INFLUENCE OF LANDSCAPE HETEROGENEITY ON *PLASMODIUM* INFECTION IN NYAKACH SUB-COUNTY, WESTERN KENYA

BY

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DECLARATION

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

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ABSTRACT

Despite scaled up intervention strategies in Kenya, existing control and treatment tools have not suppressed *Plasmodium* infection. Persistence of malaria has been attributed to submicroscopic infection with densities too low to be detected by standard diagnostic methods. Although landscape heterogeneity may contribute to the persistence of malaria, its role in persistence of submicroscopic infections is unknown. Landscape heterogeneity is defined here as variation in topography and rainfall seasonality. The topography of Nyakach Sub-County ranges from Lake Victoria's shores to the highland plateau, with habitat stability thought to influence diverse vector ecology and *Plasmodium* infection. Dynamic changes in vector ecology will always pose a challenge to intervention strategies. It is unknown, however, what effect landscape heterogeneity has on malaria entomological indices in maintaining year round vector population. Variation in the ecological landscape may result in differential risk exposures to malaria contributing to variation in febrile incidences in the community. It remains to be seen whether year-round clinical malaria persistence is influenced by landscape heterogeneity. The current study investigated the influence of landscape heterogeneity on *Plasmodium* infection in Nyakach Sub-County, western Kenya. The specific objectives were to determine the influence of topography and seasonality on prevalence of submicroscopic malaria, entomological indices of malaria, and incidence of clinical malaria in Nyakach Sub-County, western Kenya. A cross-sectional study design was used to collect data on prevalence of submicroscopic infection and entomological survey while longitudinal study design was used to collect data on the incidences of clinical malaria in lakeshore, hillside and highland plateau throughout wet and dry seasons. 1,777 finger prick blood smears and dry blood spots on filter paper were collected for microscopic inspection and real time-PCR diagnosis of *Plasmodium* infection over the wet and the dry season of 2019 and 2020. Larval sampling was conducted in all larval habitats using a standard dipper and adult *Anopheles* mosquitoes sampled using Pyrethrum Spray Catches. Finger-prick blood samples were collected from 2,205 febrile cases and tested for malaria parasites using ultra-sensitive Alere® malaria rapid diagnostic tests. Mixed effect model, negative binomial, and binary logistic regression determined the influence of topography and seasonality on: prevalence of submicroscopic infection; *Anopheles* larval and adult vector densities and abundance; and incidence of clinical malaria. The prevalence of submicroscopic infection was 14.2% (253/1,777). The likelihood of submicroscopic infection was higher in the lakeshore in both the wet and dry seasons (AOR: 2.71, 95% CI=1.85-3.95; $p<0.0001$) and hillside (AOR: 1.74, 95% CI=1.17–2.61, $p=0.007$) than in the highland plateau zones. *Anopheles* larval densities were 3.23 (95% CI=2.50-4.18, $p<0.0001$) and 1.81 (95% CI=1.32-2.48, $p<0.0001$) times higher in the lakeshore and hillside zones, respectively, than on the highland plateau and 4.59 (95% CI=3.61-5.83, $p<0.0001$) higher in wet season than dry season. Adult *Anopheles* abundance were 1.72 (95% CI=1.02-2.90, $p=0.041$) times higher in the lakeshore zone than on the highland plateau, and 2.17 (95% CI=1.48-3.20, $p<0.0001$) times higher in wet season than in dry season. Clinical malaria incidences were 2.02 (95% CI=1.62-2.50, $p<0.0001$) times higher lakeshore and 1.4 (OR: 1.42, 95% CI=1.13-1.79, $p=0.002$) times higher in hillside zone than on the highland plateau, and 1.49 (95% CI=1.24-1.80, $p<0.0001$) times higher in wet season, than in dry season. Landscape heterogeneity influenced prevalence of submicroscopic infection, entomological indices of malaria, and incidence of clinical malaria. Empirical evidence on the role of landscape heterogeneity on malaria, emphasizes importance of: developing strategies for identifying malaria transmission determinants in diverse landscapes; tailoring malaria control interventions to specific landscape attributes and improve the accuracy of malaria diagnosis and treatment.

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ACRONYMS AND ABBREVIATIONS

ACT	Artemisinin-based combination therapy
AL	Artemether-lumefantrine
CHV	Community health volunteer
CI	Confidence Interval
DBS	Dried blood spots
DNA	Deoxyribonucleic acid
ICEMR	International Center of Excellence for Malaria Research
IRS	Indoor residual spray
ITN	Insecticide-treated net
IVM	Integrated vector management
LLIN	Long-lasting insecticide-treated nets
LSM	Larval source management
Pf	*Plasmodium falciparum*
Pm	*Plasmodium malariae*
PMI	President's Malaria Initiative
Po	*Plasmodium ovale*
RDT	Rapid diagnostic test
RT-PCR	Real-time polymerase chain reaction
WHO	World Health Organization

OPERATIONAL DEFINITION OF TERMS

Endemic: Affecting a specific region or population on a regular basis

Entomological indices: *Anopheles* vector indicators characterized by *Anopheles* larval habitat availability, composition and abundance, and larval density, as well as adult *Anopheles* vector composition and abundance.

Febrile case: An individual having fever (axillary temperature ≥37.5ºC) during examination or complaints of fever and other nonspecific malaria related symptoms 1-2 days before examination.

Fever: An increase in body temperature above normal, for example above 37.5°C.

ITN: Net that has been factory-treated and does not need to be treated further.

Landscape heterogeneity: The variation in topography and rainfall seasonality.

***Plasmodium*:** A genus of protozoan vertebrate blood parasites that includes the malaria parasites. Malaria is caused by *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium vivax*.

Seasonality: Predictable changes that occur every calendar year and are characterized by wet and dry periods.

Sporozoites: Inoculated by a feeding female *Anopheles* mosquito, motile malaria parasites that are infective to humans.

Submicroscopic infections: Infection with *Plasmodium* detected by real-time polymerase chain reaction (RT-PCR) but not by microscopy.

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CHAPTER ONE

INTRODUCTION

1.1. Background Information

Malaria continues to be a major public health concern as well as a leading cause of disease and death around the world (WHO, 2021). Worldwide, an estimated 241 million cases of malaria occur each year, resulting in approximately 627,000 deaths (WHO, 2021). Sub-Saharan Africa is the worst-affected region, with an estimated 228 million people infected and 602,000 deaths each year (WHO, 2021). Approximately 75% of Kenya's 47.5 million people (Kenya National Bureau of Statistics, 2019) are at risk of malaria, with malaria prevalence in the endemic zone of Lake Victoria basin at 19% (Kenya Malaria Control Programme, 2019). The parasite *Plasmodium falciparum* is the most common cause of malaria (Division of National Malaria Program, 2021).

Kenya is currently ramping up malaria control interventions in order to reduce disease burden and eventually eliminate the disease (Kenya Malaria Control Programme, 2019). Despite increased interventions, existing control and treatment tools have not sufficiently suppressed *Plasmodium* infection in western Kenya (Degefa et al., 2017; Khagayi et al., 2019; Ng'ang'a et al., 2021; Orondo et al., 2021). This could be due to the fact that a substantial proportion of *Plasmodium* infections are submicroscopic, with densities too low to be detected using standard diagnostic methods like blood smear microscopy (Omondi et al., 2022). Concerns have been raised on whether submicroscopic infections sustain ongoing *Plasmodium* transmission, particularly if microscopy is used exclusively for *Plasmodium* parasite detection (Ochwedo et al., 2021; Zhiyong et al., 2016). However, no research has been conducted in Nyakach Sub-County to link malaria in the area to submicroscopic *Plasmodium* infection.

The risk of malaria transmission and infection varies across Kenya because of differences in topography across geographic zones (Division of National Malaria Program, 2021). *Plasmodium* infection is very common in the western Kenya lowland below 1300m above sea level because of the flat plains, which are prone to water pooling due to frequent flooding and have swampy areas that provide good breeding grounds for mosquito vectors (Dabaro et al., 2021; Githeko et al., 2006; Wanjala et al., 2010). In the highlands of western Kenya above 1300m above sea level, on the other hand, the prevalence of infection is low, most likely due to the hilly terrain with unstable productive larval habitats (Amek et al., 2012; Cohen et al., 2008; Laforet et al., 2017; Mwakalinga et al., 2018; Stevenson et al., 2015). In western Kenya's lowlands and highlands, the primary focus of malaria studies has been on high density parasitemia and the impact of topography on these infection burden (Afrane et al., 2014; Cohen et al., 2010; Degefa et al., 2017; Essendi et al., 2019; Mwakalinga et al., 2018; Wanjala et al., 2010; Zhou et al., 2020). The landscape of Nyakach Sub-County is diverse, ranging from the upper Nyakach Nyabondo plateau in a highland with an elevation of 1600m above sea level to the lower Nyakach near Lake Victoria basin with a lower elevation of 1,100m. However, it is unknown how the diverse landscape of Nyakach Sub-county contributes to variation in the submicroscopic *Plasmodiun* infections. Furthermore, no study has linked the prevalence of malaria infection in western Kenya's lowlands and highlands to submicroscopic *Plasmodium* infection. A high number of submicroscopic *Plasmodium* may contribute to the persistence of parasite infection transmissions (Zhiyong et al., 2016). As a result, it is necessary to investigate whether landscape heterogeneity influences submicroscopic infection levels and whether such infections are relevant to current parasite control interventions and contribute to the infectious reservoir.

Seasonality has been shown to affect *Plasmodium* infection, with infection rates increasing after wet seasons and decreasing during dry seasons (Dabaro et al., 2021; Reiner et al., 2015; Selvaraj et al., 2018). During the wet season, environmental conditions are ideal for mosquito breeding, which is critical for the life cycle of malaria vectors and parasites (Amek et al., 2012; Billingsley et al., 2007). In the dry season, when parasite transmission is low, microscopy may miss a larger portion of parasites that are responsible for infection maintenance. The high number of submicroscopic *Plasmodium* may contribute to the persistence of parasite infection transmissions (Zhiyong et al., 2016). Low density parasitemia may be maintaining endemicity in areas with low and seasonal transmissions (Agbana et al., 2022; Ochwedo et al., 2021; Vareta et al., 2020). Submicroscopic infections will always maintain infection levels if they are not accurately diagnosed, reversing the gains and goals of malaria eradication. However, there has been no research linking the submicroscopic infection to seasonal variations in rainfall. It is necessary to determine whether the wet and dry seasons promotes the year-round persistence of submicroscopic *Plasmodium* infection across heterogeneous landscape of Nyakach Sub-County in western Kenya. Understanding the role of seasonality in submicroscopic *Plasmodium* infection sustenance within geographically diverse landscapes may thus aid in addressing major challenges in malaria control and maintaining progress toward elimination.

The key determinants of entomological indicators of malaria include *Anopheles* larval habitat availability, composition and abundance, larval density, and *Anopheles* adult vector composition and abundance, as well as sporozoite rates (Himeidan et al., 2009; Kristan et al., 2008; Mzilahowa et al., 2012; Ndenga et al., 2011; Omalu et al., 2015). The availability and

productivity of breeding habitats determine the abundance of *Anopheles* vectors (Atieli et al., 2011; Gouagna et al., 2012; Minakawa et al., 2012). Altitude, as a determinant of malaria transmission and endemicity, can influence local hydrology, stability and productivity of vector larval habitats, and thus the spatial distribution of malaria exposure in the human population (Githeko et al., 2006). Western Kenya's lowland is characterized by flat plains with frequent flooding, whereas the highland regions are characterized by hills, valleys, and plateaus (Mutero et al., 2020; Mwakalinga et al., 2018; Ng'ang'a et al., 2019). The elevation of the landscape may influence hydrology as well as the stability and productivity of larval habitats and vector densities. Heterogeneity and dynamic changes in the malaria landscape and vector ecology will change vector bionomics, influencing malaria epidemiology (Mutero et al., 2020; Mwakalinga et al., 2018; Ng'ang'a et al., 2019). These changes in vector ecology may alter mosquito host-seeking behavior (Mwakalinga et al., 2018). Since diverse malaria eco-epidemiological settings and local vector ecologies necessitate interventions that work best for each setting as malaria epidemiology fluctuates over time (Mutero et al., 2020). Understanding the vector ecology and persistence *Plasmodium* infection in western Kenya across heterogeneous landscape will allow for appropriate target specific integrated vector management limiting vector breeding and human-vector contact. As a result, the focus of *Anopheles* larval habitat characterization, composition, and abundance should include landscape heterogeneity in order to understand its contribution to the persistence of *Anopheles* vector populations within a defined geographic landscape of Nyakach Sub-County in western Kenya, along an altitudinal transect of lowland lakeshore, hillside, and highland plateau.

Rainfall seasonality fluctuations may alter timing of vectors seasonal activity and host demographic processes, influencing malaria vector survival as well as parasite development rates (Anumudu et al., 2019; Cairns et al., 2015; Mawejje et al., 2021; Sallah et al., 2021). Rainfall seasonality has been shown to have an effect on mosquito population size, which in turn has an effect on sporozoite rates (Dabaro et al., 2021; Reiner et al., 2015; Selvaraj et al., 2018). Rainfall pattern influences vector occurrence indirectly by determining larval habitat quality and stability (Dabaro et al., 2021; Matsushita et al., 2019). Fluctuations in the wet and dry seasons may change the timing of *Anopheles* mosquito seasonal activity and affect the survival and transmission of malaria vectors as well as the parasites' development rates (Reiner et al., 2015; Selvaraj et al., 2018). Changes in the rainfall pattern are expected to modulate warming-related changes, with wetter conditions likely favoring vector survival and reproduction whereas dry conditions having no effect on vectors (Bouvier et al., 1997a; Dabaro et al., 2021; Kifle et al., 2019; Matsushita et al., 2019; Reddy et al., 2011). As malaria vector and pathogen transmission cycles respond to increasing variability and changes in rainfall pattern, a short transition period between the dry and wet seasons may increase the risk of endemic vector-borne diseases (Bouvier et al., 1997b; Soma et al., 2021). Larval habitat availability with adequate precipitation that provide a favorable environment for vector proliferation has a significant impact on mosquito vector reproduction success (Atieli et al., 2011; Conde et al., 2015; Grech et al., 2013; Hardy et al., 2013; Munga et al., 2009; Mutero et al., 2020). However, it is unclear how changes in rainfall patterns will affect entomological indices of malaria in Nyakach Sub-County, western Kenya, sustaining *Anopheles* vector populations and thus year round *Plasmodium* infection year round.

Despite the intensification of malaria interventions, clinical malaria occurs throughout the year as the scale-up interventions are insufficient to eradicate the disease in Western Kenya (Kenya Malaria Control Programme, 2019). Malaria febrile illness is the most common clinical manifestation of *Plasmodium* infection and is used to assess the public health impact of malaria (Drakeley et al., 2017; Lutambi et al., 2014; Selvaraj et al., 2018). Western Kenya's lowlands around lake Victoria are relatively hot and dry, with high *Plasmodium* infection transmission, whereas western Kenya's highlands have low temperatures, which affects *Plasmodium* infection (Amek et al., 2012; Essendi et al., 2019; Matsushita et al., 2019; Munga et al., 2009; Mutero et al., 2020; Selvaraj et al., 2018). Variation in the ecological landscape may result in differential risk exposures to malaria contributing to variation in clinical malaria incidences in the community (Mwakalinga et al., 2018). Clinical malaria incidences has been linked to housing type, vector availability and abundance, bed net use, and outdoor exposure-related activities (Bannister-tyrrell et al., 2018; Guerra et al.,2018). The risk factors to clinical malaria have serious public health implications in terms of ensuring adequate antimalarial supply and optimizing vector control interventions. The persistence of clinical malaria in the community, as well as the dynamics of transmission, will always pose a challenge, causing malaria elimination to be delayed. To our knowledge, no research has linked year-round clinical malaria persistence to variations in topography and rainfall in areas with heterogeneous landscapes such as Nyakach Sub-County in western Kenya. As a result, it is unclear whether landscape heterogeneity will contribute to the persistence of clinical malaria.

Variation in the rainy and dry season may influence malaria prevalence, and high clinical malaria cases have been observed during the wet season (Anumudu et al., 2019). Changes in rainfall

patterns and rising climate variability may affect the environment's suitability for vectors and vector-borne pathogens to thrive (Moiroux et al., 2014; Patz et al., 1998; Takken & Verhulst, 2013). The rainfall pattern indirectly influences vector occurrence by determining habitat qualities, which in turn influences vector numbers, resulting in high human vector contact, and thus increased incidences of malaria (Dabaro et al., 2021; Matsushita et al., 2019). Variation in rainfall may have an impact on vector ecology hence altering here and when environments conducive to the growth of vectors and vector-borne pathogens (Moiroux et al., 2014; Patz et al., 1998; Takken & Verhulst, 2013). Risk of endemic vector-borne diseases may increase as malaria vector and pathogen transmission cycles respond to increasing variability and changes in rainfall pattern (Bouvier et al., 1997a; Soma et al., 2021). However, no study has found a direct link between seasonality and the persistence of clinical malaria febrile illness across a heterogeneous landscape. Understanding the contribution of seasonality on maintaining incidences of clinical malaria is critical for improving the effectiveness of malaria intervention tools.

Fever is the most common symptom of clinical malaria, and its severity prompts people to seek hospital treatment (Carlucci et al., 2017). Monitoring clinical malaria at the community level is challenging in endemic areas because coincidental febrile illness episodes can be caused by causes other than malaria (U.S. President's Malaria Initiative, 2022). The differential risk exposures to malaria may contribute to variation in the distribution of clinical malaria in the community (Mwakalinga et al., 2018). Although algorithms of clinical symptoms indicative of malaria are readily available along with expertise in reading blood smears and malaria RDTs (Siahaan et al., 2018a), health care providers may still rely on presumptive clinical diagnosis (Graz et al., 2011). This is problematic since, for example, malaria symptoms such as fever,

prostration, and myalgia are similar to those of other common infectious diseases caused by viral and bacterial pathogens (Mfuh et al., 2019). It remains to be seen whether variations in clinical malaria incidences and treatment are influenced by topography and seasonality. As a result, studies that look at topography and seasonality as predictors of clinical malaria persistence in a heterogeneous landscape such Nyakach Sub-County, western Kenya, need to be expanded.

Along the Lake Victoria basin, Kisumu County's highland of Nyakach Sub-County have been reported to have a high malaria burden (Mutero et al., 2020). Therefore, *Plasmodium* infection cannot be generalized within a heterogeneous landscape such as Nyakach Sub-County, western Kenya. Malaria research has been primarily focused on the highland plateau region of Nyakach Sub-County (Imbahale et al., 2012; Mutero et al., 2020; Nganga et al., 2019; Sultana et al., 2017). Nyakach Sub-County was chosen because of its distinct landscape variation, which ranges from 1100m along Lake Victoria's shore, which is prone to flooding during the wet seasons, to 1700m on the highland plateau, which has more stable larval habitats. (Kenya Supplemental Environmental Assessment Amendment, 2013).

1.2. Statement of Research Problem

Despite the increased efforts in Kenya to scale up intervention strategies, the existing control and treatment tools have not suppressed mosquito-borne transmission of *Plasmodium* infection and disease. Malaria vector ecology and *Plamodium* infection remain highly variable, with serious implications for vector control interventions and malaria burden. However, contribution of landscape heterogeneity on persistent submicroscopic infections throughout the year in western Kenya is limited. Although landscape heterogeneity may contribute to the persistence of

Plasmodium infections, the role of landscape heterogeneity proxies such as topography and seasonality on submicroscopic infection persistence is unknown. Second, despite increased vector control interventions, the impact of landscape heterogeneity on malaria entomological indices in maintaining high year-round vector populations is unknown. Finally, it is unclear how landscape heterogeneity influence the persistence of clinical malaria incidences. Nyakach Sub-County has distinct geographical variation, with seasonal variations in rainfall and year-round malaria burden. As a result, the study sought to investigate the influence of landscape heterogeneity as defined as variation in topography and rainfall seasonality on *Plasmodium* infection in Nyakach Sub-County, western Kenya.

