100 but the cost of other antimalarial drugs varies depending on the dosage.

Potential covariates to treatment seeking behavior such as easy access to hospital, ability to pay hospital bills, the nature of symptoms (severe or mild), age group, whether the house was sprayed (IRS), knowledge of malaria symptoms, region of residence (irrigated or non-irrigated), household head level of education and use of a bed net were considered and included in a predictive model. Assessment of knowledge of malaria symptoms was based on the ability of the household head to link fever or headache to malaria.

5.3.3 Data analysis

The data were coded, entered in Excel sheet, and analyzed using GMP Pro 16. Demographic characteristic of participants, knowledge, attitude and practice towards malaria were summarized

using descriptive statistics. Differences in proportions were compared using either Fisher's exact test or Chi-square test. To assess treatment-seeking behavior, we first used descriptive analysis to determine the difference among those who sought treatment at health facility versus those who used antimalarial drugs bought from the chemist or those who did not seek treatment to manage the fever. We then used multiple logistic regression to identify the factors that influence treatment-seeking behavior.

5.4 Results

5.4.1 Socio-demographic characteristics of study participants in household survey

Two hundred and forty (240) households with a total population of 1,142 people (574 from irrigated and 568 from non-irrigated areas) were visited. Children under the age of 5 constituted 20.2% (231/1142), followed by children aged 5 to 14 years (32.9%, 376/1142), and those aged 15 years and older constituted 46.8 (535/1142). The mean household size was 4.8 people, with a range of 1-11individuals per household. The majority of the respondents were females (76.3%, n =183) as indicated in table 5.2. Most of the respondents (58.8%, n = 141) had a primary education, while the least (4.2%, n = 10) had a college education. The overall bed net ownership by study participants was 93.3% (224/240). Irrigated area had significantly higher bed net ownership of 98.3% compared to that of non-irrigated area (88.3%) (Fisher's exact test = 0.003). The proportion of households having 1 LLIN for two people was 37.5% (45/120) in irrigated compared to 24.2% (29/120) in non-irrigated area (χ^2= 5.0, df = 1, p = 0.03). Bed net usage survey indicated that in most households (83.3% in irrigated and 73.7% in non-irrigated area), all family members used bed net. Although bed net ownership and usage was above average in the study area, the majority of the households (59.2% in irrigated and 57.5% in non-irrigated areas) had nets which were torn.

Indoor residual spray coverage was at 66.7% (80/120) in irrigated and 69.2% (83/120) in non-irrigated areas.

Table 5.1 Demographic information of the households visited and the household heads

Variables	Irrigated (n =120) n (%)	Non-irrigated (n = 120) n (%)	p-value
Sex of respondent			
Male	26 (21.7)	31 (21.8)	0.5
Female	94 (78.3)	89 (74.2)	
Education			
No education	14 (11.7)	10 (8.3)	0.5
Primary	61 (50.8)	80 (66.7)	0.02
Secondary	36 (30.0)	29 (24.2)	0.38
College	9 (7.5)	1 (0.8)	0.019
Bed net ownership	118 (98.3)	106 (88.3)	0.003
All family members sleep under bed net?			
Yes	100 (83.3)	88 (73.7)	0.08
No	20 (16.7)	32 (26.7)	
1 LLIN for 2 people	45 (37.5)	29 (24.2)	0.03
IRS in the last 6 months?			
Yes	80 (66.7)	83 (69.2)	0.8
No	40 (33.3)	37 (55.8)	

5.4.2 Monthly clinical malaria and diagnosis routine at the healthcare facilities

The majority of public healthcare facilities 70% (7/10) in the study area are dispensaries which are headed by clinical officers and provide only outpatient services. Health centers and a hospital which are headed by at least one doctor and provide inpatient services accounts for 20% (2/10) and 10% (1/10) respectively. A total of 1264 suspected malaria cases in the 10 healthcare facilities were recorded during a period of 3 months. Out of the 1264 cases, 76.7% (972/1264) were diagnosed by microscopy, 4.2% (53/1264) by RDT kits while 18.9% (239/1264) were clinically diagnosed. The overall clinical malaria cases were 19.7% (65/330), 18.2% (109/600), and 21% (70/334) during October, November and December respectively as illustrated in table 5.3

Table 5.2 Monthly suspected and confirmed malaria cases from the 10 selected health facilities within the study area

	October 2019		November 2019		December 2019	
Type of diagnosis	Suspected malaria	Positive n (%)	Suspected malaria	Positive n (%)	Suspected malaria	Positive n (%)
Microscopy	241	39 (16.2)	466	70 (15.0)	265	47 (17.7)
RDT	27	3 (11.1)	21	5 (23.8)	5	0 (0)
Clinical	62	23 (38.0)	113	34 (30.1)	64	23 (35.6)

During the three months of the study, three dispensaries (Nyarut, Omboga, and Kodula), did not have RDT kits and thus relied on clinical symptoms to confirm suspected malaria cases. The number of cases confirmed by clinical diagnosis was always greater than 30% during the 3 months of the study. Microscopy confirmed cases, however, accounted for less than 20% of all cases during the same time period. For example, during the month of October 2019, the proportion of suspected malaria which were confirmed by microscopy was 16.2% (39/241) compared 38% (23/62) clinically confirmed cases ($p = 0.0005$). During the month of November 2019, the proportion of microscopy confirmed cases was 15% (70/466) compared to 30.1% (34/113) cases confirmed clinically ($p = 0.0003$). Similarly, during the month of December 2019, cases confirmed by microscopy were 17.7% (47/265) while those confirmed clinically were 35.6% (23/64) ($p = 0.0025$) (Table 5.4).

Table 5.3 Comparison between Slide positivity rate (SPR) and clinically confirmed cases at health care systems

	October 2019		November 2019		December 2019	
	Confirmed cases n (%)	p-value	Confirmed cases n (%)	p-value	Confirmed cases n (%)	p-value
SPR	39 (16.2)	0.0005	70 (15.0)	0.0003	47 (17.7)	0.0025
Clinical	23 (38.0)		34 (30.1)		23 (35.6)	

5.4.3 Knowledge, attitude and practice of household heads towards malaria

Table 5.5 summarizes the knowledge, attitude and practice of household heads towards malaria. The majority of household heads (77%), indicated that malaria is transmitted by the bite of any mosquito. Other means of malaria transmission mentioned included the bite of infected mosquito (11.3%), and contaminated water (5.4%). About 8.3% however, indicated that they did not know how malaria is transmitted. With regards to malaria symptoms, the following symptoms were identified by the household heads; fever 177 (73.8%), headache 171 (71.3%), joint pains 120 (50%) and vomiting 95 (39.6%). Children under the age of 5 years, were mentioned as the most vulnerable 174 (72.5%) group. Other vulnerable groups included, pregnant women 108 (45%), the elderly 50 (20.8%), everyone 63 (26.3%), and older children 4 (1.7%). The majority of participants 288 (95%) indicated that the use of treated bed-nets was the best way to control malaria. Other control methods mentioned included: mosquito coils/ repellants 47 (19.6%), indoor residual spray 44 (18.3%), chemoprophylaxis 32 (13.3%), and keeping food clean 22 (9.2%).