1.3. Objectives

1.3.1. General Objective

To investigate the influence of landscape heterogeneity on *Plasmodium* infection in Nyakach Sub-County, western Kenya.

1.3.2. Specific Objectives

i. To determine the influence of topography and seasonality on prevalence of submicroscopic *Plasmodium* infection in Nyakach Sub-County, western Kenya.
ii. To determine the influence of topography and seasonality on entomological indices of malaria in community of Nyakach Sub-County, western Kenya.
iii. To determine the influence of topography and seasonality on incidence of clinical malaria cases in Nyakach Sub-County, western Kenya

1.3.3. Hypotheses

i. Topography and seasonality do not influence prevalence of submicroscopic *Plasmodium* infection in Nyakach Sub-County, western Kenya.
ii. Topography and seasonality do not influence entomological indices of malaria in community of Nyakach Sub-County, western Kenya.
iii. Topography and seasonality do not influence incidence of clinical malaria cases in Nyakach Sub-County, western Kenya.

1.4. Significance of the Study

Landscape heterogeneity is a major correlate of submicroscopic malaria infection in the Lake Victoria area of western Kenya. The findings of the study have the potential to: first, supplement and support the development of strategies for identifying submicroscopic malaria transmission determinants in diverse landscapes in order to maintain progress toward parasite reservoir elimination. Secondly, emphasizes the importance of tailoring target specific adaptive environmental management interventions to specific landscape attributes to have a significant impact on transmission reduction. Lastly, improve the accuracy of malaria diagnosis and treatment by introduction of more sensitive and reliable diagnostic tools in order to maintain progress toward elimination and strengthen community case management of malaria by providing supportive supervision of community health volunteers to advocate for community awareness, early diagnosis, and treatment of malaria across heterogeneous landscape.

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

Malaria control and elimination necessitate interventions that focus on both prevention and reduction of malaria transmission. In Kenya, despite increased vector control efforts, malaria remains a year-round problem in rural communities. This is due, in part, to the fact that, despite the government's directive to provide free malaria treatment at public hospitals, the vast majority of malaria cases go unreported because they are missed at the health facility and at the community (Kenya Malaria Control Programme, 2019; U.S. President's Malaria Initiative, 2022). In addition, landscape heterogeneity role in maintaining submicroscopic infections remains unknown. This chapter reviews the literature on the role of landscape heterogeneity in the persistence of submicroscopic infections and clinical malaria cases, as well as malaria entomological indices.

2.2. Submicroscopic *Plasmodium* infection

Malaria is still a major public health issue and a leading cause of morbidity and mortality. Globally, about 241 million cases of malaria occur worldwide with about 627,000 deaths reported annually (WHO, 2021). Sub-Saharan Africa is the hardest hit region with an estimated 228 million people infected, resulting in 602,000 deaths reported annually; children under five years are mostly affected and account for about 272,000 malaria deaths globally (WHO, 2021). In Kenya, approximately 75% of people are at risk of malaria; the disease's burden is disproportionately higher in the Lake Victoria region, where 9.8 million people are at risk (Kenya Malaria Control Programme, 2019). In sum, with an estimated 13% to 15% of malaria

outpatient consultations in Kenya, malaria disease still remains a major public health concern (Division of National Malaria Program, 2021).

The risk of malaria transmission and infection varies across Kenya because of differences in altitude and rainfall patterns across geographic zones (Division of National Malaria Program, 2021). The country is divided into four epidemiological zones which have been determined based on malaria prevalence, weather (temperature, rainfall, altitude), and topography: endemic (lake and coast), epidemic (highland), seasonal (semi-arid), and low risk zones (U.S. President's Malaria Initiative, 2022). Endemic Lake Victoria basin has a malaria prevalence of 18.9% and the Kenyan coast region has a prevalence of 4.5%, the highland area has a prevalence of 0.7%, the semiarid area has a prevalence of 1.8%, and the low risk areas have a prevalence of 0.4% (Division of National Malaria Program, 2021).

Lake Victoria and its surrounding areas have suitable ambient temperatures for malaria transmission, as well as the necessary amounts of rainfall to sustain long periods of transmission (Bhatt et al., 2015; Drakeley et al., 2017; Weiss et al., 2019). Malaria transmission is stable around Lake Victoria in western Kenya, at altitudes ranging from 0 to 1,300 meters (Degefa et al., 2017). The highland areas of western Kenya, which are above 1,500 meters above sea level, are an epidemic prone area with seasonal malaria transmission (Atieli et al., 2011; Githeko et al., 2006). Malaria in the highland areas occur when weather conditions are favorable for sustained vector breeding and successful sporogony, resulting in an increased intensity of malaria transmission that occasionally reaches epidemic proportions (Division of National Malaria

Program, 2021). However, the factors that maintain *Plamosdium* transmission year-round remain unknown.

Malaria research has primarily focused on the Upper Nyakach Sub-County's highland plateau region, and the burden of infection cannot be generalized across the heterogeneous landscape (Imbahale et al., 2012; Mutero et al., 2020; Nganga et al., 2019; Sultana et al., 2017). Nyakach Sub-County's landscape varies from the lowlands along Lake Victoria's shores to the highland plateau zone, despite its small geographic setting(Mutero et al., 2020). As a result, generalizing the infection burden may not be justified because it informs intervention measures. To the best of our knowledge, mapping of *Plasmodium* infection burden across heterogeneous landscapes such as Nyakach Sub-County and whether these variations may be sustaining malaria infection despite blanket intervention measures implemented by the Ministry of Health are not yet available.

In western Kenya's lowlands and highlands, the primary focus of malaria infection has been on high density parasitemia and the impact of topography on these infection burden (Afrane et al., 2014; Cohen et al., 2010; Degefa et al., 2017; Essendi et al., 2019; Mwakalinga et al., 2018; Wanjala et al., 2010; Zhou et al., 2020). *Plasmodium* infection is very common in the western Kenya lowland because of the flat plains, which are prone to water pooling due to frequent flooding and have swampy areas that provide good breeding grounds for mosquito vectors (Dabaro et al., 2021; Githeko et al., 2006; Wanjala et al., 2010). In the highlands of western Kenya, on the other hand, the prevalence of infection is low, most likely due to the hilly terrain with unstable productive larval habitats (Amek et al., 2012; Cohen et al., 2008; Laforet et al., 2017; Mwakalinga et al., 2018; Stevenson et al., 2015). However, no study has linked the

prevalence of malaria infection in western Kenya's lowlands and highlands to submicroscopic *Plasmodium* infection.

Infections detected by PCR but not by microscopy are known as submicroscopic infections (Ochwedo et al., 2021; Vareta et al., 2020; Whittaker et al., 2021; Zhiyong et al., 2016). Submicroscopic infections are distinguished by low parasitemia density, which may aid in infection transmission maintenance. The transmission of parasites from humans to mosquitos occurs frequently in low densities (Gaye et al., 2015) which may be missed by microscopy due to their low sensitivity (Ochwedo et al., 2021). Microscopy, when compared to molecular diagnostic tools, misses half of all Plasmodium infections in endemic areas. These low parasite densities missed by microscopy could act as silent reservoirs, allowing malaria transmission to continue (Waltmann et al., 2015; Yimam et al., 2021; Zhao et al., 2018). The high number of submicroscopic *Plasmodium* may contribute to the persistence of parasite infection transmissions (Zhiyong et al., 2016). It is unclear whether landscape heterogeneity causes variation in submicroscopic infection levels, and whether such infections are relevant to current parasite control interventions. Utility of more sensitive diagnostics for identifying submicroscopic infections in heterogeneous landscapes within endemic settings should be prioritized.

Plasmodium infection is an important indicator for tracking the progress of malaria control interventions in endemic areas (Okell et al., 2012). Microscopy is the primary diagnostic tool for malaria. Since submicroscopic parasite densities are lower than the detection limit of microscopy (Acquah et al., 2021), enhanced detection tools such as polymerase chain reaction are required. There is evidence that people with submicroscopic malaria can infect mosquitos and that only molecular methods can detect submicroscopic infections (Lin et al., 2014). However, the true

Plasmodium infection burden may be called into question if only microscopy is used to quantify the disease burden, as sensitive molecular tools detect a higher number of *Plasmodium* infections. Impact of topography on submicroscopic infection should be studied as malaria control is scaled up, as well as whether submicroscopic carriers should be prioritized for intervention. Further research should be conducted to determine the extent to which landscape heterogeneity influences submicroscopic malaria that contributes to the infectious reservoir, and thus what diagnostic detection threshold is required to effectively interrupt transmission.

Persistent, submiscroscopic *Plasmodium* infections have been linked to a variety of health risks, including chronic anemia, pregnancy complications, impaired immune competence, co-infections with invasive bacterial diseases, and cognitive impairment (Nguyen et al., 2018; Okell et al., 2012). Furthermore, how much submicroscopic parasite infections missed during medical diagnosis contribute to the persistence of transmission, particularly in low-transmission settings, is unknown. It is therefore critical to understand where and when submicroscopic *Plasmodium* infections is most likely to occur.

Malaria has been shown to be affected by seasonality, with rainfall having a positive correlation with infection prevalence in both the wet and dry seasons (Imbahale et al., 2012; Ng'ang'a et al., 2019). Transmission declines observed during prolonged dry seasons are most likely due to a reduction in the number of vector breeding sites (Matsushita et al., 2019). The western Kenyan Nyakach Sub-County highland plateau has an elevation of 1600m above sea level, whereas the elevation near Lake Victoria basin in the lower Nyakach is much lower at 1,100m above sea level. Furthermore, between the Lake Victoria shores and the highlands, there are numerous

valleys and basin-like depressions in hillside regions, which could explain the difference in malaria (Degefa et al., 2017; Matsushita et al., 2019; Ndenga et al., 2011; Wandiga et al., 2010; Zhou et al., 2010). However, it is unknown how seasonality affects the prevalence of submicroscopic *Plasmodiun* infections across a heterogeneous landscape, resulting in the persistence of malaria diseases. Understanding the role of seasonality in *Plasmodium* infection sustenance within geographically diverse landscapes may thus aid in addressing major challenges in malaria control and maintaining progress toward elimination.

Low density parasitemia may be maintaining endemicity in areas with low and seasonal transmissions when mosquito numbers are low and malaria transmission is almost non-existent (Agbana et al., 2022; Ochwedo et al., 2021; Vareta et al., 2020). Seasonality has an effect on mosquito population size, which in turn has an effect on sporozoite rates, with mosquito populations increasing after rainy seasons and decreasing during dry seasons (Dabaro et al., 2021; Reiner et al., 2015; Selvaraj et al., 2018). During the wet season, environmental conditions are ideal for mosquito breeding, which is critical for the life cycle of malaria vectors and parasites (Amek et al., 2012; Billingsley et al., 2007). People infected with submicroscopic malaria will always pose health risks as they may act as reservoirs of malaria parasite infection, sustaining *Plasmodium* transmission (Bousema et al., 2010; Ouédraogo et al., 2016). However, it is unknown whether the rainy and dry seasons contribute to the year-round prevalence of malaria submicroscopic infection in the Nyakach Sub-County.

Insecticidal treated nets (ITNs) and the type of household structure are important determinants of malaria infection. There is a high level of access to the ITN but a low level of utilization

(Division of National Malaria Program, 2021; Ng'ang'a et al., 2021; Ochwedo et al., 2021). The perception of serious *Plasmodium* infection and high vector numbers occurring only during the wet seasons, on the other hand, serve as barriers to net use (Division of National Malaria Program, 2021; Ng'ang'a et al., 2021; Santos et al., 2019; U.S. President's Malaria Initiative, 2022). Household structure type, such as the wall type, and floor type, and roof type, influences malaria transmission patterns (Kleinschmidt et al., 2018; Ng'ang'a et al., 2021; Tokponnon et al., 2019; Wanjala et al., 2015). The majority of rural households have iron sheet roofing and mud floors, with mud/dirt and stone as the primary wall materials (Division of National Malaria Program, 2021). These characteristics of households are strong predictors of malaria transmission. However, no research to our knowledge has linked malaria risk as potential confounders of submicroscopic *Plasmodium* infection across topography and seasonality.

2.3. Entomological Indices of Malaria

The key determinants of entomological indicators of malaria include *Anopheles* larval habitat availability, composition and abundance, larval density, and *Anopheles* adult vector composition and abundance, as well as sporozoite rates (Himeidan et al., 2009; Kristan et al., 2008; Mzilahowa et al., 2012; Ndenga et al., 2011; Omalu et al., 2015). *Anopheles gambiae* and *Anopheles funestus* are the primary malaria vectors in western Kenya (Himeidan et al., 2009; Imbahale et al., 2011; Minakawa et al., 2002; Zhou et al., 2007). The availability and productivity of breeding habitats determine the abundance of *Anopheles* vectors (Atieli et al., 2011; Gouagna et al., 2012; Minakawa et al., 2012). *Anopheles gambiae* larvae prefer clear, sunlit shallow water habitats in drainage ditches, natural swamps, tire trucks, hoof prints, and man-made ponds for breeding. *Anopheles funestus* prefers shady habitats with vegetation covers

to breed, particularly man-made ponds and swamps (Conde et al., 2015; Hardy et al., 2013; Himeidan et al., 2009; Kweka et al., 2012).

Altitude, as a determinant of malaria transmission and endemicity, can influence local hydrology, the stability and productivity of vector larval habitats, and thus the spatial distribution of malaria exposure in the human population (Githeko et al., 2006). The lowland of western Kenya is characterized by flat plains with frequent flooding, whereas the western Kenya's highland regions is characterized by hills, valleys, and plateaus. These variation in topography will have an impact on the *Anopheles* larval habitats distribution and abundance. The uneven distribution of larval breeding sites may have an impact on vector spatial distribution and the risk of human exposure to infective mosquitos (Mutero et al., 2020; Mwakalinga et al., 2018; Ng'ang'a et al., 2019). It is unknown whether variation in topography and seasonality within a defined geographic setting influences malaria entomological indices, thereby maintaining vector populations and *Plasmodium* infection across heterogeneous landscapes like Nyakach Sub-County in western Kenya.

Kenya is currently stepping up malaria control interventions such as long-lasting insecticide-treated nets (LLIN) and indoor residual spraying (IRS) (Kenya Malaria Control Programme, 2019). Despite the increasing intervention efforts, existing control and treatment tools have not suppressed mosquito-borne *Plasmodium* infection and disease transmission (Kenya Malaria Control Programme, 2019). The rate of progress in reducing malaria cases in Kenya has slowed in recent years, and malaria prevalence and vector abundance have resurfaced (Kenya Malaria Control Programme, 2019) due to behavioral changes in the *Anopheles* vectors with outdoor

transmission becoming more common and occurring earlier in the evenings and mornings (Kleinschmidt et al., 2018; Monroe et al., 2019; Moshi et al., 2018); rise in insecticide resistance (WHO, 2019); and reduced funding for vector interventions, competing public health challenges beyond malaria (Ajayi et al., 2020; Heuschen et al., 2021; Weiss et al., 2021).

With the scaling-up of malaria control interventions to reduce disease burden in Kenya, variation in malaria landscape will always pose a challenge to intervention methods. Due to varying human exposure to transmission risks, changes in altitude may influence mosquito distribution and infection (Imbahale et al., 2012). The LLINs and IRS commonly used vector control interventions only target to eradicate indoor resting mosquitos (Zhou et al., 2020). Outdoor transmission, on the other hand, is becoming more common as malaria vector biting behavior changes (Degefa et al., 2017; Moshi et al., 2018; Reddy et al., 2011). As outdoor malaria transmission increases, outdoor resting vectors may be contributing to the challenge of malaria control (Thomsen et al., 2017). To aid in the monitoring of outdoor transmission, it may be necessary to identify *Anopheles* vectors from their larval habitats and determine whether topography and seasonality contribute to variation in habitat types and *Anopheles* vector densities. Such information could be used to aid in the control of outdoor resting mosquitos, particularly during breeding, thereby reducing the malaria vector population.

There are three eco-epidemiological zones in Nyakach Sub-County, western Kenya: the lowland lakeshore area, the hillside along the slope, and the highland of the Nyabondo plateau. These ecological zone distinctions may have an impact on habitat distribution and species composition. *Plasmodium* infection is common in the lowlands of western Kenya because of the flat

topography, which allows water to pool as a result of frequent flooding and swamps (Cohen et al., 2010; Hardy et al., 2013; McCormack et al., 2019; Mutero et al., 2020; Mwakalinga et al., 2018). The plateaus, on the other hand, are nearly flat with swamps caused by numerous streams, which may act as potential larval habitats, whereas the hillside zone has efficient drainage provided by the valley slope (Wanjala et al., 2010) with reduced opportunities for water to pool. The water pooling has the potential to support vector breeding (Cohen et al., 2010; Hardy et al., 2013; McCormack et al., 2019; Mutero et al., 2020; Mwakalinga et al., 2018). The elevation of the landscape may influence hydrology as well as the stability and productivity of larval habitats and vector densities.

Vector density, species composition, and distribution of vector populations may be affected by ecological variation (Afrane et al., 2012). Availability of water, vegetation cover, presence of predation, and the presence of algae may influence larval habitat productivity (Bartilol et al., 2021; Debrah et al., 2021; Gouagna et al., 2012). Furthermore, distance of the household to breeding habitat, and land use type surrounding the larval habitat may affect mosquito distribution resulting in the heterogeneous human exposure to transmission risks (Cohen et al., 2010; Hardy et al., 2013; McCormack et al., 2019; Mutero et al., 2020; Mwakalinga et al., 2018). Variations in these risk determinants may influence mosquito distribution and infection (Imbahale et al., 2012). Larval ecological variation may cause an uneven distribution of vectors, resulting in changes in vector bionomics and parasite transmission and disease burden (Afrane et al., 2014; Githeko et al., 2006). These changes in vector ecology may alter mosquito host-seeking behavior, influencing malaria epidemiology (Mwakalinga et al., 2018). The ecology of larval vectors may have an impact on vector control (Zhou et al., 2020). As a result, the focus of

habitat characterization, composition, and abundance should include topography and seasonality in order to determine the variation in distribution of adult Anopheles vectors within a defined geographic setup such as Nyakach Sub-County in western Kenya. Such knowledge will be essential for accurately identifying malaria transmission dynamics and implementing target site specific integrated vector management.

Changes in the rainfall pattern are expected to modulate warming-related changes, with wetter conditions likely favoring vector survival and reproduction whereas dry conditions having no effect on vectors (Bouvier et al., 1997a; Dabaro et al., 2021; Kifle et al., 2019; Matsushita et al., 2019; Reddy et al., 2011). Indirectly, the rainfall pattern influences vector occurrence by determining habitat quality (Dabaro et al., 2021; Matsushita et al., 2019). Seasonal fluctuations may alter timing of vectors seasonal activity and host demographic processes, influencing malaria vector survival as well as parasite development rates (Anumudu et al., 2019; Cairns et al., 2015; Mawejje et al., 2021; Sallah et al., 2021). Adult malaria vector distribution is influenced in part by the distribution of vector mosquito larvae's aquatic habitats (Mutuku et al., 2009; Mwangangi et al., 2009; Nikookar et al., 2017; Ondiba et al., 2019). Larval habitat availability with adequate temperature and precipitation that provide a favorable environment for vector proliferation has a significant impact on mosquito vector reproduction success (Atieli et al., 2011; Conde et al., 2015; Grech et al., 2013; Hardy et al., 2013; Munga et al., 2009; Mutero et al., 2020). Since the malaria vector is temperature sensitive as an immature stage in the aquatic environment and as an adult, temporal and spatial changes in temperature and precipitation may affect the biology and ecology of mosquito vectors (Mawejje et al., 2021). Rising water temperatures after the onset of the wet season cause the larvae to mature faster and have a greater

capacity to produce more offspring, and increased rainfall increases the number and quality of mosquito breeding sites (Dabaro et al., 2021; Imbahale et al., 2012; Kifle et al., 2019; Matsushita et al., 2019). It is unclear how changes in rainfall patterns will impact vector bionomics in Nyakach Sub-County, western Kenya, and sustaining transmission during the rainy and dry seasons, ultimately influencing malaria transmission risks.

The distribution, abundance, and diversity of the major *Anopheles* vectors in western Kenya (*Anopheles funestus, Anopheles arabiensis, Anopheles gambiae s.s*., and *Anopheles coustani*, a secondary vector contributing to malaria transmission) vary (Abong'o et al., 2020; Debrah et al., 2021; Degefa et al., 2017; Essendi et al., 2019; Kweka et al., 2015; Mutero et al., 2020). Extensive use of LLINs and IRS has contributed to the shift in vector ecology such that the *An. arabiensis* is replacing the *An. gambiae s.s* as the dominant species (Abong'o et al., 2020; Zhong et al., 2020). Despite reductions in vector densities as a result of increased intervention, *An. funestus* has merged as the main malaria vector species in endemic zones of western Kenya (Debrah et al., 2021; Degefa et al., 2017; Minakawa et al., 2021; Zhong et al., 2020; Zhou et al., 2010). Since malaria infection can be maintained by even low vector densities (President's Malaria Initiative Kenya, 2019), entomological surveillance will be critical in assessing malaria transmission dynamics across heterogeneous landscape such as Nyakach Sub-County in western Kenya. Despite increased vector control interventions, the role of landscape heterogeneity in *Anopheles* mosquito sustenance and thus maintaining high malaria transmission remains unknown.

2.4. Clinical Malaria Cases

Accurate diagnosis and treatment are the primary strategies for optimizing clinical malaria management (Kenya Malaria Control Programme, 2019). Malaria patients who present with fever or malaria-like symptoms should have their diagnosis confirmed (Kenya Malaria Control Programme, 2016). In cases of uncomplicated *P. falciparum* malaria, artemisinin-based combination therapies (ACTs) are recommended, whereas artesunate and quinine are recommended for severe malaria (WHO, 2015).

Variation in the rainy and dry season may influence malaria prevalence, and high clinical malaria cases have been observed during the wet season (Anumudu et al., 2019). The rainfall pattern influences vector occurrence indirectly by determining habitat qualities, and habitat characteristics are important for vector survival, particularly during development times (Dabaro et al., 2021; Matsushita et al., 2019). Changes in rainfall patterns and rising climate variability may affect the environment's suitability for vectors and vector-borne pathogens to thrive (Moiroux et al., 2014; Patz et al., 1998; Takken & Verhulst, 2013). Changes in rainfall patterns will affect mosquito populations depending on whether they modulate changes in landscape and habitat to the benefit or detriment of available larval development sites. However, there has been little research on the role of seasonality in the sustenance of patent *Plasmodium* infection within micro-geographic localities under similar vector control interventions. As a result, in order to address one of the major challenges in malaria control, it is critical to investigate the influence of topography and seasonality transmission risks leading to the sustenance of clinical malaria in hyper-endemic lowland lake and highland plateau areas of western Kenya.

Seasonality affects mosquito population size, which affects sporozoite rates (Dabaro et al., 2021; Reiner et al., 2015; Selvaraj et al., 2018). Changes in temperature and precipitation over time and space may have an impact on vector ecology, increasing the risk of clinical malaria illness (Mawejje et al., 2021). Changes in rainfall patterns may alter where and when environments conducive to the growth of vectors and vector-borne pathogens (Moiroux et al., 2014; Patz et al., 1998; Takken & Verhulst, 2013). Seasonal changes may affect *Anopheles* mosquito seasonal activity, affecting malaria vector survival and transmission as well as parasite development rates (Anumudu et al., 2019; Cairns et al., 2015; Mawejje et al., 2021; Sallah et al., 2021). Risk of endemic vector-borne diseases may increase as malaria vector and pathogen transmission cycles respond to increasing variability and changes in rainfall pattern due to the minimal transition between the dry and wet seasons (Bouvier et al., 1997a; Soma et al., 2021). Understanding the impact of seasonality on malaria febrile incidences is critical for furthering the understanding of local transmission determinants, improving the effectiveness of intervention tools, and limiting vector breeding and human-vector contact.

Fever is the most common symptom of clinical malaria, and its severity prompts people to seek hospital treatment (Carlucci et al., 2017). The differential risk exposures to malaria may contribute to variation in the distribution of febrile illness in the community (Mwakalinga et al., 2018). *Plasmodium* infection is transmitted via gametocytes, which are the parasites' sexual stage. Clinical malaria case can be complicated because the mosquito may pick up multiple genotypes of the parasite during transmission, which mate and recombine before transmission to the new host, resulting in mixed genotypes in the vector and parasite genetic variability, leading to the development of complex infection (Buchwald et al., 2019). The infection's complexity

may worsen febrile illness (Siahaan et al., 2018), resulting in parasite transmission and infection persistence. However, it is unknown whether clinical malaria complications are caused by variations in topography and seasonality in Nyakach Sub-County, western Kenya.

Malaria patients who present with fever or malaria-like symptoms should have their diagnosis confirmed using an RDT or microscopy (Kenya Malaria Control Programme, 2016). Rapid diagnostic tests for malaria are simple to use in remote community hospitals; however, their sensitivity decreases with low parasite densities in the host, and the chances of still testing positive even after the parasite has been cleared from the host are very high (Mfuh et al., 2019). While microscopy can differentiate between blood stages and provide pathophysiological and prognostic data that can be used to predict the severity of a malaria in expert hands (Amir et al., 2018), this is doubtful in practice at the operational level. Misdiagnosis, which leads to overtreatment and undertreatment of clinical malaria, has been identified as a growing concern that contributes to malaria persistence (Nyaoke et al., 2019; Oladosu & Oyibo, 2013; Onchiri et al., 2015). The persistence of clinical malaria in the community, as well as the dynamics of transmission, will always pose a challenge, causing malaria elimination to be delayed. There has been little research to link clinical malaria persistence to variation in topography and rainfall in areas such as Nyakach Sub-County, western Kenya. Therefore, it remains to be seen whether landscape heterogeneity will contribute to the persistence of the clinical malaria.

In Kenya, community case management of malaria is being pursued by community health volunteers who have been trained in malaria management using RDTs and AL (U.S. President's Malaria Initiative, 2022). Although algorithms of clinical symptoms indicative of malaria are

readily available along with expertise in reading blood smears and malaria RDTs (Siahaan et al., 2018a), health care providers may still rely on presumptive clinical diagnosis (Graz et al., 2011). This is problematic since, for example, malaria symptoms such as fever, prostration, and myalgia are similar to those of other common infectious diseases caused by viral and bacterial pathogens (Mfuh et al., 2019). For effective clinical malaria management and control, an accurate, reliable, and affordable diagnosis must be followed by effective and timely treatment. However, malaria symptoms overlap with those of other infectious diseases, making clinical diagnosis unreliable (Mfuh et al., 2019). It remains to be seen whether variations in clinical malaria manifestation are influenced by topography and seasonality.

Health care providers adhere to the "test and treat" policy to a high degree based on the availability of malaria diagnostics and antimalarial drugs. Antimalarial drug shortages and reduced parasitological diagnostics, have an impact on case management guidelines adherence (Kenya Malaria Control Programme, 2016). The goal of the Kenya National Malaria Control Programme is to reduce malaria febrile illness by 75%, with the ultimate goal of protecting 100% of residents living in malaria risk areas through malaria control interventions and treatment in accordance with guidelines (Kenya Malaria Control Programme, 2016), build malaria-eradication systems in targeted counties, improve malaria surveillance and information, and develop policies for the most effective malaria intervention implementation (Kenya Malaria Control Programme, 2019). At the community, residents with recent fever, 20% seek treatment at a health facility, while the remaining over 80% self-medicate at home (Kenya Malaria Control Programme, 2016). Despite the fact that the majority clinical malaria treatment is at the health facility, the proportion varies depending on the febrile residents treatment options (National

Malaria Control Programme, 2016). Whether variation in topography could be contributing to variation in clinical malaria treatment seeking patterns need to be determined.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Site

Nyakach Sub-County, Kisumu County, Western Kenya is at Latitude -.333333 and Longitude 34.9916667, near the shores of Lake Victoria. Based on previous research, the study area was divided into three eco-epidemiological zones and two seasons: wet and dry (Zhou et al., 2020).The study area was divided into three eco-epidemiological zones based on malaria prevalence and topographical features: lakeshore, hillside, and Highland plateau (Figure 3.1.1). The Lakeshore zone is located in the Lake Victoria basin and is distinguished by a flat plain and lower elevation of 1100-1200 m above sea level, which is frequented by flooding in the wet seasons. The Highland plateau zone is at an elevation of 1450 – >1600 m where stable larval habitats are more common. The Hillside zone is located between the Lakeshore and Highland plateau zones, at an elevation of 1300-1450 m. Hillside zone larval breeding habitats are uncommon.

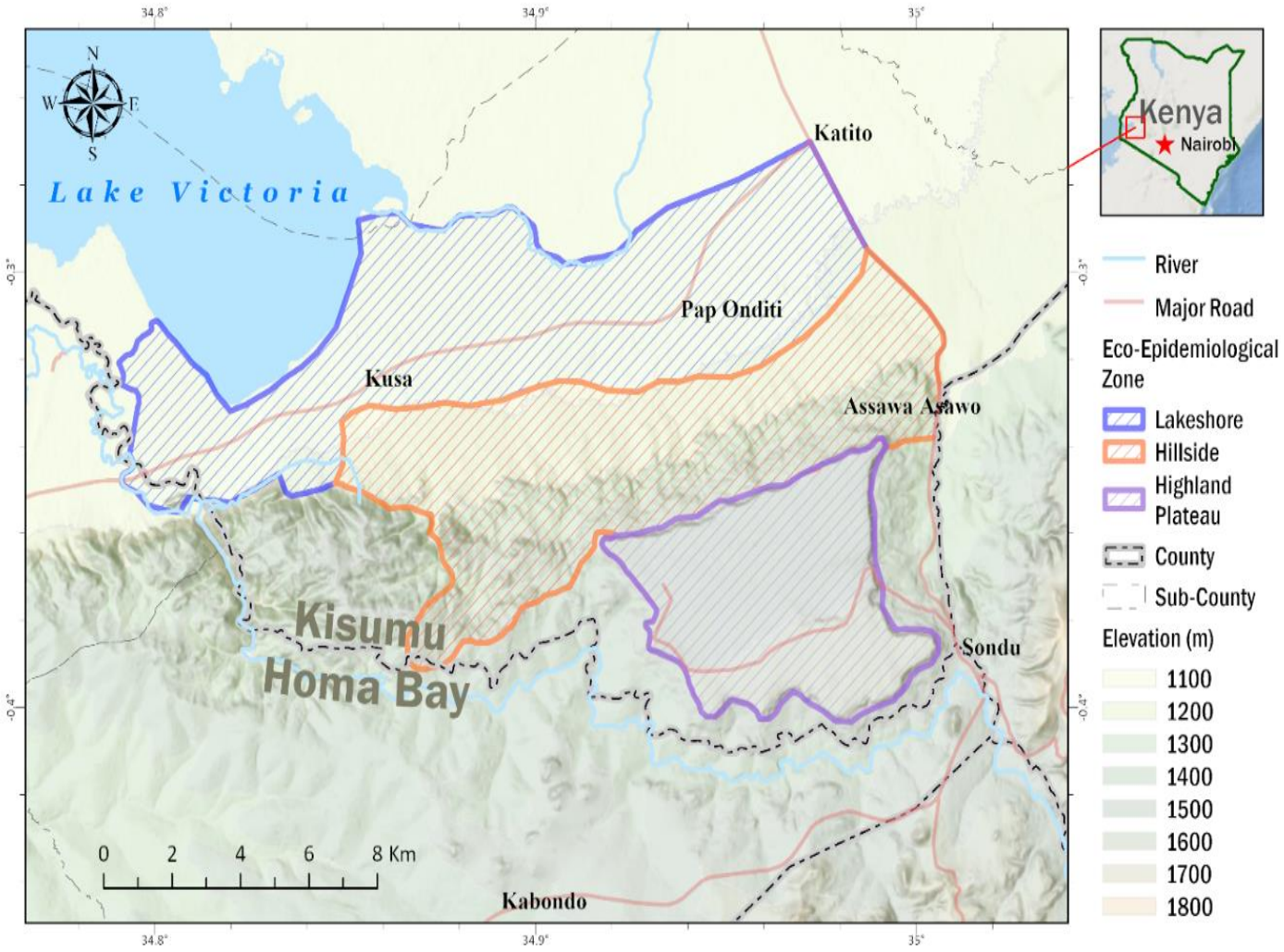

Figure 3.1.1. Map of Nyakach Sub-County, Western Kenya.
Blue shaded background: Lakeshore zone; brown shaded background: Hillside zone; and purple shaded background: Highland plateau zone.

3.2. Study Population

Nyakach Sub-County has an area of approximately 327 square kilometers and a projected population of 168,140 people living in 35,553 households at a population density of 460 people per square km (Kisumu County, 2018). This region's economic activities are primarily fishing, subsistence farming, rock mining, and small-scale trading. The malaria prevalence in Nyakach Sub-County is estimated to be 35%, with *Anopheles gambiae* and *Anopheles funestus* being the

primary vectors of Plasmodium infection (Mutero et al., 2020; Ng'ang'a et al., 2021). Malaria transmission generally peaks after the long rainy season in June. The dry season in November is associated with minimal transmission in western Kenya (Atieli et al., 2011; Essendi et al., 2019). The study participants for the influence of landscape heterogeneity on the prevalence of submicroscopic infection was 1,777, while the study population for the influence of landscape heterogeneity on the incidence of clinical malaria was 2,205.

3.3. Study Design

A cross-sectional study design was used to collect data on prevalence of submicroscopic infection in three Eco-epidemiologically distinct zones in Nyakach Sub-County, Kisumu in June and November in 2019 and 2020 during wet and dry season. Adjacent regions were topologically characterized as lakeshore, hillside and highland plateau zones.

A cross-sectional entomological survey was conducted along an altitudinal transect in three eco-epidemiological zones: lakeshore along the lakeside, hillside, and highland plateau in July (wet season) and November (dry season) of 2020 in Nyakach Sub-County, western Kenya.

A longitudinal study design was used to collect data on the incidences of clinical malaria through active case detection of malaria in three eco-epidemiologically distinct zones: Lakeshore, Hillside, and Highland plateau zones, between March 2020 and March 2021 in Nyakach Sub-County, western Kenya.

3.4. Sample size determination

The Fisher (1998) formula was used to determine the sample size.

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

Whereby: n = desired sample size and Z = 1.96 (95% confidence level). P = proportion of confirmed malaria while q = proportion confirmed malaria negative (1-P). d = standard error at 95% CI (0.05). The proportion confirmed malaria was estimated at 50%. Therefore, based on the given figures, the following will be the calculated sample size:

$$n = \frac{1.96^2 0.50(1-0.50)}{0.05^2}$$

$$n = 384$$

The minimum sample size for each study zones in each wet and dry season for the prevalence of submicroscopic infection was 384. There was no definitive approach for determining sample size for entomological indices; however, effect size was computed, and any significant differences reflected the true biological phenomenon. A minimum of 384 study participants were surveyed longitudinally for one year, to determine the incidence of clinical malaria among residents in each study zone during the wet and dry seasons. The Cohen's *f*2 measure of effect size was calculated, and any significant differences reflected the influence of seasonality and topography on entomological indices and clinical malaria incidence. The Cohen's *f*2 measure of effect size for multiple regression was defined as:

$$f^2 = \frac{R^2}{1-R^2}$$

Where R^2 is the squared multiple correlation (Statistics Solutions, 2013).

3.5. Inclusion and Exclusion Criteria

Only residents of Nyakach Sub-County were eligible for the study, and they were recruited with the help of Community Health Volunteers who work within the study areas. All the study participants provided written informed consent. Minors (under age of 18) provided assent with informed consent from the parents or guardians. Individuals who did not consent to participate in the study were excluded, as were children whose parents/guardians did not consent to participate.

Individuals of any age or gender were eligible to participate in the survey for the prevalence of submicroscopic *Plasmodium* infection. Individuals who did not consent to participate in the study were excluded, as were children whose parents/guardians did not consent to participate.

Individuals eligible to participate in the incidence of clinical malaria survey were only those with fever (body temperature $\geq 37.5^{o}C$) at the time of examination or feverish symptoms (chills/shivering, malaise, fatigue, muscle pain, joint pain, headache, irritability, nausea, vomiting, diarrhea, abdomen pain, loss of appetite, breathing difficulty, dizziness, coughing, and stomachache) and severe symptoms (impaired consciousness, prostration, multiple convulsions, respiratory distress (metabolic acidosis), circulatory collapse, jaundice, hemoglobinuria, abnormal bleeding, and pulmonary edema), 1-2 days before the exam, according to Ministry of Health Kenya (Kenya Malaria Control Programme, 2016) and WHO definition (WHO, 2013b). Residents who did not have a fever or malaria symptoms were excluded from the study. Individuals who did not consent to participate in the study, as well as febrile children whose parents/guardians did not consent to participate, were excluded.

3.6. Submicroscopic Infection Prevalence

3.6.1. Data collection

Field assistants and community health volunteers (CHVs) were recruited from the local community and trained on the use of Open Data Kit (ODK) application on an android tablet with a questionnaire for entering data requirements following an interview with the household head in the lakeshore, hillside and the highland plateau zone (Appendix I). A total of 1777 study participants took part in the study during the wet seasons of June 2019 and June 2020 (n=458 and n=388 study participants, respectively) and the dry seasons of November 2019 and November 2020 (n=456 and n=475, respectively).

Certified laboratory technicians collected blood samples from consenting individuals in the study with the assistance of CHVs for mobilization and field assistants for data entry into the tablet. A total of 1,777 finger-prick blood smears and filter paper dry blood spots (DBS) samples were collected for parasite examination via microscopy and RT-PCR, respectively. Blood samples were transported to the International Centre of Excellence for Malaria Research (ICEMR) laboratories in Homa Bay, Kenya for further analysis.

As potential confounding factors, questionnaire collected self-reported demographic data such as age, gender, LLIN use, household structure, primary occupation, and fever history. The LLIN use was defined as sleeping under an insecticide treated net. The wall material type was used to assess house structure categorized as brick/block, mud & wood, and mud & cement. Occupation was divided into four categories (farmer, commercial sales, child younger than working age, and unemployed). A febrile case, according to the WHO definition, was defined as an individual with

a body temperature of ≥37.5°C or complaints of fever and malaria like-symptoms prior to examination (WHO, 2013b).

3.6.2. Collection and processing of blood smear

A total of 1,777 blood smear samples were collected and examined (WHO, 2016a). Thick and thin blood films were stained with 10% Giemsa for 15 minutes before being examined (WHO, 2016b). Two certified microscopists examined the slides in oil immersion to identify and count the parasite species. If at least one asexual blood-stage malaria parasite was found on a slide, it was considered positive. Discrepancies in slide readings were confirmed by a third, more experienced technician for quality control.

3.6.3. Dried blood spots DNA extraction

The Chelex resin (Chelex-100) saponin method (Plowe et al., 1995) was used with minor modifications for *Plasmodium* parasite DNA extraction of 1,777 samples. In brief, GE Whatman Protein Saver card (Whatman 903 Protein Saver Card, LOT: 7102118W171) was used for the Dry Blood Spot (DBS). The DNA from the blood samples was extracted using Chelex resin (Chelex®-100) (Strøm et al., 2014). Using a sterile craft store puncher, a 3mm piece of blotted filter paper containing blood sample was punched and placed into a sterile 1.5ml Eppendorf tube. Before incubating at 4°C for 4 hours, 950µl of 1× Phosphate buffer saline (PBS) and 50µl of 10% saponin were added and mixed thoroughly. The mixture was centrifuged at 12000 rpm for 10 minutes at room temperature before the liquid content was discarded. Remaining saponin was washed away with 1ml PBS and spun at 12000 rpm for 5 minutes. The PBS was discarded, and the tube contents were spun for 15 seconds before the liquid component was removed with a

P200 pipette. At room temperature, the DNA was dried on filter paper for 15 minutes. Two hundred and fifty microliters (20%) of Chelex suspension were added, and the mixture was incubated for 10 minutes in an 85°C water bath. During the incubation period, the DNA-containing mixture was vortexed every 2 minutes to keep the extracted DNA suspended. The DNA was then transferred to a clean 0.5ml Eppendorf tube and stored at -20°C after the mixture was spun for 1 minute at 12000rpm.