The knowledge about mosquito breeding habitat was assessed and the findings indicate that 95% (228/240) of the study participants were aware that stagnant water is the most suitable breeding place of mosquitos. The other breeding place mentioned was bushes at 66.7% (166/240). Attitude towards malaria was also assessed and 95.4% (229/240) of the participants indicated that malaria was a serious disease while 4.6% (11/240) mentioned that malaria is a mild disease. Approximately 93.8% (225/240) indicated that it was very important to follow malaria treatment prescription given by the doctor, while 6.2% (15/240) indicated that it was not important.

Table 5.4 Knowledge, attitude, and practice towards malaria

Characteristics	Frequency (%)
How can malaria be transmitted (n= 240)	
Bite of any mosquito	185 (77)
Bite of infected mosquito	27 (11.3)
Contaminated water	13 (5.4)
Do not know	20 (8.3)
Malaria symptoms known (n= 240)	
Fever	177 (73.8)
Headache	171 (71.3)
Joint pains	120 (50)
Vomiting	95 (39.6)
Chills	19 (7.9)
Loss of appetite	15 (6.3)
Common cold	44 (18.3)
Do not know	2 (0.8)
Who are most vulnerable towards malaria (n = 240)	
Everyone	63 (26.3)
Children below 5 years old	174 (72.5)
Pregnant women	108 (45)
Children above 5 years	4 (1.7)
Adults	50 (20.8)
Don't know	8 (3.3)
Malaria control methods (n = 240)	
Insecticide treated nets	228 (95)
Indoor residual spray	44 (18.3)
Mosquito coils/ repellants	47 (19.6)
Window screen	4 (1.7)
Chemoprophylaxis	32 (13.3)
Avoid playing in the cold	14 (5.8)
Do not know	6 (2.5)
Keeping food clean	22 (9.2)
Breeding places of mosquito (n = 240	
Stagnant water	228 (95)
Bushes	160 (66.7)
Do not know	8 (3.3)
How serious is malaria to human health (n = 240)	
Very serious	229 (95.4)
Mild	11 (4.6)

5.4.4 Treatment-seeking pattern among study participants

Treatment-seeking behavior among fever patients is described in table 5.6. Out of the 240 households visited, 44% (106/240) households reported to have had fever cases in the previous 2 weeks. The total number of people who experienced fever cases were 15.1% (173/1142). The irrigated areas had a significantly higher number of fever patients (17.6%, n = 101) compared to that of non-irrigated (12.8%, n = 72) ($\chi^2 = 5.0$, df = 1, p = 0.03). Among these 173 individuals, 37.0% (n = 64) sought treatment at the health facility, 50.3% (n = 87) used antimalarial drugs bought from the chemist while 12.7% (n = 22) did nothing to manage the fever. The proportion of individuals seeking treatment at a health facility for fever cases were more common in irrigated (45.5%, n = 46) than in non-irrigated areas (25%, n = 18) ($\chi^2 = 6.8$, df = 1, p = 0.009) as shown in table 5.6. Malaria was confirmed in 65.6% (42/64) of the febrile patients who sought treatment at healthcare facilities. Due to drug stock-outs in some healthcare facilities, 87.7% (30/42) of the malaria-confirmed patients were directed to purchase antimalarial drugs from a chemist. In non-irrigated areas, most fever patients who did not seek treatment at healthcare facilities, bought drugs at the local chemists. For instance, pharmacy-purchased medications were used by 59.7% (43/72) of fever patients in non-irrigated areas compared to 43.6% (44/101) of fever patients in irrigated ($\chi^2 = 3.8$, df = 1, p = 0.05). Similarly, the proportion of those who did nothing or waited to get better when they experienced fever was 10.9% in irrigated and 15.3% in non-irrigated areas ($\chi^2 = 0.4$, df = 1, p = 0.5).

Treatment seeking behavior differed significantly among age groups. The majority of children aged < 5 years (77.7%) were taken to a health facility for treatment compared to 31.4% and 20.9% of children aged 5-14 years old and those aged ≥15 years respectively ($\chi^2 = 37.07$, df = 2, p < 0.0001). However, use of antimalarial drugs purchased from a chemist for fever-related cases was

highest among adults (59.7%), followed by 58.6% of children aged 5-14, and lowest among children under the age of 5 (16.7%) ($\chi^2 = 20.6$, df = 2, p <0.0001). Approximately 5.6%, 10% and 19.4% of children under the age of 5, children aged 5-14 and those aged ≥15 years old, respectively, did not receive any form of treatment and instead their caregivers just waited for the fever to subside ($\chi^2 = 4.8$, df = 2, p = 0.09) .

Table 5. 5 Malaria treatment seeking behavior (n = 173)

	Hospital	p-value	Chemist	p-value	Do nothing	p-value
Region						
Irrigated	46/101 (45.5%)	0.009	44/101 (43.6%)	0.05	11/101 (10.9%)	0.5
Non-irrigated	18/72 (25%)		43/72 (59.7%)		11/72 (15.3%)	
Age group						
<5 yrs.	28/36 (77.7%)	< 0.0001	6/36 (16.7%)	< 0.0001	2/36 (5.6%)	0.09
5-14 yrs.	22/70 (31.4%)		41/70 (58.6)		7/70 (10%)	
≥ 15 yrs.	14/67 (20.9%)		40/67 (59.7%)		13/67 (19.4%)	

5.4.5 Factors related to treatment-seeking

Access to a health facility was one of the factors that influenced participants' decision to seek treatment at a health facility. Those who had easy access to a hospital were more likely to seek treatment there (OR = 16.23, p = 0.002) as compared to those who had a challenge accessing the health facility as indicated in table 5.7. Moreover, hospital costs, such as the cost of laboratory tests, influenced whether or not the patient sought treatment at the hospital. For example, those who indicated that paying medical bills was not a challenge to them, were more likely to seek treatment at the health facility (OR = 10.6, p = 0.006) than those who could not afford such costs. Those who experienced severe symptoms in the previous 2 weeks were more likely to seek treatment at the hospital (OR = 7.5, p = 0.037) than those who had mild symptoms. Care givers of

under 5-year-old fever patients were also more likely to take their children to health facility for treatment (OR = 17.8, p = 0.008) than those caregivers of older children and adults.

With regard to use of antimalarial drugs bought at the chemist, family members from houses which were not sprayed (IRS), were more likely to buy antimalarial drugs from the chemist (OR = 3.14, p = 0.025) than those from sprayed households. Study participants who experienced mild fever or headache, were more likely to buy antimalarial drugs from the chemist (OR = 10.09, p = 0.001) than those who experienced severe fever or headache. Knowledge of malaria symptoms also played key role towards buying of antimalarial. Those who believed that fever or headache was due to malaria, were more likely to buy drugs at the chemist (OR = 5.72, p = 0.002) than to go to hospital for treatment. Caregivers of older children or adults were 4 times more likely to buy antimalarial drugs at the chemist (OR = 4.82, p = 0.03) than seeking treatment at the hospital.