3.6.4. Multiplex screening for *Plasmodium* parasite

As previously described (Shokoples et al., 2009; Veron et al., 2009), real-time polymerase chain reaction (RT-PCR) with species-specific 18s rRNA and probes was used to identify *P. falciparum* infections from 1,777 samples. The probe sequences of *P. falciparum* probe 5'FAM-CATAACAGACGGGTAGTCAT–MGB3', *P. malariae* probe 5'Hex-ATGAGTGTTTCTTTTAGATAGC-MGB3' and the *P. ovale* probe 5'NED-CGAAAGGAATTTTCTTATT-MGB3' were used. The primer sequences *P. falciparum* -forward-primer ATTGCTTTTGAGAGGTTTTGTTACTTT and *P. falciparum* reverse-primer GCTGTAGTATTCAAACACAATGAACTCAA *P. malariae* forward-primer AGTTAAGGGAGTGAAGACGATCAGA and *P. malariae* reverse-primer CAACCCAAAGACTTTGATTTCTCATAA *P. ovale* forward-primer AACCCAAAGACTTTGATTTCTCATAA and *P. ovale* reverse-primer CCGACTAGGTTTTGGATGAAAGATTTTT. In brief, RT-PCR was performed in a final volume of 12µl containing 2µl of sample DNA, 6µl of PerfeCTa® qPCR ToughMix™, Low ROX™ (Veron et al., 2009). Master mix (2X), 0.5µl of the species-specific probe, 0.4µl of the species-specific forward primers (10µM), 0.4µl of the species-specific reverse primers (10µM)

and 0.1µl of double-distilled water. The thermal profile used was 50 °C for 2 minutes, followed by 45 cycles of (95 °C for 2 minutes, 95 °C for 3 seconds, and 58°C for 30 seconds). As positive and negative controls for RT-PCR, three positive samples from the laboratory strain and three negative samples from blank filter paper were used. The RT-PCR amplification was carried out using the QuantStudio 3 Real-Time PCR System (ThermoFisher, Carlsbad, CA, USA), and positive results were displayed in the form of curves (Once the sample contains *Plasmodium* parasite DNA, the *Plasmodium*-specific primer will attach and terminate the amplification, emitting fluorescent light to indicate a positive sample) (Appendix II).

Submicroscopic infection was defined as an infection with *Plasmodium* detected by RT-PCR results but not by microscopy (Ochwedo et al., 2021; Vareta et al., 2020; Whittaker et al., 2021; Zhiyong et al., 2016).

3.6.5. Data analysis

The data was analyzed using the Statistical Package for Social Science (SPSS) Software Version 21.0. Chi-square test of independence was used to examine the relationship between submicroscopic malaria infection prevalence by topography and seasons. Fisher's exact test was used to compare *Plasmodium* species prevalence across seasons. Adjusted agreement between microscopy and RT-PCR results were measured using Cohen's kappa statistic, sensitivity, specificity, positive predicted value, negative predicted value, and diagnostic accuracy. The influence of topography and seasonality on the prevalence of submicroscopic infection was determined using univariate binary logistic regression and multivariate mixed effect binary logistic regression analyses. All risk factors were tested in the univariate analysis, and only those

with $p \leq 0.5$ were chosen and included in the multivariate analysis. In the multivariate analysis, variables with $p \leq 0.05$ were considered significant risk factors. Topography, seasonality, gender, age, bed net usage, wall type, bed net type, and symptoms were among the variables investigated. The Chi-square test, relative risk, and odds ratio (OR) with $p \leq 0.05$ were regarded as statistically significant.

3.7. Entomological Indices of Malaria

Entomological indices of malaria assessed were: *Anopheles* larval habitat availability, composition, and abundance, larval density, *Anopheles* vector composition and abundance, and sporozoite rates.

3.7.1. Larval habitat sampling

All potential larval habitats were sampled using a standard dipper (350 ml capacity, BioQuip Products, Inc., Compton, CA, USA) during the dry and wet sea- son. The scooped water was poured in a white plastic tray and carefully examined for Anopheles larvae. The sampling took place in the morning (09:00–12:00). The larvae were taxonomically identified using referenced keys, and the Anopheles larvae were separated from the Culicine larvae and counted separately [19, 20]. All larvae instars and pupae were sampled, counted, and recorded. The larvae collected were classified as early instars (L1 and L2) or late instars (L3 and L4). The larval density was estimated as the average number of larvae collected per dip. Anopheline larvae were transported to the ICEMR in Homa Bay and reared into adults in the insectary. The larvae were fed TetraMin® fish meal and kept at 27 ± 2 °C. Of all the larvae that survived to adults, further

identification was performed using taxonomic keys (Gillies & Coetzee, 1987). No sibling species identification by PCR analysis was performed.

3.7.2. Characterization of larval habitats

Larval habitats were categorized based on: landscape zones (lakeshore, hillside, highland plateau), larval habitat type (drainage ditch, river edge/reservoir shoreline, swamp, animal hoof print, tire track, manmade pond, natural pond, rock pool, water container, and brick pit), seasonality (dry and wet), land use type (i.e. environment surrounding the larval habitat), vegetation cover, substrate type, proximity to the nearest household, presence of predators and algae in the larval habitats, and larval habitat size as measured by larval habitat length, width, and depth. The habitats were further characterized as: drainage ditch, river edge, swamp, animal hoof print, tire track, manmade pond, natural pond/rain pool, rock pool, water container, and brick pit (Appendix III). The substrate was classified as sand/gravel, mud, or container. The distance to the nearest household was divided into four categories (less than 100 meters, 100-200 meters, over 200 meters). Habitat length and width were measured and recorded in meters. Depending on the size of the habitat, the average depth of water was measured with a meter stick at various locations. The presence or absence of algae was visually assessed in the habitat. The vegetation coverage and shade cover were calculated based on visual observation by estimating the percentage of the larval habitat covered. The natural vegetation and activities that occur on the land surrounding the larval habitats were used to categorize the land-use type, which included forest/shrub land for bushy areas with short trees, cultivated areas, pasture for grazing areas, and swamps.

3.7.2. Adult mosquito collection

Field assistants were trained before fieldwork on the use of ODK application in an android tablet with a questionnaire for entering data requirements (Appendix IV). Adult mosquito collection Adult mosquitos were collected using the pyrethrum spraying catch (PSC) method in each dry and wet season. After obtaining consent from the household head, mosquito collection and questionnaire survey were conducted. The PSC were carried out between 08:00 and 12:00 pm (Ndiath et al., 2011). The mosquitoes were collected and stored in 1.5-ml Eppendorf tubes with silica gel desiccant and cotton wool before being transported to ICEMR laboratory in Homa Bay for further analysis. *Anopheles* mosquitoes were identified taxonomically according to Coetzee (Coetzee, 2020; Gillies & Coetzee, 1987) and females stored on silica gel at room temperature pending further sibling species ID analysis. Data on potential confounders of mosquito abundance, such as LLIN ownership, household structure, land-use type surrounding the household, and distance from the household, were also collected. Self-reported data on, LLIN ownership, household structure, and land-use type surrounding the household, and distance from the household to the nearest mosquito habitat were collected in the study questionnaire (Appendix IV). The LLIN ownership was defined as owning at least one LLIN per house. The wall material type was used to evaluate the house's structure, and it categories into (stone/block/brick, mud & wood, and mud & cement types). The land use type around the house was classified as forest and shrub land, grassland, and cultivated land. The house's distance from the nearest breeding habitat was divided into three categories (100 meters, 100-200 meters, and >200 meters).

3.7.3. Mosquito DNA extraction

To separate the head, thorax, and abdomen of adult mosquitos, they were cut in half. As previously described, the Chelex resin (Chelex®-100) method was used to extract mosquito DNA (Musapa et al., 2013). In summary, the collected female *Anopheles* mosquitos' heads and thoraxes were cut off with a sterilized scalpel and placed in a sterile 1.5ml Eppendorf tube. In a 1.5ml tube, about 190ul of 1XPBS + 10ul of Saponin was added to the mosquito head + thorax, ground, and incubated at room temperature for 20 minutes. The mixture was centrifuged for 10 minutes at 12000 rpm before the supernatant was discarded. The pellets were suspended in 200ul of 1XPBS, centrifuged for 30 seconds at 12000 rpm, and the supernatant was removed with a P200 pipette before air drying for 15 minutes. The pellets were suspended in 100ul of 20 percent w/v Chelex 100 resin suspension in Chelex suspension in double distilled water, and the mixture was incubated for 10 minutes in an 85°C water bath on a floating rack before being vortexed for 2 minutes to suspend the extracted DNA. The mixture was then spun for 1 minute at 12000rpm before being transferred to a clean 0.5ml vial tube and stored at -20°C.

3.7.4. *Anopheles gambiae* and *An. funestus* identification

Speciation was accomplished using multiplex PCR in T100^{TM} Thermal Cycler (Bio-Rad, Hercules, CA, USA) with the primers previously listed (Cohuet et al., 2003; Scott et al., 1993). The species-specific primer sequences for *Anopheles gambiae* CTGGTTTGGTCGGCACGTTT, *Anopheles arabiensis* AAGTGTCCTTCTCCATCCTA, and Universal reverse primer GTGTGCCCCTTCCTCGATGT for all the forward primer specific for *An. gambiae* and *An. arabiensis.* The species-specific primer for *An. funestus* GCATCGATGGGTTAATCATG and the universal reverse primer TGT GAA CTG CAG GAC ACA T. Following protocols developed

for *An. gambiae s.l.* (Scott et al., 1993) and *An. funestus s.l.* (Koekemoer et al., 1999, 2002), members of the *An. gambiae* complex were identified to the species level using the polymerase chain reaction (PCR) method. About 3µl of parasite DNA was mixed with 6.5µl of DreamTaq Green PCR Master Mix (2X), 0.5µl of species-specific primer (10µM), 0.5µl of universal reverse primer (10µM), and 0.5µl of double-distilled water to make a final reaction volume of 12µl (varies based on the number of primers added). The PCR conditions were as follows: 3 minutes of initial denaturation at 94°C, 34 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, primer extension at 72°C for 45 seconds, and final extension at 72°C for 6 minutes. For PCR amplification, the T100^{TM} Thermal Cycler was used. The amplicons from *An. gambiae* (390 base pair), *An. arabiensis* (315 base pair), and *An. funestus* (505 base pair).were analyzed using gel electrophoresis in 1.5% w/v agarose gel.

To visualize the PCR products, 1.5 percent w/v gel solution was made by dissolving 0.6g of agarose in 40ml of 1X Tris-borate-EDTA (TBE) buffer, then boiled, cooled, and 2µl of 1.0M SmartGlow® was added, swirled, and dispensed in the gel tank. The loading wells were formed by placing the gel comb in the gel tank. Allowing the casted gel to set for 35 minutes before pipetting PCR product or amplicons and ladder into each well. For 25 minutes, electrophoresis was performed at a constant voltage of 120V. The amplicons were then visualized with the SmartDoc 2.0 System (E5001-SD by Accuris^{TM}) under a UV transilluminator (Barnes, 1994).

All mosquito specimens were tested for *Plasmodium* sporozoite infection using real-time PCR with species-specific 18s rRNA. Plasmodia infections (*P. falciparum, P. ovale*, and *P. malarae*) were detected in DNA samples using the TaqMan assay, as previously described (Shokoples et al., 2009; Veron et al., 2009).

3.7.5. Data analysis

Anopheles larval density was calculated by dividing the number of *Anopheles* larvae by the number of dips taken from each larval habitat. Negative binomial regression was run to predict *Anopheles* larval density based on topography and seasonality with habitat type, habitat size, vegetation cover, shade cover, predator presence, and algae presence as potential confounding factors. The negative binomial regression was run to predict number adult *Anopheles* vector abundance based on topography and seasonality with household structure type, and LLIN usage as potential confounders. The sporozoite infection rate was calculated by dividing the number of positive *Plasmodium* sporozoite mosquitos by the total number of mosquitos tested for sporozoite infections (Kilama et al., 2014; Mzilahowa et al., 2012; Soma et al., 2021; Tanser et al., 2003). To compare sporozoite rates across topography and among mosquito species, the Fisher's exact test was used. The predictors of malaria infections were identified using logistic regression. For all tests and all regression independent variables, the significant level was set at $p \leq 0.05$.

3.8. Clinical Malaria Cases

3.8.2. Study participation and data collection

Community health volunteers (CHVs) were trained on how to identify and record clinical malaria cases in each household, as well as how to take blood sample for ultra-sensitive malaria RDT and RT-PCR analysis. Clinical malaria case was defined as an individual with fever (body temperature $\geq 37.5^{o}$C) at the time of examination or feverish symptoms and other nonspecific uncomplicated symptoms (chills/shivering, malaise, fatigue, muscle pain, joint pain, headache, irritability, nausea, vomiting, diarrhea, abdomen pain, loss of appetite, breathing difficulty,

dizziness, coughing, and stomachache) and severe symptoms (impaired consciousness, prostration, multiple convulsions, respiratory distress (metabolic acidosis), circulatory collapse, jaundice, hemoglobinuria, abnormal bleeding, and pulmonary edema), 1-2 days before the examination, according to Ministry of Health Kenya (Kenya Malaria Control Programme, 2016) and WHO definition (WHO, 2013b). The Community Health Volunteer did a bi-weekly household visits to identify the febrile residents. The body temperature was taken and recorded taken. There were no recurrent cases of clinical malaria during the bi-weekly ACD of malaria survey. All incidence of clinical malaria incidences reported after the biweekly survey were considered new cases of clinical malaria.

The study questionnaire collected self-reported information on age, gender, and active fever, treatment-seeking method, primary occupation, and LLIN use as potential confounders (Appendix V). Individuals with active fever had an axillary temperature of ≥37.5^{o}C at the time of examination. The treatment seeking method (health seeking pattern) of the participants were divided into five categories (public hospital, private hospital, drug shops, traditional medication, and do nothing). Occupation was divided into seven categories (farmer, small-scale business, office worker, unemployed, student, non-school child, and others. The LLIN use was defined as sleeping under an insecticide treated net night before the survey.

3.8.3. Collection and processing of blood sample

Every resident's body temperature was taken by the CHVs in each household, and those with a body temperature of ≥37.5^{o}C or who complained of fever at the time of examination or clinical presentation of uncomplicated or severe malaria 1-2 days before the examination were recruited

for clinical malaria examination. Finger-prick blood samples were collected from febrile patients for parasite analysis using the ultra-sensitive Alere® malaria RDT (Reference number: 05FK140, Republic of Korea) and RT-PCR on dry blood spots. The DBS were transported to laboratory in ICEMR in Homa Bay, for further analysis.

Blood samples for the ultra-sensitive Alere® malaria RDT were collected and tested in accordance with the manufacturer's instructions (Standard Diagnostic, Inc. Republic of Korea). In brief, an alcohol swab was used to clean the finger area to be lanced. The lateral side of the finger was pricked with a sterile lancet. The initial drop of blood was wiped away with sterile gauze. The blood sample was placed in the circular end of a new disposable inverted cup (5µl)

The whole blood was poured into the round specimen well, making contact with the pad. In the square assay diluent well, four drops of assay diluent were dispensed. After 20 minutes, the test was read. The presence of one colored line within the result window at the "C" control violet line of the RDT cassette indicated a negative result. A negative result was indicated by the presence of two colored lines within the result window, one at the "C" control violet line and one at the "P.f" test navy blue line. The presence of a faint line, no matter how faint the result, was considered positive. If the "C" control violet line was not visible within the result window after the test, the result was deemed invalid.

In accordance with Ministry of Health Kenya guidelines, the CHVs prescribed antimalarial to confirmed malaria cases and referred residents who tested negative for malaria to the nearest health facility for further treatment. (Kenya Malaria Control Programme, 2016).

3.8.4. Influence of topography on incidences of clinical malaria diagnosis

Influence of topography on incidences clinical malaria diagnosis was conducted at health facilities frequented by febrile residents from the lowland lakeshore, hillside, and highland plateau. Participants in the study were given study cards with codes that were used to track febrile residents seeking treatment at the three health facilities across the topographical landscape. At the health facility, Patients were interviewed using a structured questionnaire related to fever and other malaria symptoms. This information was recorded on digital tablets using REDCap Survey software (Vanderbilt University) (Appendix VI). Patients were referred to the hospital laboratory for diagnostic testing, and study technicians stationed at the hospitals collected clinical and demographic data from them.

3.8.5. Processing of blood smears

At the health facilities, finger prick blood smears were collected from febrile residents of the lowland lakeshore, hillside, and highland plateau and microscopically examined. One blood smear was examined at the health facility laboratory and the other at ICEMR laboratory in Homa Bay for independent expert reading of blood smears. To identify and count the parasite species, thick and thin blood films were stained with 10% Giemsa for 15 minutes and examined using oil immersion under magnification x 1,000. If a slide contained at least one asexual blood-stage *Plasmodium* parasite, it was considered positive.

3.8.6. DNA extraction and screening for *Plasmodium* infection

Dried blood spots for DNA extraction was used to test sensitivity and specificity of the ultrasensitive malaria RDT. Chelex resin (Chelex-100) saponin method was used with slight

modifications (Plowe et al., 1995). *Plasmodium* species-specific primers and probes targeting 18S ribosomal RNA were used (Veron et al., 2009). PCR reaction volume was constituted as follows; 6 µl of PerfeCTa® qPCR ToughMix™, Low ROX™ Master mix (2X), 0.4 µl forward and reverse species-specific primers (10 µm), 0.5 µl of the species-specific probe, 0.1 µl of double-distilled water and 2 µl of parasite DNA. Thermocycler conditions were 50°C for 2 minutes, (95°C for 2 minutes, 95°C for 3 seconds, and 58°C for 30 seconds) for 45 cycles (QuantStudio™ 3 Real-Time PCR System). The RT-PCR amplification was carried out using the QuantStudio 3 Real-Time PCR System (ThermoFisher, Carlsbad, CA, USA).

Positive results were displayed in the form of curves (Appendix II). Once the sample contains Plasmodium parasite DNA, the *Plasmodium*-specific primer will attach and terminate the amplification, emitting fluorescent light to indicate a positive sample.

3.8.7. Data Analysis

The SPSS software Version 21.0 was used to analyze the data. The demographic profiles of the study participants were described using descriptive statistics. Multiple regression was used to predict clinical malaria cases across topography and seasonality. Sensitivity, specificity, positive predicted value, negative predicted value, diagnostic accuracy and Cohen's kappa were used to determine the adjusted agreement between ultrasensitive malaria RDT and RT-PCR results. The significant level was set at $p \leq 0.05$ for all tests and all regression independent variables.

3.9. Ethical Considerations

Academic approval was obtained from the School of Graduate Studies at Maseno University. Maseno University's Ethics Review Committee granted ethical approval for the study (Appendix VIII). The County Director of Health in Kisumu (Appendix IX) and Nyakach Sub-County commissioner (Appendix X) granted permission for the study to be carried out. The survey was open to all residents who were willing to participate in the study, regardless of their demographics. Residents who declined to participate or changed their willingness to participate at any point during the study were excluded. Prior to the study, all respondents provided written informed consent, and minors provided consent through their parents/guardians. The survey was carried out during the Covid-19 period, and all infection prevention and control protocols were followed (Ministry of Health, 2020).

3.9.2. Informed consent

Respondents provided written informed consent, and minors (Under 18 years of age) provided assent in a language they were comfortable with (English or Luo) before the study began (Appendix XI). The study participants were given ample time to make their decision, including time to consult with family members, as well as ample opportunity to consider whether or not to participate. Once participants agreed to participate in the study, they were given a copy of the consent form in the language of their choice to read or read out, and the purpose of the consent form was explained to them. They signed in the appropriate place on the informed consent form. On the form, the witness wrote their name, signature, and date.

3.9.3. Discomfort and risks

The finger-prick blood collection method causes slight discomfort. Sterile blood lancets (followed with sterile ethanol) was used. The procedures posed very minimal risk of being infected by other pathogens. The study participants' privacy was protected, and the confidentiality of all information provided by them during data collection was maintained. Additionally, the CHVs and the field assistants were training on culturally sensitive ways of reducing discomfort and embarrassment during the data collection.

3.9.4. Benefits

The study participants did not receive financial benefit from participation in the study, however, residents with fever or were ill, were redirected to the local clinic for care.

3.9.5. Confidentiality

The information obtained was kept in strict confidence and protected from unauthorized access. The identities of study participants were not used in any of the study's reports or publications. Study data recorded was entered into a secure database.

3.9.6. Potential problems and solutions

Concerns may be raised about the quality of routine public health data that may be used in the study. Technical assistance and quality control help over the study period was provided. First, the CHVs were trained during active case detection of malaria to improve malaria diagnosis accuracy. Second, technicians and the CHVs were helped in the community to collect cluster-specific clinical malaria incidence data.

CHAPTER FOUR

RESULTS

4.1. Influence of Topography and Seasonality on Submicroscopic Infection

4.1.1. Demographic characteristics of the study population

A total of 1777 study participants took part in the study during the wet seasons of June 2019 and June 2020 (n=458 and n=388 study participants, respectively) and the dry seasons of November 2019 and November 2020 (n=456 and n=475, respectively). There were no significant differences in the number of study participants residing in the lakeshore, hillside and highland plateau topographical areas ($\chi^2 = 4.142$, df = 6, $p = 0.657$) (**Table 4.1.1**). Study participants were more frequently female than male ($\chi^2 = 8.448$, df = 3, $p=0.038$), equal to and older than 15 years ($\chi^2 = 34.583$, df = 6, $p<0.0001$), and occupied a household that included three or less residents ($\chi^2 = 8.448$, df = 3, $p=0.038$). Reported use of long lasting insecticide treated bed nets was 95.4% at the time of study enrolment. There were minor changes in bed net use according to season and year, but self-reported bed net use was greater than 90% in all four cross-sectional surveys (**Table 4.1.1**).

Table 4.1.1. Demographic information of study population across season

Factor		Enrolment		Season								χ^2 Value	*p*-value
				Rainy (June-19)		Dry (Nov-19)		Rainy (June-20)		Dry (Nov-20)			
				N= 458		N= 456		N= 388		N= 475			
		n	%	n	%	n	%	n	%	n	%		
Topography	Lakeshore	622	35.0	155	33.8	167	36.6	144	37.1	156	32.8	4.142	0.657
	Hillside	595	33.5	151	33.0	158	34.6	123	31.7	163	34.3		
	Plateau	560	31.5	152	33.2	131	28.7	121	31.2	156	32.8		
Sex	Male	674	37.9	160	65.1	181	39.7	167	43	166	34.9	8.448	0.038
	Female	1103	62.1	298	34.9	275	60.3	221	57	309	65.1		
Age	<5 years	241	13.6	75	16.4	50	11.0	36	9.3	80	16.8	34.583	< 0.0001
	5 - <15 years	533	30.0	99	21.6	158	34.6	135	34.8	141	29.7		
	≥15 years	1003	56.4	284	62.0	248	54.4	217	55.9	254	53.5		
Household size (Individuals)	≤ 3	849	47.8	267	58.3	154	33.8	164	42.3	264	55.6	133.525	< 0.0001
	4-5	691	38.9	163	35.6	180	39.5	175	45.1	173	36.4		
	>5	237	13.3	28	6.1	122	26.8	49	12.6	38	8.0		
Education level	Never attended school	88	5.0	10	2.2	31	6.8	28	7.2	19	4.0	87.675	< 0.0001
	Younger than school age	164	9.2	54	11.8	40	8.8	23	5.9	47	9.9		
	Primary school	952	53.6	187	40.8	283	62.1	223	57.5	259	54.5		
	Secondary school	458	25.8	173	37.8	78	17.1	93	24	114	24.0		
	College & above	115	6.5	34	7.4	24	5.3	21	5.4	36	7.6		
Occupation/ income generating activity	Farmer	521	29.3	177	38.6	135	29.6	70	18.1	139	29.3	695.252	< 0.0001
	Commercial sales	252	14.2	70	15.3	54	11.8	74	19.1	54	11.4		
	Unemployed	104	5.9	36	7.9	24	5.3	21	5.4	23	4.8		
	Child younger than working age	900	50.6	175	38.2	243	53.3	223	57.4	259	54.5		
Bed net ownership	Yes	1699	95.6	418	91.3	448	98.2	367	94.6	466	98.1	36.151	< 0.0001
	No	78	4.4	40	8.7	8	1.8	21	5.4	9	1.9		

Chi square test of independence was used to determine difference in the demographic characteristics across the seasonality. Comparison based on demographic traits. Given the differences in the wet and dry seasons, the seasons were examined separately.

4.1.2. Prevalence of submicroscopic malaria infection by topography and seasonality

Out of the 1,777 samples, the prevalence of submicroscopic infection was 14.2% (253/1,777). The prevalence of malaria infection was 3.7% (66/1,777) by microscopy and 18% (319/1,777) by RT-PCR, respectively. Submicroscopic infections were significantly different across seasonality (χ^2 = 17.374, df =3, p<0.0001) with high infection in the dry season of November 2019 (19.7%, n=456), followed by the rainy season of June 2020 (13.9%, n=458). Submicroscopic *P. falciparum* infection prevalence showed significant topographical variation (χ^2 =39.344, df=2, p<0.0001), with highest infection in the lakeshore zone (20.6%) followed by the hillside zone (13.6%) and highland plateau zone (9.1%) **(Figure 4.1.1)**. The highest sub-microscopic prevalence was observed in residents of the lakeshore zone in the 2019 dry season (29.9%, n=167) and 2020 and 2019 rainy seasons (21.5%, n=144 and 18.1%, n=155, respectively) as compared to the hillside and the highland plateau zones **(Figure 4.1.1)**. The prevalence of submicroscopic infection was higher in the 2019 dry season due to changes in weather patterns, with the dry season being shorter than expected.

Blood smear microscopy exclusively identified *P. falciparum* infections, whereas RT-PCR identified *P. malariae* and *P. ovale* mono-infections and co-infections with *P. falciparum*. *Plasmodium malariae* and *P. ovale* were detected in less than 1% of blood samples **(Table 4.1.2)**.

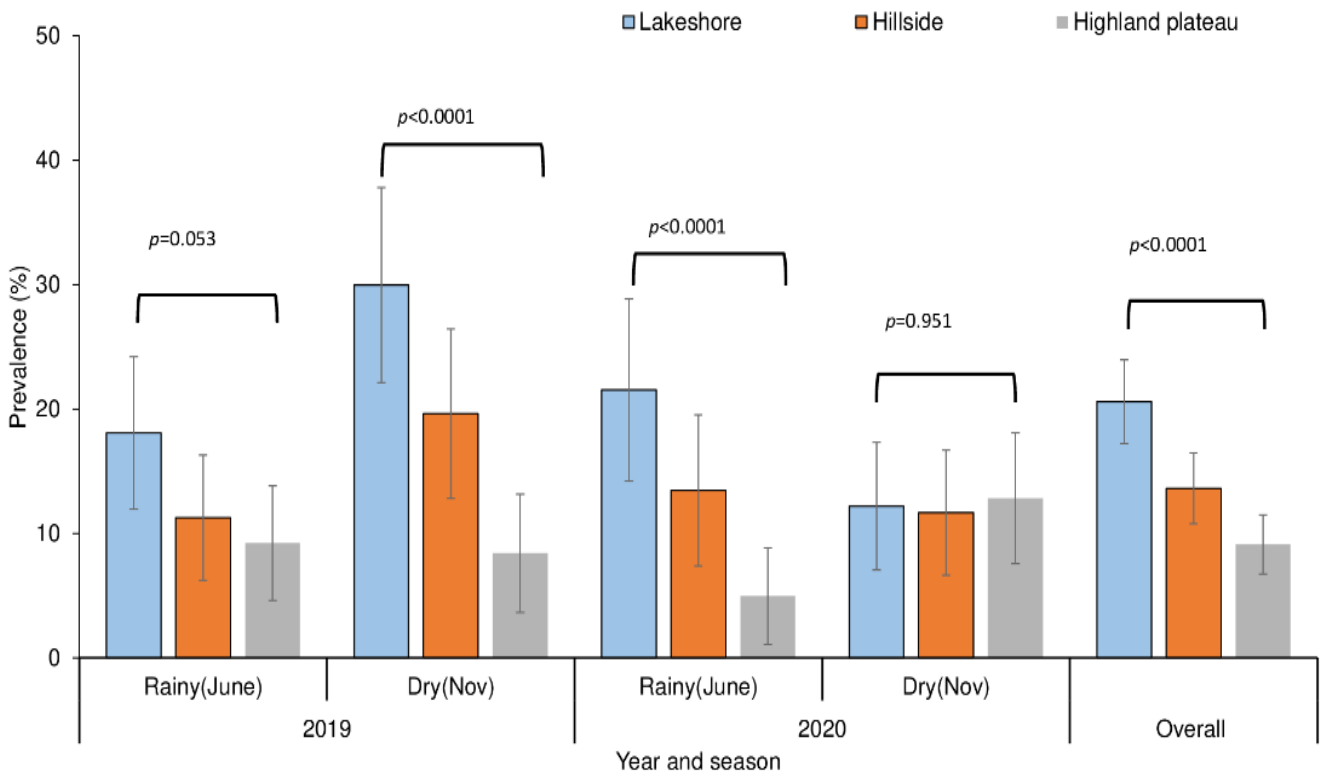

Figure 4.1.1. Submicroscopic infection by season and topographical zone.
The prevalence of submicroscopic infection was based on the seasons across topography and the overall infection across topography. The error bars represent 95% confident intervals. Chi square test of independence tested for differences in submicroscopic *Plasmodium* infection across topographical zones and seasons

Table 4.1.2. *Plasmodium* infection prevalence by season and diagnostic test

Diagnosis	*Plasmodium* species	Season				χ^2-value	*p*-value
		Wet (June-19) Infection (95% CI)	**Dry (Nov-19)** Infection (95% CI)	**Wet (June-20)** Infection (95% CI)	**Dry (Nov-20)** Infection (95% CI)		
Enrolment		458	456	388	475		
Microscopy	***P. falciparum***	3.9 (2.1, 5.7)	4.8 (2.9, 6.8)	3.8 (1.9, 5.7)	2.3 (1.0, 3.7)	4.252	0.236
RT-PCR	***P. falciparum***	15.3 (12.0, 8.6)	23.9 (20.0, 27.8)	15.7 (12.1, 19.4)	11.4 (8.5, 14.2)	27.823	< 0.0001
	P. malariae	0.4 (0.17, 1.04)	0.2 (0.21, 0.65)	0.4 (0.17, 1.04)	0.2 (0.20, 0.62)	1.154	0.817
	P. ovale	0.2 (0.21, 0.64)	0.4 (0.16, 1.00)	0.6 (0.09, 1.40)	0.6 (0.08, 1.35)	1.612	0.704
	P. falciparum* + *P. malariae	0.4 (0.16, 1.04)	0	0.2 (0.21, 0.65)	0.4 (0.16, 1.01)	2.163	0.659
	P. falciparum* + *P. ovale	0.4 (0.16, 1.04)	0	0.5 (0.20, 1.23)	0.2 (0.20, 0.62)	2.571	0.491
	Total	16.8 (12.2, 19.8)	24.6 (20.4, 28.2)	17.8 (15.6, 22.9)	12.8 (9.8, 16.0)	21.821	< 0.0001
Submicroscopic infections		12.9 (9.8, 16.0)	19.7 (16.0, 23.4)	13.9 (10.5, 17.4)	10.5 (7.8, 13.3)	17.374	< 0.0001

Fisher's exact test was used to compare *Plasmodium* infection prevalence across seasons.

4.1.3. Influence of topography and seasonality to submicroscopic infection

The topography and seasonality associations to submicroscopic infection were found to be significant in multivariate analysis (**Table 4.1.3**). In both the rainy and dry seasons, the likelihood of submicroscopic infection was higher in the lakeshore (AOR: 2.71, 95% CI=1.85-3.95; p<0.0001) and hillside (AOR: 1.74, 95% CI=1.17–2.61, p=0.007) than in the highland plateau zones. The dry season of 2019 (AOR: 1.69, 95% CI=1.12–2.54, p=0.012) had a higher likelihood of submicroscopic infection than the dry season of 2020. Lakeshore zone residency and the wet season were associated with an increased risk of submicroscopic malaria infection (**Table 4.1.3**).

Table 4.1.3. Predictive factors associated with submicroscopic malaria infection

Risk factor	Category	Infection n (%)	Univariate		Multivariate	
			OR (95% CI)	*p*-value	AOR (95% CI)	*p*-value
Topography	Lakeshore	128 (20.6)	3.04 (2.11, 4.37)	<0.0001	2.71 (1.85, 3.95)	<0.0001
	Hillside	81 (13.6)	1.85 (1.26, 2.72)	0.002	1.74 (1.17, 2.61)	0.007
	Plateau	44 (7.9)	1			
Season	Wet (2019)	59 (12.9)	1.26 (0.84, 1.88)	0.263	1.20 (0.78, 1.83)	0.416
	Dry (2019)	90 (19.7)	2.09 (1.44, 3.03)	<0.0001	1.69 (1.12, 2.54)	0.012
	Wet (2020)	54 (13.9)	1.37 (0.91, 2.07)	0.129	1.41 (0.85, 2.35)	0.182
	Dry (2020)	50 (10.5)	1			
Age group	<5 years	19 (7.9)	0.58 (0.35, 0.96)	0.034	0.54 (0.322, 0.89)	0.017
	5-<15years	105 (19.7)	1.66 (1.25, 2.20)	<0.0001	1.57 (1.17, 2.09)	0.002
	≥15 years	129 (12.9)	1			
Sex	Female	141 (12.8)	0.74 (0.56, 0.96)	0.025	0.74 (0.56, 0.96)	0.025
	Male	112 (16.6)	1			
Bed net usage	No net	14 (17.9)	1.34 (0.74, 2.42)	0.339	1.66 (0.88, 3.10)	0.115
	Use net	239 (14.1)	1			
Wall type	Brick/block	40 (11.4)	0.61 (0.39, 0.95)	0.030	0.64 (0.41, 1.00)	0.049
	Mud	160 (14.2)	0.78 (0.55, 1.09)	0.144	0.63 (0.42, 0.98)	0.044
	Mud & cement	53 (17.6)	1			
Symptoms	Yes	189 (12.7)	0.51 (0.37, 0.70)	<0.0001	0.69 (0.48, 0.99)	0.048
	No	64 (22.1)	1			

Univariate binary logistic regression and multivariate mixed effect binary logistic regression model predicted influence of topography and seasonality on the prevalence of submicroscopic infection. Since more females than males were recruited in the study, the analysis was adjusted for gender

4.1.4. Influence of topography on submicroscopic *Plasmodium* infection

The wet and dry seasons had a significant influence on submicroscopic infection across topographic zones. In the lakeshore zone, the rainy season of 2020 had 3.4 times the odds of submicroscopic infection than the dry season (AOR=3.40, 95% CI=1.57-7.36, p=0.002) **(Table 4.1.4)**.

Children under the age of 5 years old in the lakeshore zone had a lower risk of submicroscopic infection than residents over the age of 15 years old (AOR: 0.23, 95% CI=0.10-0.57, p=0.002). Furthermore, school-aged children (5-<15 years old) in the hillside (RR: 0.56, 95% CI=0.39-0.82, p=0.002) and highland plateau zones (RR: 0.25, 95% CI=0.14-0.45, p<0.0001) had a lower risk of submicroscopic infection than those in the lakeshore zone. In the lakeshore zone, females had a lower likelihood of submicroscopic infection than males (AOR: 0.67, 95% CI=0.45-0.98, p=0.042), but the hillside (p=0.102) and highland plateau (p=0.812) zones there was no significant difference in the risk of submicroscopic infection between the males and the females **(Table 4.1.4)**.

The relative risk of submicroscopic infection among females was lower in the hillside (RR: 0.66, 95% CI 0.46-0.93, p=0.018) and highland plateau (RR: 0.45, 95% CI=0.30-0.68, p<0.0001) zones compared to the lakeshore zone. Males in the hillside (RR: 0.67, 95% CI=0.46-0.97, p=0.030) and highland plateau (RR: 0.30, 95% CI=0.30-0.68, p<0.0001) zones were less likely to be infected than males in the lakeshore zones **(Table 4.1.4)**.

Although people who did not use ITNs in the highland plateau zone had higher likelihood of submicroscopic infections than those who did (AOR: 3.37, 95% CI=1.19-9.54, p=0.022), there was no significant difference in the likelihood of infection between ITN users and non-users in the lakeshores (p=0.523) and hill side zones (p=0.958)**.** The risk of sub-microscopic infection among ITN users was lower in the hillside (RR: 0.67, 95% CI=0.52-0.87, p=0.002) and highland plateau zones (RR: 0.35, 95% CI=0.25-0.50, p<0.0001), than in the lake zone **(Table 4.1.4)**.

Residency in mud wall houses on the hillside and highland plateau had a lower risk of submicroscopic infection than those who lived in lakeshore zones, with risk factors of 0.48 (RR: 0.48, 95% CI=0.34-0.67, p<0.0001) and 0.38 (RR: 0.38, 95% CI=0.26-0.57, p<0.0001), respectively **(Table 4.1.4)**.