Table 5. 6 Nominal logistic fit for treatment seeking behavior

Hospital treatment			
Variable	Level	Odds ratio 95% CI	p-value
Easy access to hospital	Yes	16.23 (2.74-96.12)	0.002
	No	1.00	-
Ability to pay hospital bills	Yes	10.60 (1.97-57.1)	0.006
	No	1.00	-
Severe symptoms	Yes	7.55 (1.12-50.80)	0.037
	No	1.00	-
Age group	< 5yrs	17.81 (2.12-149.7)	0.008
	≥5 yrs.	1.00	-
Buy drugs at the chemist			
House sprayed (IRS)	Yes	1.00	-
	No	3.14 (1.15-8.57)	0.025
Mild symptoms	Yes	12.09 (2.70-54.24)	0.001
	No	1.00	-
Knowledge of malaria symptoms	Yes	5.72 (1.87-17.49)	0.002
	No	1.00	-
Age group	<5yrs	1.00	-
	≥5yrs	4.82 (1.19-19.61	0.03

1 = Reference value

5.5 Discussion

This study assessed the rural population's knowledge, attitude, and practice regarding malaria, as well as treatment-seeking behavior and predictors of treatment-seeking in malaria endemic zone. Malaria knowledge and appropriate treatment seeking behavior are critical components of malaria clinical management (Hasabo *et al.*, 2022). Our findings revealed a community that is well-versed in malaria transmission, symptoms, and control methods.

Despite widespread knowledge of malaria control methods, study's findings revealed that only a small percentage of households met the requirement of "one LLIN for every two people". A minimum coverage of 60% is required for LLINs to provide optimal protection (Killeen *et al.*, 2017). The present study's 30.8% universal coverage ownership of LLINs was lower than the coverage (45%) recorded right after the completion of the 2017 mass distribution of nets. (MoH, 2019a). The survey was done three years after the previous mass distribution, thus most nets must have worn out and stopped being used, lowering the proportion of persons who had nets. Furthermore, the majority of nets surveyed had holes, decreasing nets' ability to offer effective protection.

The findings further indicated that the majority of participants consider malaria to be a serious disease, acknowledge stagnant water as the suitable breeding place for mosquitoes, and are aware of the groups of people who are most vulnerable to malaria. This high level of knowledge in malaria disease may be attributed to the community's location in a malaria endemic region where the disease is common, and also the fact that the majority of the respondents (90%) had at least a primary education. This is comparable to other studies conducted in southeastern Iran (Ahmed *et al.,* 2009), in Mozambique (de Sousa Pinto *et al.*, 2021), Northwestern Ethiopia (Flatie *et al.*, 2021), and Bangladesh (Saha *et al.*, 2019).

According to the study findings, 44% of the total households surveyed had at least one fever patient. More than a half of these febrile cases were treated with antimalarial drugs obtained from a local chemist. This is similar to studies in Indonesia (Guntur *et al.*, 2022), Ghana (Awuah *et al.*, 2018), India (M. P. Singh *et al.*, 2017), Myanmar (Thandar *et al.*, 2015), and Bangladesh (Ahmed *et al.*, 2009) where majority of febrile patients reportedly preferred self-medication over hospital-based treatment. In the current study, key predictors of self-treatment included knowledge of malaria symptoms, severity of symptoms, and age of febrile patients. The study area being a malaria endemic region, the majority of people are frequently exposed to *Plasmodium* infections. As a result, many participants who were familiar with malaria symptoms, were more likely to delay or fail to seek treatment at a health facility when fever developed. Similarly, some studies conducted in Ethiopia (Hwang *et al.*, 2010; Tesfahunegn *et al.*, 2019) reported possible delays or failure to seek malaria treatment at a health facility due to knowledge of malaria symptoms. However, the current study findings, contradicted previous research that found that knowledge about malaria increased the likelihood of early treatment-seeking in a health facility (Mitiku *et al.*, 2017; Workineh *et al.*, 2018). The study further demonstrated that patients with mild symptoms were more likely to buy drugs from a chemist or do nothing. In all regions where malaria transmission is perennial, most people, particularly older children and adults, may be asymptomatic or exhibit mild clinical symptoms of malaria due to acquired immunity to the disease (Badiane *et al.*, 2021; McQueen *et al.*, 2013; White, 2017). Therefore, people with mild malaria symptoms may delay or avoid seeking treatment at the hospital as previously reported (Iwelunmor *et al.*, 2010).

Access to healthcare facilities, ability to pay hospital bills, severity of symptoms, and age of fever patients were factors that predicted treatment-seeking at healthcare facilities. The ease of access to healthcare facilities has been reported in previous studies to encourage treatment-seeking at healthcare facilities (Guntur *et al.*, 2022; Kizito *et al.*, 2012; Musoke *et al.*, 2014; Xu *et al.*, 2012). Easily accessible health facilities may not require transportation or may require only a small amount of money; as a result, the majority of people find it easy to access them. Similarly, unavailability of money to pay for hospital bills such as laboratory tests charges discouraged some participants to seek treatment at these public healthcare facilities. Financial constraints have been reported in previous studies to have a significant impact on treatment-seeking at healthcare facilities (M. P. Singh *et al.*, 2017; Suswardany *et al.*, 2015).

The study's findings also demonstrated that young children with fevers, were more likely to be taken to the healthcare facility by their caregivers than older children or adults. This could be due to the majority of respondents being aware that children under the age of five are more vulnerable to malaria than older children or adults, as previously reported (Adedokun *et al.*, 2020; Cassy *et al.*, 2022). In the current study, most participants indicated that malaria is a serious disease. This could be the most plausible explanation for why the majority of people with severe symptoms sought treatment at healthcare facilities. This is comparable to a study in Cameroon in which people could only visit healthcare facilities if they experienced severe symptoms (Makoge *et al.*, 2017).

According to survey findings, some dispensaries were resource constrained. Chronic challenges that plagued the day-to-day operations of most of these dispensaries included a lack of well-equipped laboratories, RDT kits stock-outs, and antimalarial drug shortages. These challenges if not addressed, may have an impact on treatment seeking behavior. For example, patients who are

referred to some distant healthcare facilities for proper checkups may resort to buying drugs at local chemists due to a lack of funds for transportation. Similarly, drug shortages in healthcare facilities are likely to hamper effective treatment seeking as reported in previous studies (Chuma *et al.*, 2010). For instance, in irrigated areas, the majority of fever patients sought treatment at healthcare facilities compared to fever patients in non-irrigated areas. This could be due to a combination of factors, including limited access to healthcare facilities and the types of services provided at those facilities. For example, in irrigated areas, the majority of people have easy access to healthcare, as opposed to non-irrigated areas. Secondly, as opposed to non-irrigated areas, most healthcare facilities in the irrigated areas had well-established laboratories for conducting quality malaria diagnosis. These two factors may influence the treatment seeking behavior of local residents (Guntur *et al.*, 2022; Hasabo *et al.*, 2022). Another plausible explanation could be income disparities between irrigated and non-irrigated households. A recent study conducted by Omondi, Ochwedo, *et al.* (2022) in the same study area, discovered that household income in irrigated areas was higher than in non-irrigated areas.