Table 4.1.4. Influence of topography on factors associated with submicroscopic infection

Risk factor	Details	Lakeshore zone[1]			Hillside zone			Highland plateau zone			Risk ratio[2]			
		Infection (n, %)	AOR (95% CI)[1]	*p*-value	Infection (n, %)	AOR (95% CI)[1]	*p*-value	Infection (n, %)	AOR (95% CI)[1]	*p*-value	Hillside (95% CI)[2]	*p*-value	Plateau (95% CI)[2]	*p*-value
Season	Wet (2019)	28 (18.1)	1.5 (0.77, 3.05)	0.225	17 (11.3)	1.11 (0.50, 2.46)	0.795	14 (9.2)	0.68 (0.28, 1.70)	0.414	0.78 (0.49, 1.25)	0.296	0.30 (0.14, 0.60)	0.0002
	Dry (2019)	50 (29.9)	2.54 (1.26, 5.09)	0.009	31 (19.6)	1.69 (0.84, 3.42)	0.143	9 (6.9)	0.77 (0.32, 1.83)	0.548	0.57 (0.35, 0.92)	0.018	0.42 (0.23, 0.74)	0.002
	Wet (2020)	31 (21.5)	3.4 0 (1.57, 7.36)	0.002	17 (13.8)	1.11 (0.43, 2.90)	0.829	6 (5.0)	0.16 (0.04, 0.63)	0.009	0.56 (0.31, 1.00)	0.049	0.25 (0.11, 0.57)	0.0003
	Dry (2020)	19 (12.2)	1		16 (9.8)	1		15 (9.6)	1		0.74 (0.43, 1.25)	0.251	1.19 (0.61, 2.32)	0.610
Age	<5 years	9 (10.6)	0.23 (0.10, 0.57)	0.002	6 (7.7)	0.49 (0.18, 1.28)	0.149	4 (5.1)	0.57 (0.16, 2.01)	0.381	0.73 (0.27, 1.95)	0.522	0.48 (0.16, 1.51)	0.199
	5-<15 years	61 (31.0)	1.02 (0.57, 1.83)	0.943	32 (17.5)	1.14 (0.62, 2.13)	0.673	12 (7.8)	0.96 (0.41, 2.24)	0.915	0.56 (0.39, 0.82)	0.002	0.25 (0.14, 0.45)	<0.0001
	≥15 years	58 (17.1)	1		43 (12.9)	1		28 (8.5)	1		0.75 (0.52, 1.09)	0.128	0.50 (0.33, 0.76)	0.001
Sex	Female	69 (18.0)	0.67 (0.45, 0.98)	0.042	44 (11.8)	0.67 (0.42, 1.08)	0.102	28 (8.1)	1.08 (0.57, 2.05)	0.812	0.66 (0.46, 0.93)	0.018	0.45 (0.30, 0.68))	<0.0001
	Male	59 (24.8)	1		37 (16.6)	1		16 (7.5)	1		0.67 (0.46, 0.97)	0.030	0.30 (0.18, 0.51)	<0.0001
Bed net usage	No net	6 (24.0)	1.39 (0.50, 3.8)	0.523	2 (11.1)	1.04 (0.22, 5.00)	0.958	6 (17.1)	3.37 (1.19, 9.54)	0.022	0.46 (0.11, 2.03)	0.502	0.71 (0.26, 1.96)	0.512
	Use net	122 (20.4)	1		79 (13.9)	1		38 (7.2)	1		0.67 (0.52, 0.87)	0.002	0.35 (0.25, 0.50)	<0.0001
Wall type	Block	17 (15.9)	0.91 (0.43, 1.91)	0.798	13 (17.1)	1.18 (0.53, 2.65)	0.682	10 (6.0)	0.55 (0.15, 2.06)	0.375	1.12 (0.61, 2.25)	0.823	0.38 (0.18, 0.79)	0.007
	Mud & wood	90 (22.3)	1.42 (0.81, 2.50)	0.221	40 (10.7)	0.68 (0.37, 1.24)	0.209	28 (8.6)	0.73 (0.23, 2.37)	0.601	0.48 (0.34, 0.67)	<0.001	0.38 (0.26, 0.57)	<0.0001
	Mud &	21	1		28	1		6	1		1.02	0.920	0.50	0.152

	Cement	(18.8)			(19.2)			(9.3)			(0.61, 1.70)		(0.18, 1.36)	
Symptoms	Asymptomatic	86 (18.4)	0.85 (0.49, 1.46)	0.558	63 (12.2)	0.56 (0.27, 1.16)	0.118	34 (7.1)	1.18 (0.37, 3.78)	0.776	0.66 (0.49, 0.89)	0.125	0.54 (0.40, 0.75)	<0.0001
	Symptomatic	42 (27.1)	1		18 (23.1)	1		10 (7.1)	1		0.29 (0.19, 0.44)	<0.001	0.09 (0.03, 0.23)	<0.0001
Occupation/ income generating activity	Farmer	28 (15.9)	0.37 (0.18, 0.75)	0.006	16 (9.2)	0.54 (0.25, 1.15)	0.109	16 (9.4)	1.36 (0.55, 3.35)	0.500	0.58 (0.32, 1.03)	0.058	0.59 (0.33, 1.05)	0.067
	Commercial sales	9 (13.0)	0.27 (0.11, 0.68)	0.005	15 (16.1)	1.11 (0.48, 2.56)	0.805	6 (4.1)	0.58 (0.15, 2.06)	0.171	0.59 (0.33, 1.05)	0.584	0.32 (0.09, 1.12)	0.056
	Child younger than working age	71 (25.0)	0.31 (0.14, 0.68)	0.003	35 (15.1)	1.04 (0.42, 2.57)	0.927	16 (7.1)	3.71 (1.00, 13.79)	0.050	0.60 (0.42, 0.86)	0.006	0.28 (0.17, 0.48)	<0.0001
	Unemployed	20 (21.5)	1		15 (15.6)	1		9 (9.9)	1		0.73 (0.40, 1.33)	0.299	0.46 (0.22, 0.96)	0.030

[1]Multivariate binary logistic regression model used for risk factor analysis across topographical zones

[2]Significance by $\chi 2$ test for risk ratio of submicroscopic malaria infection according to topographic zone with Lakeshore zone as reference. Analysis adjusted for gender

4.2. Influence of Topography and Seasonality on Entomological Indices of Malaria

4.2.1. Larval habitats availability by topography and seasonality

During the study, 10 different types of larval habitats were identified, with a total of 1315 larval habitats encountered and recorded. The most common habitats were 359 (27.3%) drainage ditches, 243 (18.5%) man-made ponds, 163 (12.4%) brick pits, 147 (11.2%) tire truck, 107 (8.1%) water container, 100 (7.6%) swamp, 93 (7.1%) natural pond, 49 (3.7%) river edge, 36 (2.7%) animal hoof print, and 18 (1.4%) rock pool.

The availability of larval habitats differed significantly across topographical zones (χ^2=616.351, df=18, p<0.0001). The lakeshore zone had the most larval habitats (40.6%) of the 1315 identified, followed by the highland plateau zone (38.6%), and then the hillside zone (20.8%) (**Figure 4.2.1)**. The larval habitat availability differed significantly across seasonality (χ^2=38.815, df=9, p<0.0001). The wet season had the most larval habitats (59.8%) of the total 1315 habitats identified, followed by the dry season (40.2%) (**Figure 4.2.2)**.

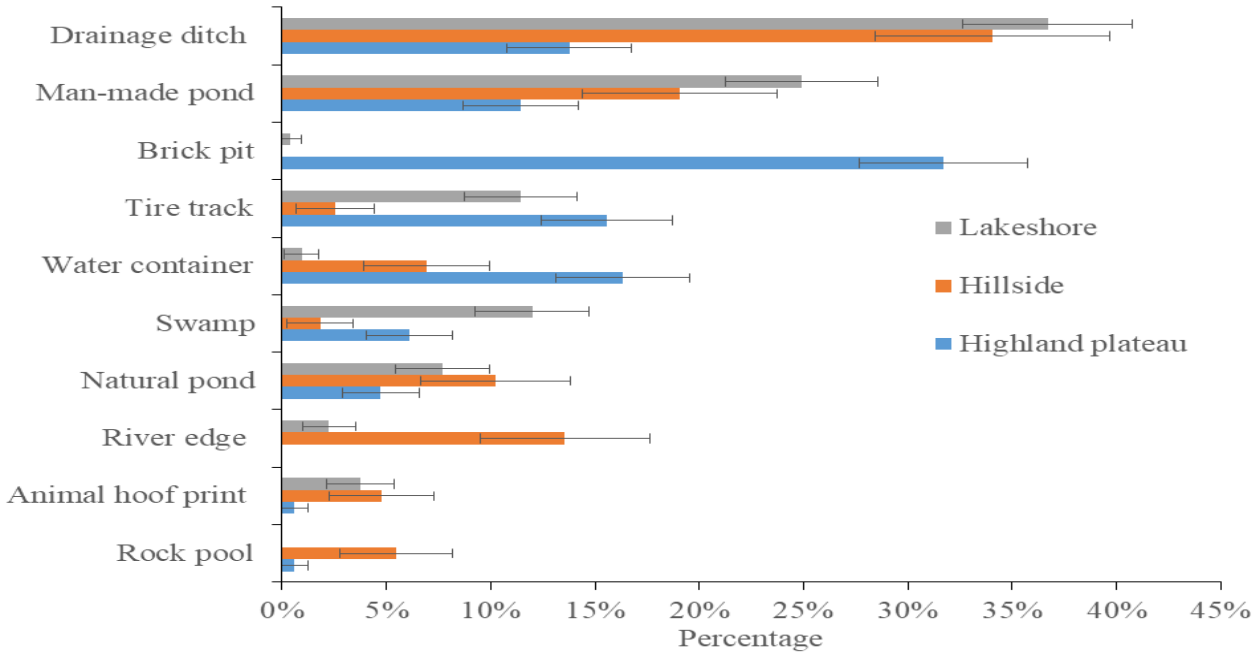

Figure 4.2.1. Larval habitat availability across topographical zones.
The error bars represent 95% confident intervals. *Anopheles* larval habitat distribution in lakeshore, hillside, and highland plateau zones of Nyakach Sub-County, western Kenya

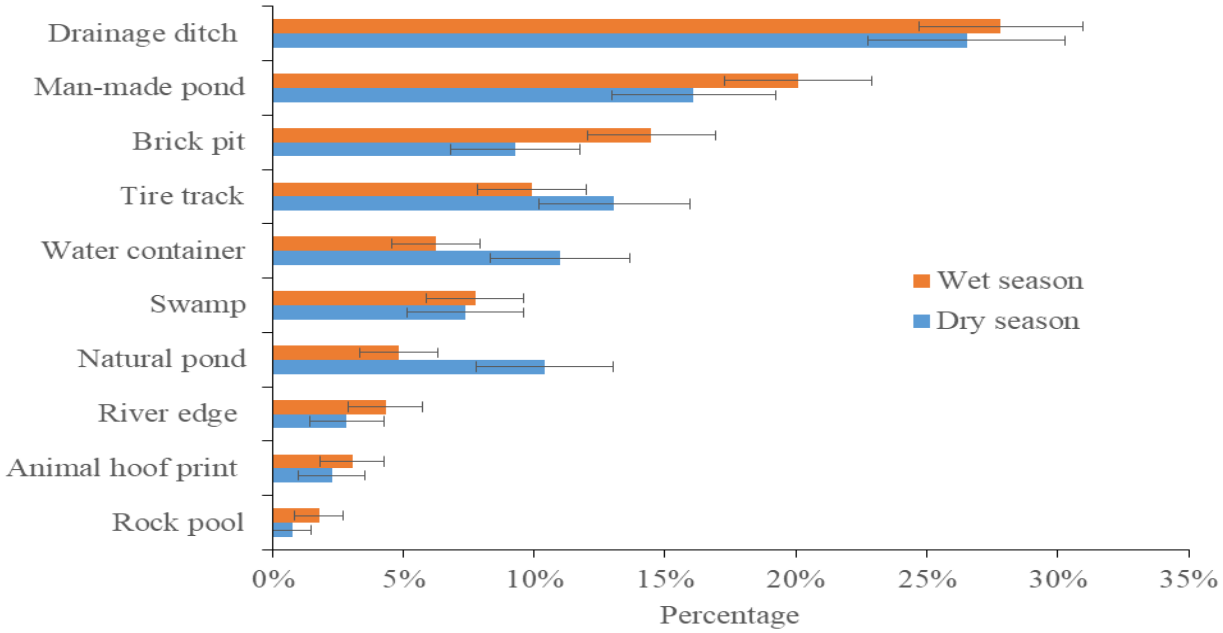

Figure 4.2.2. *Anopheles* larval habitat availability by seasonality.
The error bars represent 95% confident intervals. *Anopheles* larval habitat distribution during the wet and dry seasons in Nyakach Sub-County, western Kenya

4.2.2. Influence of topography on *Anopheles* larvae composition and abundance

There was a significant difference in the composition of *Anopheles* larvae in the larval habitat types across topography. In the lakeshore zone, the *An. gambiae s.l.,* (N=1500) composition differed significantly across the habitats types [H (9) =63.81, $p<0.0001$], with man-made ponds (31.3%), drainage ditches (29.9%) and the swamps (16.3%) having the highest composition. The composition of the *A. funestus* (N=30) was highest in man-made ponds (36.7%), drainage ditches (23.3%), and swamps (23.3%) ($p<0.0001$). *Anopheles coustani* (N=15) was found only in man-made ponds (46.7%), ($p<0.0001$) **(Table 4.2.1**).

In the hillside zone; the *An. gambiae s.l.,* (N=479) composition differed significantly across the habitats types [H (9) = 942.9, $p<0.0001$], with drainage ditches (42.1%) having the highest composition. The composition of the *An. funestus* (N=7) was highest in man-made ponds (71.4%), ($p<0.0001$). *An. coustani* (N=20) was highest in the natural ponds (55.0%) ($p<0.0001$) **(Table 4.2.1**).

In the Highland plateau zone; the *An. gambiae s.l.,* (N=419) composition differed significantly across the habitats types [H (9) =1046, $p<0.0001$], with man-made pond (18.1%) having the highest composition. The composition of the *An. funestus* (N=19) was highest in brick pit (36.8%), ($p<0.0001$). *An. coustani* (N=11) was highest in the brick pit (90.9%) ($p<0.0001$) **(Table 4.2.1**).

Drainage ditch had the highest abundance (28.9%) of the total 2,505 *Anopheles* larvae, followed by man-made pond (26.6%), swamp (12.6%), brick pit (8.3%), tire truck (7.9%), natural pond

(6.8%), animal hoof print (4.6%), river edge (3.0%), water container (0.7%), and rock pools (0.4%) **(Table 4.2.1)**. In total, 2,505 immature *Anopheles* mosquitoes were morphologically identified belonging to 4 species, which included *An. gambiae s.l.,* (95.7%, n=2398), *An. funestus* (2.2%, n=56), *An. coustani* (1.8%, n=46) and *An. pharoensis* (0.2%, n=5). The drainage ditch (29.1%) and man-made pond (26.7%) had the highest compositions of the 2398 *An. gambiae s.l.* species identified ($p<0.0001$); the composition of the 56 *An. funestus* found was highest in the man-made pond (28.6%) and the drainage ditch (27.6%) ($p<0.0001$); natural (30.4%) and man-made pond (23.9%) pond habits contributed the most to the 46 *An. coustani* composition ($p<0.0001$); and the *An. pharoensis* were only found in the drainage ditch **(Table 4.2.1)**.

Table 4.2.1. *Anopheles* larvae composition and abundance

***Anopheles* larvae identified**		**Habitat type (n, %)**										**Total**	**H-test[1]**	***p*-value**
		Drainage	**River edge**	**Swamp**	**Animal hoof print**	**Tire track**	**Man-made pond**	**Natural pond**	**Rock pool**	**Water container**	**Brick pit**			
Lakeshore	***An. gambiae***	448 (29.9)	28 (1.7)	244 (16.3)	82 (5.5)	161 (10.7)	469 (31.3)	68 (4.5)	0	0	0	1500	63.81	<0.0001
	An funestus	7 (23.3)	2 (6.7)	7 (23.3)	0	3 (10.0)	11 (36.7)	0	0	0	0	30	64.49	<0.0001
	An. pharoensis	4 (100)	0	0	0	0	0	0	0	0	0	4	39	<0.0001
	An. coustani	3 (20.0)	0	3 (20.0)	0	0	7 (46.7)	2 (13.3)	0	0	0	15	40.56	<0.0001
	Total	462 (30.3)	30 (1.9)	254 (16.4)	82 (5.3)	164 (10.6)	487 (30.6)	70 (4.5)	0	0	0	1549	413.8	<0.0001
Hillside	***An. gambiae***	202 (42.1)	45 (9.4)	29 (6.1)	25 (5.2)	17 (3.5)	95 (19.8)	55 (11.5)	9 (1.9)	2 (0.4)	0	479	942.9	<0.0001
	An funestus	1 (14.3)	0	1 (14.3)	0	0	5 (71.4)	0	0	0	0	7	35.98	<0.0001
	An. pharoensis	0	0	0	0	0	0	0	0	0	0	0	NA	NA[2]
	An. coustani	4 (20.0)	1 (5.0)	0	0	0	4 (20.0)	11 (55.0)	0	0	0	20	69.03	<0.0001
	Total	207 (40.9)	46 (9.1)	30 (5.9)	25 (4.9)	17 (3.4)	104 (20.6)	66 (13.0)	9 (1.8)	2 (4.0)	0	506	133.44	<0.0001
Highland plateau	***An. gambiae***	47 (11.2)	0	31 (7.4)	9 (2.1)	12 (2.9)	76 (18.1)	34 (8.1)	2 (4.8)	15 (3.7)	193 (46.1)	419	1046	<0.0001
	An funestus	6 (31.6)	0	0	0	6 (31.6)	0	0	0	0	7 (36.8)	19	55.21	<0.0001
	An. pharoensis	1 (100)	0	0	0	0	0	0	0	0	0	1	NA	NA[2]
	An. coustani	0	0	0	0	0	0	1 (9.1)	0	0	10 (90.9)	11	88.98	<0.0001

	Total	54 (12.0)	0	32 (7.1)	9 (2.0)	18 (4.0)	76 (16.9)	35 (7.8)	2 (0.4)	15 (3.3)	209 (46.4)	450	2100	<0.0001
Overall	***An. gambiae***	697 (29.1)	73 (3.0)	304 (12.7)	116 (4.8)	190 (7.9)	640 (26.7)	157 (6.5)	11 (0.5)	17 (0.7)	193 (8.0)	2398	580.2	<0.0001
	An funestus	14 (25.0)	2 (3.6)	9 (16.1)	0	9 (16.1)	16 (28.6)	0	0	0	6 (10.7)	56	96.34	<0.0001
	An. pharoensis	5 (100)	0	0	0	0	0	0	0	0	0	5	47.77	<0.0001
	An. coustani	7 (15.2)	1 (2.2)	3 (6.5)	0	0	11 (23.9)	14 (30.4)	0	0	10 (21.7)	46	63.73	<0.0001
	Total	723 (28.9)	76 (3.0)	316 (12.6)	116 (4.6)	199 (7.9)	667 (26.6)	171 (6.8)	11 (0.4)	17 (0.7)	209 (8.3)	2505	-17.19	<0.0001

[1] Kruskal-Wallis H test

[2] NA = Not Applicable

Kruskal-Wallis H test was used to determine the difference in the composition and abundance of larval habitat types across topographical zones.

4.2.3. Influence of topography and seasonality on *Anopheles* larval density

Negative binomial regression analysis was run to predict *Anopheles* larval density based on topography, and seasonality. Topography and seasonality were significant predictors of larval density. In comparison to the highland plateau, *Anopheles* larval densities were 3.23 (95% CI=2.50-4.18, $p<0.0001$) times higher in the lakeshore zone and 1.81 (95% CI=1.32-2.48, $p<0.0001$) times higher in the hillside zone. Larval density was 4.59 (95% CI=3.61-5.83, $p<0.0001$) higher in the wet season than in the dry season. The *Anopheles* larval densities were 5.65 (95% CI=3.48-9.17, $p<0.0001$) higher in the animal hoof prints and 1.702 (95% CI=1.14-2.55, $p<0.0001$) times higher in the tire track compared to the brick pits. **(Table 4.2.3)**.

In the lakeshore zone, the mean larval density was 3.27 larvae per dip, with high densities of immature *Anopheles* mosquitoes collected from animal hoof prints (20.50 larvae per dip). The mean *Anopheles* larval density in the hillside zone was 2.08 larvae per dip, with a high density of immature *Anopheles* mosquitoes found in animal hoof prints habitats (8.33 larvae per dip). The mean *Anopheles* larval density in the highland plateau zone was 2.47, with a high density collected from animal hoof prints (9.00 larvae per dip) from the highland plateau **(Table 4.2.3)**.