World Health Organization recommends that all suspected malaria cases be confirmed through microscopy or the use of rapid diagnostic test kits before antimalarial drugs are administered (WHO, 2015a). Particularly in malaria-endemic areas, where the majority of febrile illnesses may not be caused by malaria (D'Acremont *et al.*, 2010; Kapito-Tembo *et al.*, 2020). Although we did not re-examine to confirm the accuracy of clinically diagnosed cases, it was apparent that dispensaries that relied on clinical diagnosis reported a significantly higher number of malaria cases than those that used microscopy. This raises concerns about over-diagnosis of malaria cases, which could lead to indiscriminate use of antimalarial drugs or non-treatment of other illnesses

with similar symptoms to malaria (Mwangi *et al.*, 2005; Parsel *et al.*, 2017; Rakotonirina *et al.*, 2008).

5.6 Conclusion

The study findings indicate that most people from this region have good knowledge about malaria. However, the majority of fever patients self-treat with drugs purchased from local chemists. Access to healthcare facilities, knowledge of malaria symptoms, age of febrile patients, severity of symptoms, and income were all likely factors influencing treatment seeking behavior. Antimalarial drugs and RDT kits stock outs were common challenges to some dispensaries. The Ministry of Health should consider launching a community-based awareness campaign to educate local population about the importance of seeking medical attention at a healthcare facility. The ministry of health should also consider improving access to healthcare facilities as well as quality services in terms of diagnosis and drug availability.

CHAPTER 6

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

Western Kenya's lake region faces numerous challenges, including malaria, anemia, and unreliable rainfall, which leads to food insecurity. The Kenyan government, in collaboration with development partners, is committed to reducing malaria through an annual indoor residual spray program and increasing food security and household income in Homa Bay County through the construction of irrigation schemes. Despite the fact that malaria cases were reported to be higher in irrigated areas than in non-irrigated areas, the annual indoor residual spray program significantly reduced malaria parasite prevalence in general. Malaria prevalence in the study area has always been greater than 20% (Bashir *et al.*, 2019; MoH, 2016b), but after two annual rounds of IRS, parasite prevalence by microscopy was less than 5%, lowering the county's malaria burden.

In contrast to malaria, anemia cases were identified as a serious public health issue in the study area. Anemia was reported to be more prevalent in non-irrigated areas than in irrigated areas. The findings of the study revealed no link between *Plasmodium* infections and anemia. This is in contrast to previous research from malaria-endemic areas, which found a link between malaria and anemia (M. R. Desai *et al.*, 2005; Foote *et al.*, 2013). This could be due to the region's tenacious fight against malaria, which significantly reduced both *Plasmodium* infections and parasite density, resulting in no association. However, study findings indicated that household income and diet were two of the factors associated with anemia.

The study participants' knowledge of malaria was excellent. Participants recognize malaria as a serious disease, particularly among children under the age of five and pregnant women. The most widely used malaria control method was the use of a bed net. Despite having a good understanding

of malaria transmission pathways, control methods, and clinical symptoms, the study findings revealed that the majority of the local population still purchases antimalarial drugs from chemists to treat malaria-related fevers. Minor children with fever, on the other hand, were mostly taken to the hospital for examination and treatment because they were considered a vulnerable group. The decision to self-treat or seek treatment at a healthcare facility when suffering from a fever was influenced by hospital accessibility, knowledge of malaria symptoms, severity of fever or other related symptoms such as headache, and ability to pay malaria diagnosis charges. The lack of antimalarial drugs in the majority of healthcare facilities is a potential factor that may have contributed to self-treatment by the local population. RDT stock-outs in some dispensaries resulted in clinical diagnosis of malaria, which may have discouraged participants from seeking treatment in such dispensaries and thus purchasing antimalarial drugs from chemists. Public awareness should be considered to educate the public on the benefits of seeking treatment at a hospital.

6.2 Limitations of the study

One of the study's limitations was its inability to investigate the morphological appearance of red blood cells. This made it impossible to distinguish between anemia caused by *Plasmodium* and other forms of anemia such as iron deficiency anemia, sickle cell anemia, and anemia caused by other parasitic infections.

Due to the cross-sectional nature of the study, determining the causal relationships between anemia and independent variables was also difficult.

In ability to use specific tools like the Likert scale to measure attitudes. The study instead used structured questionnaire to collect such information.

6.3 Conclusions

The survey sought to ascertain the likely effects of a recently constructed irrigation scheme on the malaria burden among local residents. The rate of *Plasmodium* species infection, *Plasmodium falciparum* parasite density, and anemia prevalence were compared between residents living within irrigation scheme and those living outside the irrigation scheme. The findings showed that irrigated areas had higher *Plasmodium* infection rates than non-irrigated areas, implying that this irrigation scheme contributes to malaria transmission. However, the current malaria interventions in the study area, have significantly reduced malaria cases in both irrigated and non-irrigated areas. Children between the ages of 5 and 14 were more susceptible to contracting malaria than infants. In addition, the research revealed that parasite density was comparable in irrigated and non-irrigated areas.

Anemia is a major health problem among the population under the study, with residents from non-irrigated areas being more likely to become anemic than residents from irrigated areas. Residents in irrigated areas were more likely to afford a balanced diet than residents in non-irrigated areas due to increased income from agricultural produce. This could have contributed to observed low rates of anemia in irrigated areas.

Following the implementation of the indoor residual spray program, there was a significant reduction in malaria parasite infection within the study area. In contrast, the relative proportion of asymptomatic or submicroscopic infections increased with each subsequent cross-sectional survey.

The general knowledge of malaria among study participants, as well as their attitude and practice, were good. However, the majority of fever patients prefer self-treatment to hospital-based treatment. Access to health care, ability to pay hospital bills, knowledge of malaria symptoms, and

age of fever patients were some of the significant factors that influenced treatment seeking behavior. The most common challenges faced by some of the healthcare facilities in the study area were drug and RDT stock outs.

6.4 Recommendations

1. Repairing leaking canal pathway sections and clearing blocked sections on a regular basis, can reduce pools of water suitable for mosquito breeding grounds.
2. Extending the irrigation scheme to non-irrigated areas to boost food security and increasing public awareness of iron-rich food consumption are required to manage anemia rates in the study area.
3. More detailed anemia studies are needed to accurately identify anemia causes, which is critical for guiding control interventions.
4. To reduce silent infections that maintain transmission, it is necessary to consider mass identification and administration of drugs to treat asymptomatic *Plasmodium* infected individuals.
5. There is need to strengthen healthcare system by providing adequate RDTs and drugs for accurate malaria diagnosis and prompt treatment.
6. Access to healthcare facilities is critical for malaria management and should be prioritized as an investment.
7. The Ministry of Health should consider launching a community-based awareness campaign to educate residents about the importance of seeking medical attention at a healthcare facility.

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APPENDIX 1: CONSENT/ASSENT FORM

Effects of irrigation scheme on malaria burden in Homa Bay county, western Kenya

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

Name of Volunteer:_____

Age of Volunteer:_____

Principal Investigator: Collince Jared Omondi**. Supervisors:** David Odongo, Antony Otieno and Andrew Githeko**. Contact address:** P.O. Box 30197-00100 GPO, Nairobi, Kenya. Tel +254759445642

The purpose of the study

The purpose of the study is to determine the probable effect of a newly constructed irrigation scheme on *Plasmodium* species infections, *Plasmodium falciparum* parasite density and anemia among native residents.

Procedures involved

Approximately 150 µl of blood will be collected by finger prick. Approximately 50 µl of blood will be used to prepare thick and thin smears for microscopy examination, 50 µl of blood will be used to prepare dry blood spot on filter paper for qPCR examination while about 20 µl will be drawn into the optical window of the micro-cuvette to measure hemoglobin levels using Hemo Cue HB 201+ analyzer (Hemo Cue, Angelholm, Sweden). All study participants will have a unique identifier number that links the participants with his/her laboratory test results, demography, and location. Microscopy and qPCR examination of samples will be done at the International Center of Excellence for Malaria Research laboratory located at Tom Mboya University in Homa Bay county, Kenya. Analysis of hemoghlobin levels will be done at the houses of study participants.

Additionally, we will ask the household head some questions about whether in the past two weeks any member of the household has had malaria, treatment seeking behavior (hospital, buying antimalarial drugs from the chemist, or no treatment), knowledge, attitude and control practices towards malaria. We will also seek to know about the household income, and food intake in the previous one week.

Inclusion criteria

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

Exclusion criteria

We will exclude residents who are unwilling to participate in the study or those participants who are likely to relocate from the study area before the study ends.

Discomforts and risks

The finger-prick blood collection method causes slight discomfort. Sterile blood lancet (followed with sterile ethanol) will be used for every single participant, the procedure will cause very minimal risk of being infected by other pathogens.

Benefits to participants

There will be no financial benefit due to your participation. However, in case of fever during sample collection periods, we will refer you to the nearest healthcare facility for further tests and treatment.

Confidentiality

Information related to you will be treated in strict confidence to the extent provided by the law. Your identity will be coded and will not be associated with any published findings. Your code number and identity will be safely kept in the principal investigator's lock.

Freedom to withdraw

Your participation in this study is voluntary and you may discontinue your participation at any time without prejudice and your withdrawal will not affect your future health care

Who to contact?

If you have questions about the study or your participation in this study, you may contact the investigators on the contact given above. For any questions pertaining to rights as a research participant, contact person is: **The Secretary, Maseno University Ethics Review Committee, Private Bag, Maseno; Telephone numbers: 057-51622, 0722203411, 0721543976, 0733230878; Email address:** muerc-secretariate@maseno.ac.ke; muerc-secretariate@gmail.com

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I have read and understand this consent form, and I am willing to participate in the study.

Participant's Name	Participant's Signature (consent)	Date
Parent/Guardian's Name	Parent/Guardian's Signature (Assent)	Date

Investigator's Name (type or print)	Investigator's Signature	Date

APPENDIX 2: QUESTIONNAIRE

1. Questionnaire for asymptomatic *Plasmodium* infection study

Date……

Cluster No……House No…… Study NO……

Participant Name……

Sex…… Age……

Test and health conditions

1. Blood specimen collected (µl)…….

2. a) Are you feeling sick now?

No ( ) Yes ( )

b) If yes, do you have any of these symptoms?

Fever ( ) Chills ( ) Headache ( ) Vomiting ( )

Other……

3. General health condition

a) Normal ( ) (b) Fair ( ) (c) Severe ( )

2. Questionnaire for anemia study

Sociodemographic survey: Anemia and nutrition

1. Sex: 1= (Male) 2= (Female) Age: _____
   - Total household members: Below 5 ___, 5-14 ____, ≥15 _____
   - Education level for the father: 1= (primary) 2= (secondary) 3= (college) 4= (no education)
   - Education level for the mother: 1= (primary) 2= (secondary) 3= (college) 4= (no education)
2. Your source of income: Select all that applies

   1= (Formal employment) 2= (Casual employment/ daily laborer)

   3= (Business) 4= (Farming) 5= (Fishing)

   6= (Housewife) 7= (Handcraft) 8= (None)
3. Do you have daily or monthly income? 1= (Daily) 2= (Monthly)
   - If daily, averagely how much does your household earn per day?

     1= (Less than 100) 2= (101-300) 3= (301-500) 4= (Above 500)
   - If monthly, how much do you and everyone in this household earn per month?

     1= (Less than 10000) 2= (10000-20000) 3= (above 20000)
4. Do you know a condition known as anemia? 1= (Yes) 2= (No)
   - If yes, how can you recognize someone who is anemic?

     1= (Paleness/Pallor) 2= (Weakness/less energy)

     3= (More likely to become sick) 4= (don't know)
5. How serious do you think anemia is?

1= (Not serious) 2= (Very serious) 3= (Don't know)

6. What are some possible ways to prevent anemia?

1= (Eat iron-rich foods) 2= (Take vitamin C rich foods)

3= (Take iron supplements) 4= (Seek treatment)

5= (Continue breastfeeding (infants 6-23 months old) 6= (Don't know)

7. Food intake practice (Iron rich foods)

How frequent did you eat these foods in the past one week?

Beans rice, wheat? 1= (Daily) 2= (4-6 times) 3= (1-3 times) 4= (Never)

Pumpkins or carrot? 1= (Daily) 2= (4-6 times) 3= (1-3 times) 4= (Never)

Spinach or kales? 1= (Daily) 2= (4-6 times) 3= (1-3 times) 4= (Never)

Potato? 1= (Daily) 2= (4-6 times) 3= (1-3 times) 4= (Never)

Sorghum/millet 1= (Daily) 2= (4-6 times) 3= (1-3 times) 4= (Never)

Beef, chicken or fish 1= (Daily) 2= (4-6 times) 3= (1-3 times) 4= (Never)

Vitamin C and B rich fruits

Citrus fruits 1= (Daily) 2= (4-6 times) 3= (1-3 times) 4= (Never)

Melon, papaya or orange 1= (Daily) 2= (4-6 times) 3= (1-3 times) 4= (Never)

8., How many times did you take cow milk in the last 7 days?

1= (Everyday) 2= (4-6 times) 3= (1-3 times) 4= (Never)

Farm produce

1. What type of crops do you grow? ___ Maize; ___ Sweet potato; ___ Beans; ___ Others (_____)

2. Which types of vegetables do you grow?

   ___ Pumpkins, ___ Kales, ___ Spinach, ___ Tomatoes, ___ Others (_____), ____ None_____

3. What type of fruit do you grow?

   ___ Banana; ___ Mango; ___ Avocado; ___ Orange; ___ Passion; ___ Others (_____)

3. Questionnaire for KAP and treatment seeking study

Malaria KAP survey for household head

Interviewer……...Date of interview…….

1. Sociodemographic characteristics of the Participants

Study NO……Phone NO……...Gender……Age…….