Table 4.2.2. Topography and seasonality influence on *Anopheles* larval densities

Parameter	Details	Coefficient	Odd ratio (95% CI)	p-value
Topography	Lakeshore	1.173	3.23 (2.50-4.18)	<0.0001
	Hillside	0.594	1.81 (1.32-2.48)	<0.0001
	Highland plateau	0[a]	1	
Habitat type	Drainage	0.055	1.06 (0.74-1.51)	0.761
	River edge	-0.28	0.76 (0.44-1.30)	0.309
	Swamp	-0.922	0.40 (0.24-0.66)	<0.0001
	Animal hoof print	1.731	5.65 (3.48-9.17)	<0.0001
	Tire truck	0.532	1.70 (1.14-2.55)	0.01
	Man-made pond	-0.858	0.42 (0.29-0.63)	<0.0001
	Rain natural pond	-1.09	0.34 (0.18-0.64)	0.001
	Rock pool	-1.01	0.36 (0.15-0.88)	0.024
	Water container	-2.619	0.07 (0.10-0.56)	0.012
	Brick pit	0[a]	1	
Distance to the household	<100m	-0.103	0.90 (0.35-2.27)	0.826
	100-200	0.163	1.18 (0.46-3.01)	0.735
	>200m	0[a]	1	
Season	Wet	1.523	4.59 (3.61-5.83)	<0.0001
	Dry	0[a]	1	
Predators	No	-0.303	0.77 (0.61-0.89)	0.002
	Yes	0[a]	1	
Algae	No	0.721	2.06 (0.80-5.30)	0.136
	Yes	0[a]	1	
Vegetation cover		-0.001	1.00 (0.99-1.00)	0.45
Habitat size		-0.008	0.99 (0.99-0.99)	0.034
(Scale)		1[b]		
(Negative binomial)		1[b]		

Negative binomial regression analysis predicted *Anopheles* larval density based: dependent Variable: *Anopheles* density. Model: (Intercept), Topography, habitat type, distance, season, predator, algae, vegetation cover, and habitat size. a. Reference category. b. Fixed at the displayed value. The Cohen's *f*2 measure of effect size on the influence of seasonality and topography on *Anopheles* larval density was 0.10132.

Table 4.2.3. *Anopheles* larvae density by habitat types across topographical zones

Parameters		Drainage	River edge	Swamp	Hoof print	Tire track	Man-made pond	Natural pond	Rock pool	Container	Brick pit	Total
Lakeshore	Dips	197	12	182	4	39	398	125	0	4	2	963
	larvae count	462	30	254	82	164	487	70	0	0	0	1549
	Density[1]	2.35	2.5	1.4	20.5	4.21	1.22	0.56	0	0	0	1.61
Hillside	Dips	93	37	24	3	3	170	89	15	21	0	455
	Larval count	207	46	30	25	17	104	66	9	2	0	506
	Density[1]	2.23	1.24	1.25	8.33	5.67	0.61	0.74	0.6	0.1	0	1.11
Plateau	Dips	75	0	71	1	43	137	57	3	83	238	708
	Larval count	50	0	33	9	14	76	40	2	15	211	450
	Density[1]	0.67	0	0.46	9	0.33	0.55	0.7	0.67	0.18	0.89	0.64
Overall	Dips	365	49	277	8	85	705	271	18	108	240	2126
	Larval count	719	76	317	116	195	667	176	11	17	211	2505
	Density[1]	1.97	1.55	1.14	14.5	2.29	0.95	0.65	0.61	0.16	0.88	1.18

[1] *Anopheles* larval density-number of larvae collected per dip across topographical zones

4.2.4. Influence of topography and seasonality on adult vector abundance

Negative binomial regression was run to predict adult *Anopheles* vector abundance based on topography and seasonality. The topography of the lakeshore (p<0.0001) and the rainy season (p<0.0001) were significant predictors of *Anopheles* adult vector abundance. The lakeshore zone had 1.72 (95% CI=1.02-2.90, p=0.041) more *Anopheles* adult vectors than the highland plateau, while there was no significant difference in the abundance of *Anopheles* adult vectors between the hillside and the highland plateau (p=0.917). Adult *Anopheles* vectors were 2.17 (95% CI=1.48-3.20, p<0.0001) times more likely to be found during the rainy season than during the dry season. The mud & wood households were 1.74 (95% CI=1.02-2.98, p=0.042) times more likely have adult *Anopheles* compared to the mud & cement households **(Table 4.2.4)**.

Table 4.2.4. Topography and seasonality influence on *Anopheles* adult abundance

Parameter	Details	Coefficient	Odd ratio (95% CI)	p-value
Topography	Lakeshore	0.543	1.72 (1.02-2.90)	0.041
	Hillside	-0.035	1.00 (0.50-1.87)	0.917
	Highland plateau	0[a]	1	
Wall type	Brick/stone	-0.025	1.00 (0.32-2.96)	0.965
	Mud & wood	0.556	1.74 (1.02-2.98)	0.042
	Mud & cement	0[a]	1	
Season	Rainy	0.776	2.17 (1.48-3.20)	<0.0001
	Dry	0[a]	1	
Bed net usage	No net	-0.202	.82 (0.44-1.52)	0.525
	Use net	0[a]	1	
(Scale)		1[b]		
(Negative binomial)		1[b]		

Negative binomial regression analysis predicted *Anopheles* adult abundance based: dependent variable: adult *Anopheles* number; model: (intercept), topography, wall type, season, open vent, bed net usage. a. Reference category. b. Fixed at the displayed value. The Cohen's *f*2 measure of effect size on the influence of seasonality and topography on *Anopheles* adult abundance was 0.1765.

4.2.5. Adult vector species composition and abundance across topography

The mosquito species composition differed significantly across topographical zones (χ^2 = 31.73, df =6, p<0.0001). In the lake zone, the 160 *Anopheles* samples identified composed of the *An. funestus* (39.4%, n=63), *An. arabiensis* (35.6%, n=57), *An. gambiae* (0.6%, n=1), while 24.4% (n=39) of the *An. gambiae s.l.,* were un-amplified. In the hillside zone, of the 27 *Anopheles* samples identified, *An. funestus* (66.7%, n=18), *An. arabiensis* (11.1%, n=3), and un-amplified *An. gambiae s.l.,* were 22.2% (n=6). In the highland plateau, of the 26 *Anopheles* mosquito identified, 30.8% (n=8) were *An. funestus*, 7.7% (n=2) were *An. arabiensis*, 3.8% (n=1) were *An. gambiae*, and 57.7% (n=15) were un-amplified *An. gambiae s.ls.*

There were 235 *Anopheles* mosquitos collected in total. Morphologically, *An. gambie s.l.* (138), *An. funestus* (89), *An. coustani* (7), and *An. pharaoensis* (1) were identified. Out of the 235 mosquito samples collected, 213 were subjected to PCR analysis. *Anopheles funestus* (89) was the most common primary vector, followed by *An. arabiensis* (62), then *Anopheles gambiae* (2). *Anopheles gambie* samples were un-amplified in about 60 cases.

4.2.6. *Plasmodium* species sporozoite rates across topography

Of the 213 mosquito samples tested for *Plasmodium* sporozoite rates, 9 (4.2%) were infected with *P. falciparum*. Although the difference in sporozoite rates across topographical zones was not statistically significant (χ^2=0.184, df =2, p=0.982), the lakeshore zone had the highest sporozoite rate (4.4%, N=160), followed by the highland plateau zone (3.8%, N=26), and the hillside zone (3.7%, N=27).

Anopheles funestus had the highest sporozoite rate of 5.6% (n=5), followed by *Anopheles arabiensis* (3.2%, n=2), and *An. gambiae* had only two samples with one infection, though there was no statistically significant difference in sporozoite rates between *Anopheles* species (χ^2 = 6.876, df =3, p=0.070). .

4.3. Influence of Topography and Seasonality on incidence of Clinical Malaria

4.3.1. Demographic information of the study participants

A total of 5,838 study participants were surveyed for the clinical malaria between May 2020 and May 2021. The lakeshore, hillside and highland plateau zones residents' age structure, education, and occupation were all similar. Farming was the most important source of income (21.7%).

Individuals aged ≥15 years made up approximately 56.6% of the study population and literacy rates were high, with 54.7% completing primary school and 26.2% completing secondary school education **(Table 4.3.1)**.

Table 4.3.1. Descriptive statistics of the study participant's demographic information

Parameter	Details	Enrolment	Eco-epidemiological zone (n, %)			*p-value*
			Lakeshore	Hillside	Plateau	
Total household surveyed		1599	460	501	638	
Total resident enrolled		5,838	1,652	1,605	2,581	
Sex	Male	2728	749	774	1205	0.2554
	Female	3110	903	831	1376	
Age	< 5	747(12.8)	191	209	347	0.1242
	5 - <15	1784(30.6)	520	490	774	
	≥15	3307(56.6)	941	906	1460	
Education	Never attended school	117(2.0)	40(2.4)	32(2.0)	45(1.7)	<0.0001
	Pre-school age	639(10.9)	130(7.9)	189(11.8)	320(12.4)	
	Primary	3157(54.7)	929(56.2)	930(57.9)	1298(50.3)	
	Secondary	1531(26.2)	426(5.8)	373(23.2)	732(28.4)	
	College & above	394(6.7)	127(7.7)	81(5.0)	186(7.2)	
Occupation	Farmer	1029(21.7)	222(13.4)	316(19.7)	491(33.2)	<0.0001
	Commercial sales	523(11.0)	175(10.6)	142(8.8)	206(13.9)	
	Office worker	138(2.9)	62(3.8)	36(2.2)	40(2.7)	
	Unemployed	489(10.3)	132(8.0)	169(10.5)	188(12.7)	
	Student	2879(37.5)	897(54.3)	762(47.5)	1220(8.1)	
	Non-school child	573(12.1)	103(6.2)	144(9.0)	326(22.0)	
	Others	207(4.4)	61(3.7)	36(2.2)	110(7.4)	

Chi square test of independence was used to determine difference in the demographic characteristics across the topography. The $p \leq 0.05$ was considered statistically significant.

4.3.2. Influence of topography and seasonality on clinical malaria incidence

A multivariate analysis revealed that topography and seasonality were all associated with clinical malaria. Clinical malaria cases were twice as common on the lakeshore (OR: 2.02, 95% CI=1.62-2.50, $p<0.0001$) and 1.4 times more common on the hillside (OR: 1.42, 95% CI=1.13-1.79, $p=0.002$) than on the highland plateau. Residents were 1.49 times more likely to suffer from clinical malaria cases during the wet season (OR: 1.49, 95% CI=1.24-1.80, $p<0.0001$) than

during the dry season. Residents who did not have an active fever at the time of visit and had a body temperature of <37.5°C (OR: 0.27, 95% CI=0.21-0.34, p<0.0001) were less likely to develop clinical malaria than residents who did have an active fever and had a body temperature of ≥37.5°C. **(Table 4.3.2)**.

Table 4.3.2. Topography and seasonality influence on clinical malaria

Risk factors	Category	Coefficient	Odd ratio (95% CI)	*p*-value[1]
Topography	Lakeshore	0.701	2.02 (1.62-2.50)	<0.0001
	Hillside	0.354	1.42 (1.13-1.79)	0.002
	Highland plateau		1	
Gender	Female	-0.244	0.78 (0.66-0.94)	0.007
	Male		1	
Age group	<5 years	0.683	1.98 (1.54, 2.54)	<0.0001
	5-<15 years	0.695	2.00 (1.66, 2.43)	<0.0001
	≥15 years		1	
Temperature	<37.5°C	-1.423	0.27 (0.21, 0.34)	<0.0001
	≥37.5°C		1	
Seasonality	Wet	0.399	1.49 (1.24-1.80)	<0.0001
	Dry		1	
Bed net usage	No net	0.087	0.78 (0.57, 1.07)	0.121
	Use net		1	

Multivariate logistic regression model determined the influence of topography and seasonality on clinical malaria incidence. The Cohen's f2 measure of effect size on the influence of seasonality and topography on clinical malaria incidences was 0.1109.

4.3.3. Influence of topography on clinical malaria incidences

In the study area, 2205 residents reported febrile illness out of a total of 5838 residents from May 2020 to May 2021. The Lakeshore zone had the highest malaria febrile incidence, with 24.3 cases/1,000 people/month, followed by the hillside zone (18.7 cases/1,000 people/month) and the highland plateau zone (10.3 cases/1,000 people/month). There was significant difference in the clinical malaria incidence between males and females (χ^2 =7.57; df=2, p=0.0227). In the lakeshore zone, males had higher incidence of 26.7/1,000 people per month than females, who had 22.3/1,000 people per month. In the hillside and the highland plateau, the females had the

higher incidence of infection at 21.7 and 12.7cases/1,000 people per month, respectively. There was a significant difference in the clinical malaria incidence across the age group (χ^2=58.34; df=4, p<0.0001). In the Lakeshore zone, hillside and the highland plateau the school going children aged between 5-14 years old had the highest incidence of infection at 38.8, 28.1, and 13.9 cases/1,000 people/ month, respectively. Among the females in the lake zone, the incidence risk of infection was 1.72 times higher among the 5-14 years old school going children (IR:1.72, 95% CI=1.24-2.18) and 0.51 times lower among individuals ≥ 15 years old (IR:0.51, 95% CI=0.25-0.77) compared to children under five years old. Among the males, the incidence risk of infection was 0.18 times lower among individuals ≥ 15 years old (IR: 0.18, 95% CI=0.04-0.31) compared to children under five years old in the lake zone. **(Table 4.3.3)**.

Table 4.3.3. Incidence of clinical malaria across topography

Factor	Details	Lakeshore		Hillside		Highland plateau	
		Incidence	**Incidence ratio (95% CI)**	**Incidence**	**Incidence ratio (95% CI)**	**Incidence**	**Incidence ratio (95% CI)**
Overall		24.3	Ref[1]	18.7	0.77 (0.42-1.12)	10.3	0.42 (0.17-0.58)
Gender	**Female**	22.3	Ref[2]	21.7	Ref[2]	12.7	Ref[2]
	Male	26.7	1.20 (0.74-1.65)	15.3	0.71 (0.35-1.06)	8.6	0.68 (0.22-1.13)
Age	**<5**	34	Ref[3]	17.2	Ref[3]	13.4	Ref[3]
	5-<15	38.8	1.14 (0.78-1.50)	28.1	1.63 (1.03-2.24)	13.9	1.04 (0.49-1.58)
	≥15	11.7	0.34 (0.15-0.49)	12.9	0.75 (0.34-1.16)	7.9	0.59 (0.18-0.99)
	<5	29.6	Ref[4]	26.2	Ref[4]	12.2	Ref[4]
Female	**5-<15**	50.8	1.72 (1.24-2.18)	32.7	1.25(0.82-1.67)	16.4	1.34 (0.69-1.99)
	≥15	15.1	0.51 (0.25-0.77)	14.9	0.57 (0.28-0.86)	10.8	0.89 (0.36-1.40)
Male	**<5**	37.9	Ref[5]	8.8	Ref[5]	15	Ref[5]
	5-<15	48.2	1.27(0.91-1.63)	23.4	2.66 (1.53-3.62)	12	0.80 (0.35-1.25)
	≥15	6.7	0.18(0.04-0.31)	11.2	1.27 (0.51-1.95)	3.6	0.24 (0.01-0.49)

[1] Overall comparison was between survey zones using Lakeshore as reference
[2]Gender comparison was between sexes using females as references
[3] Age comparison was between the age groups using children < 5 years old as references
[4] Females comparison was between age groups using children < 5 years old as references
[5]Males comparison was between age groups using children < 5 years old as references

4.3.4. Clinical malaria treatment patterns across topography and seasonality

The treatment patterns of residents with clinical malaria differed significantly in the lakeshore (p<0.0001) and hillside zones (p<0.0001), but not in the highland plateau zone (p=0.431). The majority of residents with clinical malaria from the lakeshore zone sought treatment at public hospitals (61.9%, N=97) and purchased medication from drug stores (51.4%, N=146). The majority of clinical malaria cases in the hillside zone were from residents who sought treatment with traditional medication (51.1%, N=45) and those who did nothing (43.2%, N=259) **(Table 4.3.4)**. Residents with clinical malaria had significantly different treatment patterns during the rainy season (p=0.009) but not during the dry season (p=0.292). During the rainy season, those who did nothing (51.1%, N=571) and those who bought medication from drug stores (30.8%, N=340) were responsible for the majority of clinical malaria cases **(Table 4.3.4)**. The treatment patterns of female residents (p=0.001) with clinical malaria differed significantly, but there were no significant differences among males (p=0.055). The majority of clinical malaria cases among the females were from residents who sought treatment with traditional medication (50.9%, N=53) and those who sought treatment from the public hospital (43.8%, N=146) **(Table 4.3.4)**.

Furthermore, ultrasensitive malaria RDT detected positivity rate of 35.8% while the RT-PCR detected positivity rate of 53.6%. Using RT-PCR as a standard reference, the ultrasensitive malaria RDT sensitivity was at 65.5% (95% CI=61.3-69.6, p<0.0001), and specificity of 98.7% (95% CI=97.0-99.5, p<0.0001). The Kappa discordance test revealed moderate level of agreement between RT-PCR and ultrasensitive malaria RDT infection detection method (Cohen's kappa=0.63, 95% CI=0.58-0.67, p<0.0001)**.** The ultrasensitive malaria RDT diagnosis is highly specific, with a good sensitivity.

Table 4.3.4. Clinical malaria cases treatment pattern across topography

Items	Category	Enrolment		Treatment Seeking method									
				Public Hospital		Private Hospital		Drug shop		Traditional Medication		Do nothing	
Subjects		Fever N (%)	Pos. n (%)	Fever N (%)	Pos. n (%)	Fever N (%)	Pos. n (%)	Fever N (%)	Pos. n (%)	Fever N (%)	Pos. n (%)	Fever N (%)	Pos. n (%)
Overall	N	2205	850 (38.5)	275 (12.5)	115 (41.8)	91 (4.1)	15 (16.5)	688 (31.2)	248 (38.1)	77 (3.5)	28 (45.5)	1074 (48.7)	387 (39.4)
Zones	Lakeshore	811 (36.8)	374 (46.1)	97 (12.0)	60 (61.9)	44 (5.4)	10 (22.7)	146 (18.0)	75 (51.4)	16 (2.0)	8 (50.0)	508 (62.6)	221 (43.5)
	Hillside	691 (31.3)	266 (38.5)	88 (12.7)	21 (23.9)	36 (5.2)	3 (8.3)	263 (38.1)	107 (40.7)	45 (6.5)	23 (51.1)	259 (37.5)	112 (43.2)
	Plateau	703 (31.9)	210 (29.9)	90 (12.8)	34 (37.8)	11 (1.6)	2 (18.2)	279 (39.7)	80 (28.7)	16 (2.3)	4 (25.0)	307 (43.7)	90 (29.3)
Sex	Male	857 (38.9)	353 (41.2)	129 (15.1)	51 (39.5)	37 (4.3)	7 (18.9)	266 (31.0)	114 (42.9)	24 (2.8)	8 (33.3)	401 (46.8)	173 (43.1)
	Female	1348 (61.1)	497 (36.9)	146 (10.8)	64 (43.8)	54 (4.0)	8 (14.8)	422 (31.3)	148 (35.1)	53 (3.9)	27 (50.9)	673 (49.9)	250 (37.1)
Age	< 5	345 (15.7)	159 (46.1)	48 (13.9)	23 (47.9)	13 (3.8)	4 (30.8)	106 (30.7)	57 (53.8)	15 (4.3)	3 (20.9)	163 (47.2)	72 (44.2)
	5 ~ 14	802 (36.3)	372 (46.4)	112 (14.0)	58 (51.8)	35 (4.4)	4 (11.4)	232 (28.9)	102 (44.0)	23 (2.9)	14 (60.9)	400 (49.9)	194 (48.5)
	≥15	1058 (48.0)	319 (30.1)	115 (10.9)	34 (29.6)	43 (4.1)	7 (16.3)	350 (33.1)	103 (29.4)	39 (3.7)	18 (46.2)	511 (48.3)	157 (30.7)
Temperature	<37.5°C	1833 (83.1)	601(32.8)	214 (11.7)	65 (30.4)	78 (4.3)	10 (12.8)	591 (32.2)	201 (34.0)	67 (3.7)	29 (87.0)	883 (48.2)	296 (33.5)
	≥37.5°C	372 (16.9)	249 (66.9)	61 (16.4)	50 (82.0)	13 (3.5)	5 (38.5)	97 (26.1)	61 (62.9)	10 (2.7)	6 (13.0)	191 (51.3)	127 (66.5)
ITN Usage	**Yes**	1987 (90.1)	770 (38.8)	253 (12.7)	111 (43.9)	78 (3.9)	15 (19.2)	610 (30.0)	232 (38.0)	75 (3.8)	33 (44.0)	971 (48.9)	379 (39.0)
	No	218 (9.9)	80 (36.7)	22 (10.1)	4 (18.2)	13 (6.0)	0	78 (35.8)	30 (38.5)	2 (0.9)	2 (100)	103 (47.2)	44 (42.7)

Pos.: Positive. Chi square test of independence was used to determine difference in the treatment-seeking pattern.