1.1 How far is your house from irrigation scheme? 1= (<200 m=Irrigated) 2= (>200 m =Non-irrigated)

1.2 If < 200m, are there challenges posed by the scheme towards malaria transmission?

1= (Yes) 2= (No)

1.3 If yes, which ones? 1= (Over flow) 2= (Water spillage) 3= (Blockage of canals)

1.4 Are there ways to mitigate these challenges? 1= (Yes) 2= (No)

1.5 If yes, can you name them? 1= (Control of released water) 2= (Clearing the blocked canals) 3= (repair of worn out canals)

1.6 How many people live in this household?

1.7 What is your level of education?

1= (No education) 2= (Primary) 3= (Secondary) 4= (College/ University)

1.8 Which animals do you own?1= (Cows) 2= (chicken) 3= (Sheep) 4= (Pigs) 5= (Goats) 6= (Others) 7= (None)

1.9. What is the main wall material?

1= (Mud) (Iron sheet) 2= (Sand and cement) 3= (Blocks)

1.10. Roof material?

1= (Iron sheet) 2= (Grass) 3= (Other, specify)

2. Knowledge about malaria and control strategies

2.1. What is the mode of malaria transmission?

1= (Bite of any mosquito) 2= (Bite of infected mosquito) 3= (Contaminated water) 4= (other …specify) 5= (Do not know)

2.2. What causes malaria?

1= (Mosquitos) 2= (*Plasmodium* species) 3= (Dirty water) 4= (Cold/wet weather)

5= (Other…specify) 6= (Do not know)

2.3. Which malaria symptoms do you know?

1= (Fever) 2= (Headache) 3= (Joint pains) 4= (Vomiting)

5= (Chills) 6= (Loss of appetite) 7= (Do not know) 8= (Common cold)

2.4. Who are most vulnerable towards malaria?

1= (Everyone) 2= (Below 5 years) 3= (Pregnant women)

4= (Children above 5 years) 5= (Adults) 6= (Do not know)

2.5. Which malaria control methods do you know?

1= (Insecticide treated bed nets) 2= (IRS) 3= (Insecticide sprays)

4= (Mosquito coils/repellants) 5= (Door/window screen) 6= (Chemoprophylaxis)

7= (Avoid playing in the cold) 8= (Do not know) 9= (Keeping food clean)

2.6. Which breeding habitats of mosquito do you know?

1= (Stagnant water) 2= (Bushes) 3= (Garbage) 4= (Other specify)

5= (Do not know)

2.7. In which places are you likely to find mosquito resting?

1= (Dark places in the house) 2= (at the edge of stream) 6= (Heaves)

3= (Bushes near the houses) 4= (Dirty areas) 5= (Containers with water)

5= (Under the bed) 6= (Don't know) 7= (On the walls/corners of the house)

2.8. How can you control breeding of mosquito within your compound?

1= (Cutting bushes around the compound) 2= (Draining all Stagnant water within the compound) 3= (Insecticide spray) 4= (Do not know) 5= (Keeping house clean)

3. Attitude towards malaria, control methods and treatment

3.1 How serious do you think malaria is to your health?	1 Very serious	[ ]
	2 Mild	[ ]

	3 Not serious	[ ]
	4 Don't know	[ ]
3.2 In your opinion, how effective are mosquito nets towards malaria prevention?	1 Moderately effective	[ ]
	2 Most effective	[ ]
	3 Not effective	[ ]
	4 Don't know	[ ]
3.3 How effective is IRS towards malaria control**?**	1 Moderately effective	[ ]
	2 Most effective	[ ]
	3 Not effective	[ ]
	4 Don't know	[ ]
3.4 How important is it to follow malaria treatment prescription given by the doctor?	1. Very important	[ ]
	2. Not important	[ ]
	3. Don't know	[ ]

4. Practices in malaria control and treatment

4.1 How many live in this household

Age	Female	Male
< 5 years old		
5 – 14 years old		
≥ 15 years old		

4.2. Do you have insecticide treated bed net? 1= (Yes) 2=(No)

One [ ]

4.3. How many insecticides treated bed net do this household own?	Two	[ ]
	Three	[ ]
	More than three	[ ]

4.4. Does every member of this household sleep under treated bed nets?

1= (Yes) 2= (No)

4.4.1. If no, which members of this household mostly sleep under treated bed net?	1. Children < 5yrs	[ ]
	2. 5-14 yrs.	[ ]
	3. Parent + children < 5 years	[ ]
	4. Only parents	[ ]

4.5. Where do you source for the bed nets?

1= (Supplied by the MOH) 2= (Purchased) 3= (Any other)

4.6. If supplied by MOH, when was the last time nets were supplied?	1. Less than 3 months	[ ]
	2. Six months ago	[ ]
	3. One year ago	[ ]
	4. More than 2 years	[ ]

4.6.1 Are the nets in good conditions 1= (Yes) 2= (No) (NB: Physical examination of nets)

4.6.2 If no, Indicate the number of holes ……

4.6.2.1 What is the size of hole/holes?

1= smaller than the thumb ( ) 2= bigger than the thumb ( ) 3= bigger than the fist ( )

4.7. Are you completely protected from mosquito bites by the available ITNs?

1= (Yes) 2= (No)

4.7.1 If no, what are the challenges?	1. Mosquito bites before going to bed	[ ]

2. Mosquito bites through the treated bed net [ ]

3. Mosquito bites through patches [ ]

4. Do not know [ ]

4.8 When should you/your family sleep under bed net?

1. Always [ ]
2. Rainy season [ ]
3. Dry season [ ]

4.9. Has your house been sprayed (IRS) for the last 6 months? 1= (Yes) 2= (No)

4.9.1If no, give a reason

1 Unpleasant odor [ ]

2 Food contamination [ ]

3 side effects to children [ ]

4 Difficulty in removing household properties [ ]

5 Results to more mosquitos in the house [ ]

6 Results to bedbugs in the house [ ]

7 Others……. [ ]

8 Not applicable [ ]

5.0. Malaria treatment seeking behavior

5.1 a) Have you or any member of this household felt sick in the last two weeks?

1= (Yes) 2=(No)

b) If yes, have you had any of these symptoms?

1= (Fever) 2= (Headache) 3= (Joint pains) 4= (Vomiting) 5= Other

NB: If no malaria related symptoms mentioned, end of the survey.

5.2. a) If yes, did you/member of this household get treatment for these symptom?

1=(Yes) 2= (No)

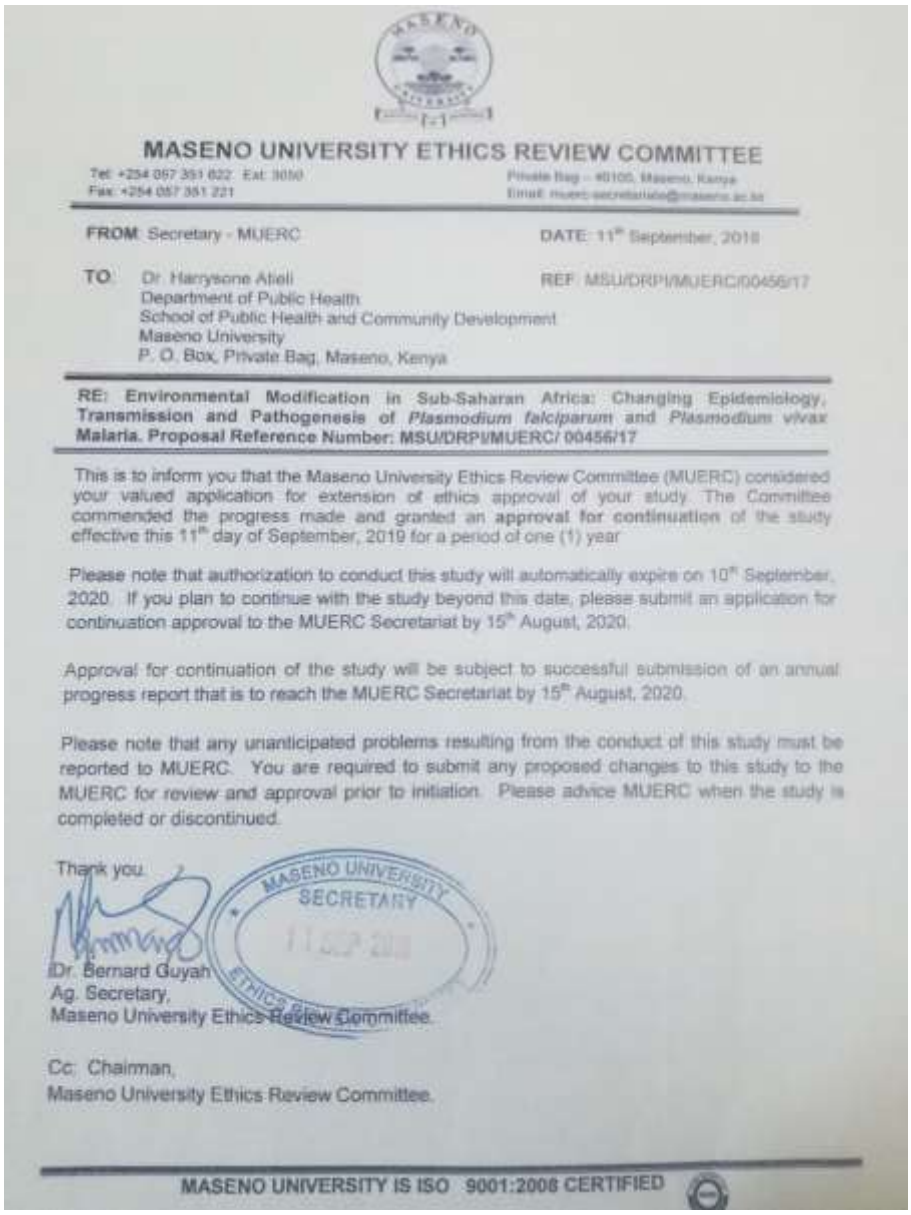
5.3 If yes, where?

1= (Health facility) 2=(Chemist) 3= (Home remedy) 4= (any other…….)

5.4. If health/chemist, which drug?

1= (Antimalarial) 2= (Any other……..)

APPENDIX 3: ETHICAL APPROVAL FORM



MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

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

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

FROM: Secretary - MUERC

DATE: 11th September, 2019

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

REF: MSU/DRPI/MUERC/00456/17

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

This is to inform you that the Maseno University Ethics Review Committee (MUERC) considered your valued application for extension of ethics approval of your study. The Committee commended the progress made and granted an **approval for continuation** of the study effective this 11th day of September, 2019 for a period of one (1) year

Please note that authorization to conduct this study will automatically expire on 10th September, 2020. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 15th August, 2020.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 15th August, 2020.

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

Thank you.

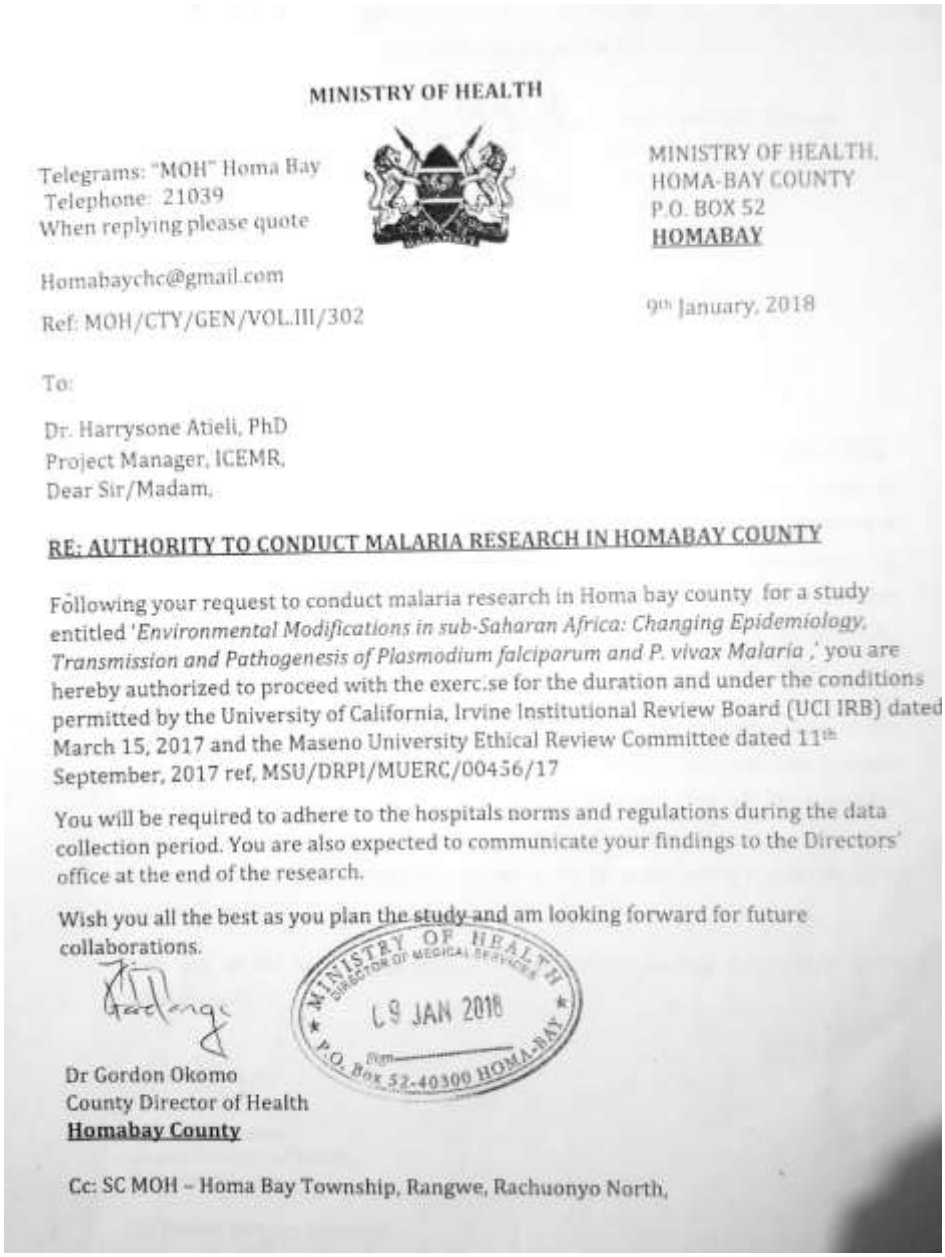
MASENO UNIVERSITY SECRETARY

Dr. Bernard Guyah
Ag. Secretary,
Maseno University Ethics Review Committee.

Cc: Chairman,
Maseno University Ethics Review Committee.

MASENO UNIVERSITY IS ISO 9001:2008 CERTIFIED

APPENDIX 4: RESEARCH APPROVAL BY COUNTY GOVERNMENT



MINISTRY OF HEALTH

Telegrams: "MOH" Homa Bay
Telephone: 21039
When replying please quote

Homabaychc@gmail.com

Ref: MOH/CTY/GEN/VOL.III/302

MINISTRY OF HEALTH,
HOMA-BAY COUNTY
P.O. BOX 52
HOMABAY

9th January, 2018

To:

Dr. Harrysone Atieli, PhD
Project Manager, ICEMR,
Dear Sir/Madam,

RE: AUTHORITY TO CONDUCT MALARIA RESEARCH IN HOMABAY COUNTY

Following your request to conduct malaria research in Homa bay county for a study entitled '*Environmental Modifications in sub-Saharan Africa: Changing Epidemiology, Transmission and Pathogenesis of Plasmodium falciparum and P. vivax Malaria* ,' you are hereby authorized to proceed with the exerc.se for the duration and under the conditions permitted by the University of California, Irvine Institutional Review Board (UCI IRB) dated March 15, 2017 and the Maseno University Ethical Review Committee dated 11th September, 2017 ref, MSU/DRPI/MUERC/00456/17

You will be required to adhere to the hospitals norms and regulations during the data collection period. You are also expected to communicate your findings to the Directors' office at the end of the research.

Wish you all the best as you plan the study and am looking forward for future collaborations.

MINISTRY OF HEALTH
DIRECTOR OF MEDICAL SERVICES
09 JAN 2018
P.O. Box 52-40300 HOMA-BAY

Dr Gordon Okomo
County Director of Health
Homabay County

Cc: SC MOH – Homa Bay Township, Rangwe, Rachuonyo North,

APPENDIX 5: PROTECTING HUMAN RESEARCH PARTICIPANTS CERTIFICATE

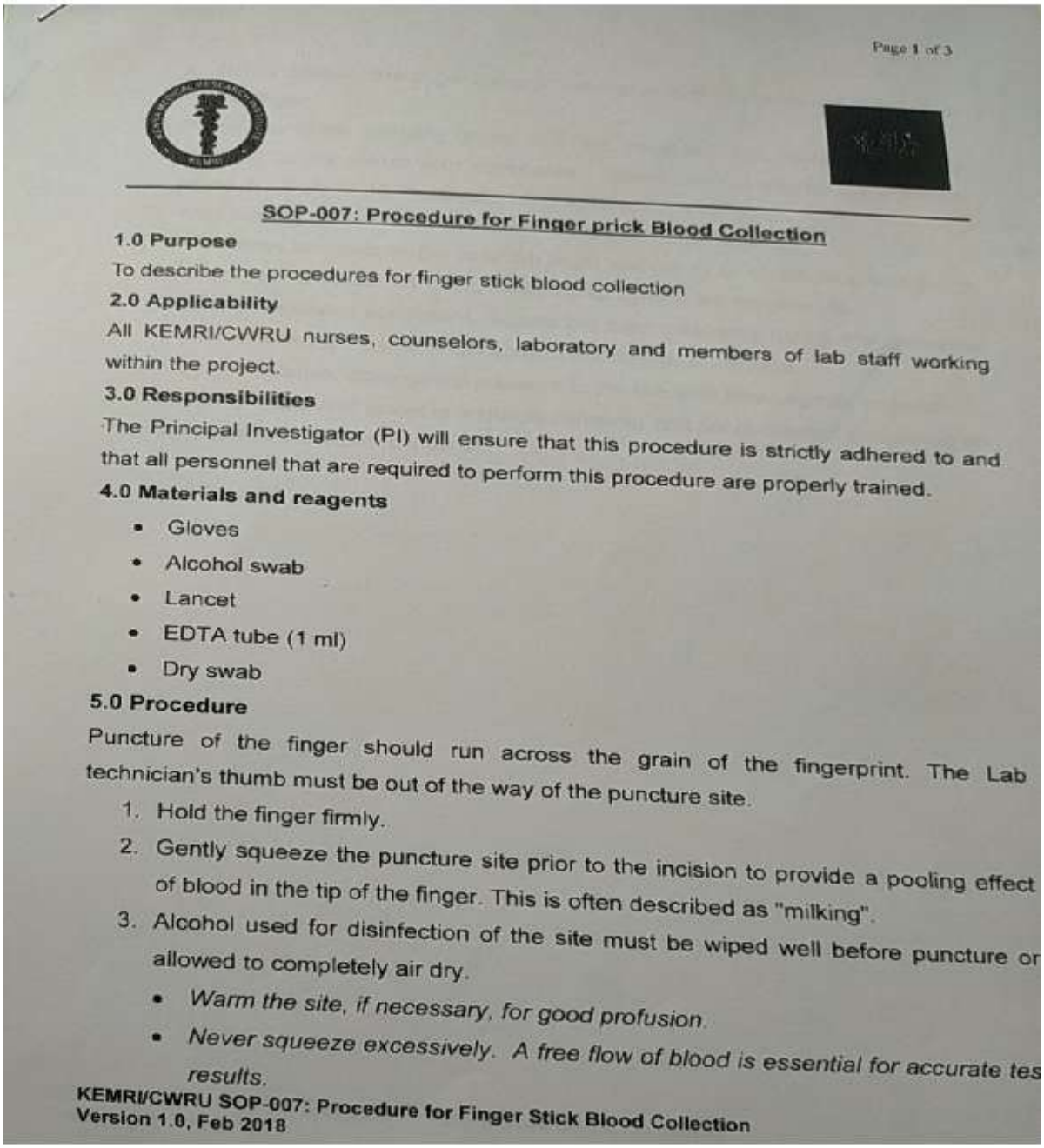
Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Collince Omondi** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 03/01/2018.

Certification Number: 2665777.

APPENDIX 6: BLOOD SAMPLING PROCEDURE



[redacted]

SOP-007: Procedure for Finger prick Blood Collection

1.0 Purpose

To describe the procedures for finger stick blood collection

2.0 Applicability

All KEMRI/CWRU nurses, counselors, laboratory and members of lab staff working within the project.

3.0 Responsibilities

The Principal Investigator (PI) will ensure that this procedure is strictly adhered to and that all personnel that are required to perform this procedure are properly trained.

4.0 Materials and reagents

- Gloves
- Alcohol swab
- Lancet
- EDTA tube (1 ml)
- Dry swab

5.0 Procedure

Puncture of the finger should run across the grain of the fingerprint. The Lab technician's thumb must be out of the way of the puncture site.

1. Hold the finger firmly.
2. Gently squeeze the puncture site prior to the incision to provide a pooling effect of blood in the tip of the finger. This is often described as "milking".
3. Alcohol used for disinfection of the site must be wiped well before puncture or allowed to completely air dry.
   - *Warm the site, if necessary, for good profusion.*
   - *Never squeeze excessively. A free flow of blood is essential for accurate tes results.*

- *Gently squeeze the finger using a "milking" motion to pool blood in the tip of the finger.*

4. Pierce the finger applying gentle, but firm pressure. The correct amount of pressure to use comes with experience. Newer devices should automatically pierce to a defined depth, usually 1.5 to 2.0 mm.
5. Wipe the first drop of blood to reduce tissue fluid contamination.
6. Allow drops to collect on the tip of the finger and gently touch the drop with the lip of the specimen tube. Try not to scrap the lip against the incision site.
7. For anticoagulated specimens, agitate the tube frequently during the collection process by snapping your finger against the bottom of the tube.
8. Upon completion, apply gentle pressure to the site until bleeding has ceased.
9. Properly dispose of lancet in a sharps container and contaminated supplies in an appropriate biohazard container.