4.3.6. Influence of topography on incidences of clinical malaria diagnosis

Blood smears were prepared and read for 936 febrile cases of the outpatient study participants across the health facilities in the three zones lakeshore, hillside and highland plateau zone. The overall positivity rate for *P. falciparum* was 28% (257/936). However, there was no significant association between topography and blood smear positivity rates in the health facilities (χ^2 = 5.589, df. = 2, p=0.0612). Treatment of patients with fever who were blood smear positive or blood smear negative was with AL, antibiotics and analgesics.

CHAPTER FIVE

DISCUSSION

5.1. Influence of Topography and Seasonality on Submicroscopic Infection

The current study provides evidence on submicroscopic infection carriage in western Kenya from the lowland Lake Victoria basin through the hillside to the highland plateau zones, as well as the influence of seasonality and topography association with submicroscopic infection. Submicroscopic infection in the study area was high at 14.2% (N=1,777). The infection showed topographical and seasonal variations, with submicroscopic infection carriage being highest in the lowland lakeshore zone and during the wet seasons.

The lakeshore zone, which is adjacent to Lake Victoria, had a threefold higher likelihood of submicroscopic infection than the highland plateau zone. The current study findings of high prevalence of submicroscopic infection in the lowland of lakeshore and a low prevalence in the highland plateaus, is consistent with a study in western Kenya that found that lowland areas on the northern shore of Lake Victoria have the highest submicroscopic malaria infection rate, while highlands areas have relatively low infection (Lo et al., 2015). The study, on the other hand, emphasizes that not all lowland sites near the lakeshore have high transmissions, particularly in the eastern and southern shore areas (Lo et al., 2015). Aside from topography, there could be other factors influencing submicroscopic *Plasmodiun* infections in the lowlands, such as vector composition and abundance, human travel, access to health care, and the effectiveness of control measures (Baum et al., 2015; Lo et al., 2015; Niang et al., 2017; Ochwedo et al., 2021).

The lowland lakeshore zone is distinguished by a flat plain frequented by flooding, resulting in stagnant water bodies that are potential mosquito breeding habitats, whereas malaria transmission may be less distinct in the highland plateau area due to flat topography and more diffuse hydrology caused by numerous streams, resulting in better drainage and lower malaria transmission stability (Balls et al., 2004; Githeko et al., 2006; Mutero et al., 2020; Nganga et al., 2019). Similarly, residents of the hillsides zones were found to be twice as likely as those of the highland plateau zone to have submicroscopic infections (AOR: 1.74, 95% CI=1.17-2.61, p=0.007). The hillside zone has unstable larval habits because rivers and streams run along the valley bottoms and the majority of breeding habitats are confined to the valley bottoms because hillside gradients provide efficient drainage. As a result, heterogeneous distribution of larval breeding habitats is likely to affect adult vector distribution and the human population's exposure to *Plasmodium* infection (Atieli et al., 2011; Githeko et al., 2006).

Submicroscopic *Plasmodium* infections are thought to be the source of human-to-mosquito transmissions, particularly in low-endemic areas with low transmission rates (Okell et al., 2012). Despite the low transmission rate in the highland plateau zone, the current study discovered a persistent prevalence of submicroscopic *Plasmodium* infection. The prevalence of infection in the highlands may be attributed to the fact that during the rainy season, vectors migrate from valley bottoms to the tops of hills, resulting in increased malaria prevalence at higher altitudes. Because of the prevalence of submicroscopic *Plasmodium* infections in low-transmission areas (Nguyen et al., 2018), using molecular tools to assess parasite infection will be critical in monitoring malaria elimination programs where microscopy measures are insufficient to assess the scale of public health measures required.

Despite the fact that the wet seasons have a higher likelihood of submiscrosccopic *Plamosdium* infections, the infections persist during the dry seasons, according to the current study. Concurrently, submicroscopic infections serve as a parasite reservoir for malaria transmission, and in areas with low and seasonal transmission, persistent asymptomatic parasite carriage bridges the dry season, when mosquito numbers are very low and malaria transmission is almost non-existent, thereby sustaining endemicity (Nguyen et al., 2018). Furthermore, as previously reported, the current study's submicroscopic prevalence during the rainy season may be explained by high vector densities during this season, which correlates with high infection rates (Atieli et al., 2011; Selvaraj et al., 2018; Soma et al., 2021).

The current study's influence of seasonality on submicroscopic *Plasmodium* infections is consistent with a study in southern Vietnam that discovered submicroscopic *Plasmodium* reservoirs may play an important role in sustaining malaria transmission and may explain the seasonal malaria increase after the dry seasons (Nguyen et al., 2018). As a result, screening interventions that rely on standard diagnostic tools will miss the majority of the low-density *Plasmodium* reservoir, and targeting the low density as a *Plasmodium* reservoir is likely to be critical to accelerating malaria elimination.

During the rainy season, the current study found a high prevalence of *Plasmodium* infection. Similarly, majority of *Plasmodium* infections in western Kenya, have been linked to seasonality, with seasonal peaks during the rainy season (Cairns et al., 2015; Essendi et al., 2019; Kipruto et al., 2017; Kweka et al., 2012; Matsushita et al., 2019; Reiner et al., 2015). The wet and dry seasons, which have been shown to affect *Plasmodium* infection rates in endemic areas, may

contribute to infection persistence as vector populations' increase under favorable weather conditions, *Plasmodium* infection rates stabilize (Degefa et al., 2017; Matsushita et al., 2019; Munga et al., 2009; Nkumama et al., 2017; Reiner et al., 2015; Shah et al., 2019; Shanks et al., 2005).

Individuals who live in or near lowland areas have a large reservoir of infectious gametocytes, whereas those who live in the highlands have a high proportion of people who are susceptible to *Plasmdium* infections. The lower transmission pressure on the highland plateau may make residents more susceptible to *Plasmodium* infection, as children on the highland plateau may have a slower ability to suppress parasite density than children in lowland areas with well-established transmission (Walldorf et al., 2015). School-aged children from the hillside and highland plateau zones had a lower risk of submicroscopic infection than those from the lakeshore zones. The current study supported previous research that found that school-going children had an increased risk of submicroscopic infection, acting as reservoirs of infectious gametocytes and thus maintaining infection in the community (Zhiyong et al., 2016). Furthermore, a study in a low endemic setting in Ethiopia found high likelihood of low density parasitemia among school-aged children (Tadesse et al., 2017).

This submicroscopic *Plasmodium* infections in school-age children could be attributed to increased *Plasmodium* exposure as a result of inadequate bed net use (Ochwedo et al., 2021). Individuals in malaria-endemic areas as a result of repeated exposure to the *P. falciparum* parasite develop adaptive immunity and this is related to age (Agwu et al., 2009). Adults are asymptomatic parasite carriers because they have developed strong immunity to malaria

parasites through repeated exposures (Chelimo et al., 2011; Mawili-Mboumba et al., 2013; Nankabirwa et al., 2014; Roberts & Matthews, 2016; Walldorf et al., 2015). However, the current study did not investigate the immunogenic suppression of *Plasmodium* infection across topography or age group.

In the highland plateau zone, unlike the lakeshore and hillside zones, non-ITN compliance was associated with an increased risk of submicroscopic infection. The ITN use has been shown to significantly reduce *Plamodium* infection (Kamau et al., 2017; Rek et al., 2020; Thomsen et al., 2017). However, the current study found no difference in submicroscopic infection between ITN users and non-ITN users across the topographical zones, which could be due to behavioral changes in malaria vectors, with outdoor transmission occurring earlier in the evenings and mornings away from the protection by the vector control interventions (Kleinschmidt et al., 2018; Monroe et al., 2019; Moshi et al., 2018).

Additional factors that may be contributing to increased submicroscopic infection across the lowland lakeshore could be attributed to the zone's economic activities. The main economic activity in the Lakeshore zone is fishing and reed cutting for mats, which are mostly done by males, causing residents to stay near water for longer periods of time, exposing themselves to mosquito breeding sites, resulting in higher biting rates and infection levels. Gender bias Plasmodium infection among males has been linked to socioeconomic differences that keep males awake late at night and early in the morning in studies conducted in Malindi and Homabay, Kenya (Diiro et al., 2016; Ochwedo et al., 2021).

The current study discovered that as a potential confounder to submicroscopic infection regardless of season or topography, house wall type was associated with submicroscopic infection. The majority of residents in the lakeshore zone had poor housing quality, with traditionally constructed housing being common. Poor housing quality may correlate with the use of personal protection measures, resulting in an increased risk of infection. As previously reported (Essendi et al., 2019; Mutero et al., 2020; Nganga et al., 2019), houses built with mud or mud and cement increased the risk of *Plamodium* infection in the current study area.

According to the current community survey, variations in landscape and seasonality contribute to submicroscopic infections in the community. In the current study microscopy exclusively identified P. falciparum infections, while RT-PCR identified *P. malariae* and *P. ovale* mono- and co-infections with *P. falciparum*. Microscopic diagnosis of blood smears from community mass blood surveys or patients in clinics, according to a study conducted in Tak, Thailand, underestimated *Plasmodium* infection prevalence (Baum et al., 2015). Microscopy sensitivity has been reported to be low in other parts of western Kenya (Afrane et al., 2014; Essendi et al., 2019; Minakawa et al., 2021; Ochwedo et al., 2021). The routine microscopy severely underestimates the burden of infections as the submicroscopic infection may act as a reservoir of infectious gametocytes (Rovira-Vallbona et al., 2017) and in some instances may develop to clinical infection (Waltmann et al., 2015; Zhao et al., 2018).

The findings current study revealed that topography and seasonality contribute to persistent malaria transmission and have an impact on susceptibility to submicroscopic infection. Further epidemiological surveillance of malaria infection persistence should concentrate on more

sensitive diagnostic tests in the monitoring of *Plasmodium* infection at the community level, tracking and identifying geographic areas where additional vector interventions are needed to reduce malaria burden.

5.2. Influence of Topography, and Seasonality on Entomological Indices of Malaria

The current study looked at the influence of landscape heterogeneity on malaria entomological and parasitological indices. *Anopheles* mosquito larvae were mostly found in the drainage ditch, man-made pond, and swamps. The lowland lakeshore zones had more drainage ditches habitats and swamps, hill side zone more rock pool habitats while highland plateau zone having more brick pits habitats. Further investigation revealed that *Anopheles* larvae density were associated with topography, and seasonality. The most abundant primary vectors in all the zones were *An. funestus* and *An. arabiensis*. Determinants of adult vectors relative abundance were topography and seasonality.

The spatial and temporal distribution of malaria vectors in various ecological settings is primarily responsible for malaria distribution. This explains the infection rates found throughout the topographical zone. The primary breeding habitats for *Anopheles* mosquitoes along the lowland lakeshore were man-made ponds, drainage ditches, and swamps. Flat plain that is frequently flooded distinguishes the lowland lakeshore zone, resulting in stagnant water bodies serve as mosquito breeding grounds. Despite the fact that numerous streams cause efficient drainage and diffuse hydrology, resulting in unstable vector breeding sites, the composition of *Anopheles* larval species and vector abundance remain high in the highland zones. Other studies

have found similar levels of malaria transmission in the highlands (Atieli et al., 2011; Cohen et al., 2008; Githeko et al., 2006; Wanjala et al., 2010). The persistence of vector population and malaria infection in the hillside zone could be attributed to human activity on the land, such as the construction of ponds for water reservoirs, which are potential larval habitat for *Anopheles* vector. Man-made ponds and constructed water pans take time to dry out, providing potential stable breeding sites for the infection and vector population.

The high density of mosquito larvae occurred during the wet season, indicating that *Anopheles* mosquitoes are more abundant during the rainy seasons. The current study supports previous findings that, during the rainy season, the reported productivity of larval habitats correlates with increased vector density and species richness (Atieli et al., 2011; Selvaraj et al., 2018; Soma et al., 2021). Furthermore, *Anopheles* vectors and malaria transmission differences between the lakeshore, hillside, and highland plateau reflect the study area's micro-ecological significance. The findings are consistent with another study that found that micro-ecology and rainfall patterns influenced the dynamics and seasonal abundance of malaria vectors (Dery et al., 2010), however, the transmission patterns did not vary seasonally. Even though the proportion of *Anopheles* mosquito larvae may increase during and immediately after the rainy season, intense rainfall may interfere with mosquito larvae densities due to larvae flushing from habitats (McCann et al., 2014). According to the current research, larval habitats are primarily in the valley bottom, with high infection in lowland areas and only a few breeding habitats on the highland plateau. This has resulted in a heterogeneous distribution of the vector and parasite burden, and similar findings have been published (Atieli et al., 2011; Githeko et al., 2006; Nmor et al., 2013). This eco-epidemiological variation has implications for vector control programs, interventions that are

effective in one setting may not be effective in another. Continuously integrating larval source management with LLINs and IRS will reduce the vector population, resulting in a reduction in disease burden.

The current study found that vector densities were high in wet season than dry season. Such findings are consistent with findings that malaria transmission is highly seasonal, with the *Plasmodium* parasites declining in dry seasons and peaks during wet season due to increase in vector population (Selvaraj et al., 2018). Due to the seasonal nature of vector abundance and *Plasmodium* infection transmission, children should be targeted for seasonal malaria chemoprevention, which presents opportunities for reducing clinical episodes and potentially eliminating malaria.

Hydrological processes that govern the formation and stability of various habitat types influence the distribution and abundance of Anopheles larvae. The *Anopheles* larval density was found to be significantly related to habitat type, land use type surrounding the habitat, seasonality, and predation. In Western Kenya, such a relationship between *Anopheles* and larval habitat characteristics has been reported (Atieli et al., 2011; Debrah et al., 2021; McCann et al., 2014; Ndenga et al., 2011). Larval survival and development are influenced by biological and physicochemical properties of the habitat, such as cannibalism and predation of larvae, as well as greater dispersal of early instars by river and stream flow (Hinne et al., 2021). Aquatic predators influence the abundance of mosquito larvae in breeding environments and are effective biological mosquito larvae control agents. The swamp habitats may support a high number of predators and competitors, and since the longer *Anopheles* larvae spend in such habitats, the

more likely they are to be preyed upon (Getachew et al., 2020) and the current study reported lower *Anopheles* larval density in these habitats. Low *Anopheles* larval density has been reported in animal hoof prints, which has been attributed to habitats that are less stable and frequently dry out quickly after rains, rarely lasting longer (Hinne et al., 2021). In contrast, the highest larval densities were found in animal hoof print habitats in the current study. Cattle hoof prints and Hippo-hoof prints were the most common animal hoof prints, and they were mostly found along the shores of Lake Victoria, where they stayed longer before drying. Such animal hoof print habitats should be targeted for larval management due to their specific and unique nature of sustaining the vector population.

Malaria prevalence and transmission risk increase in lowland zone due to higher vector abundance than in highland plateau zone. The current study's findings of increased mosquito breeding habitats leading to increased vector density, which in turn leads to increased malaria transmission are consistent with other findings (Githeko et al., 2006; Hinne et al., 2021; Mzilahowa et al., 2012; Omalu et al., 2015). The most abundant adult vector species identified in this study were *An. arabiensis* and *An. funestus*, which is consistent with findings from other studies in western Kenya, including Kisumu (Imbahale et al., 2012), Homa Bay (Orondo et al., 2021), Kombewa and Bungoma (Debrah et al., 2021).

The majority of the households in the lakeshore zones were near bodies of water that could serve as potential breading grounds. In the current study, the distance from the breeding habitat to the nearby house was associated with the abundance of adult *Anopheles* because the distance to breeding habitats can determine the density of adult mosquitos. Environmental modifications for

economic activity, such as brick-making, fish farming, irrigation, rock mining, constructed water pans and man-made ponds that leave depressions on the ground filled with water, as seen in the current study sites result in more aquatic habitats, which may alter the current ecological setting, resulting in changes in mosquito vector ecology (Hawaria et al., 2020) and variations in malaria transmission (Kibret et al., 2017). A study in Homa Bay found that agricultural insecticides primarily contained pyrethroids and organophosphates, which could be contributing to *Anopheles* species resistance to pyrethroids (Orondo et al., 2021). Moreover, despite increased vector control interventions, low levels of transmission persist, necessitating an integrated vector control that includes LLIN, IRS, and larval source management in accordance with WHO guidelines (WHO, 2013a).

5.3. Influence of Topography and Seasonality on Clinical Malaria Incidence

The current study looked at influence of topography and seasonality on malaria incidences in western Kenya. Malaria incidence (cases/1000people/month) was highest in the lakeshore zone (24.3), followed by the hillside (18.7) and the highland plateau zone (10.3). Clinical malaria cases were twice as common in lakeshore zone (OR: 2.02, 95% CI=1.62-2.50, $p<0.0001$) and 1.4 times more common on the hillside (OR: 1.42, 95% CI=1.13-1.79, $p=0.002$) than on the highland plateau. Residents were 1.49 times likely to have clinical malaria during wet seasons (OR: 1.49, 95% CI=1.24-1.80, $p<0.0001$) than during the dry season. The treatment patterns of residents with clinical malaria differed significantly in lakeshore zone ($p<0.0001$) and hillside zones ($p<0.0001$), but not in the highland plateau zone ($p=0.431$). Residents with clinical malaria had significantly different treatment patterns during the rainy season ($p=0.009$) but not during the dry season ($p=0.292$). Doing nothing and purchasing antimalarials from local drug stores without a

confirmed diagnosis was the most common treatment profile among residents across topographical zones and seasonality. These treatment patterns may be contributing to the community's persistence of malaria febrile illness. Furthermore, the current study found that ultrasensitive malaria RDT diagnosis is highly specific (98.7%) with a sensitivity of 65.5%.

The current study found high clinical malaria incidences in the Lakeshore zone, which could be attributed to the area's flat plains and frequent flooding during rainy seasons, resulting in water stagnation and the presence of permanent mosquito breeding habitats,. The current study's high malaria incidences along the lowland lakeshore corroborate previous research from western Kenya that found a high malaria cases along the lake basin (Kapesa et al., 2018; Matsushita et al., 2019; Ng'ang'a et al., 2021). The low malaria febrile illness rates in hillside zones could be attributed to the area's hilly terrain and unstable larval habits. The water pans always dry out faster in these hilly areas, resulting in fewer mosquito breeding sites and the fewer febrile cases. The current study's findings are consistent with those of previous studies on altitudinal variation in malaria prevalence (Balls et al., 2004; Dabaro et al., 2021; Wanjala et al., 2010).

The current study found that variation in topography and seasonality are the most important factors influencing clinical malaria incidences. Similar findings were made in Ethiopia, where rainfall and temperature were discovered to be major drivers of malaria (Dabaro et al., 2021). The weather variables (rainfall and humidity) may affect the bionomics of malaria vectors, thereby determining malaria febrile illness. In the current study, malaria incidence was found to be highly associated with wet season. During the wet season, there is also an increased risk of malaria transmission in Ethiopia (Dabaro et al., 2021), Kenya (Kipruto et al., 2017), and Eritrea

(Kifle et al., 2019). The effect of seasonality on clinical malaria incidences varies according to topography, and such inconsistencies can be linked to variations in risk factors for malaria febrile illness. As a result, using an adaptive combination of interventions to reduce malaria incidence in communities and control mosquito vectors will be critical.

Malaria febrile illness were seasonal, according to the current study's findings, with lowland zones having the highest infection burden and highland plateau zones having the lowest infection burden. Concurrently, malaria transmission in western Kenya's highlands has been reported to be low and seasonal, and has been attributed to local environmental factors (Zhou et al., 2015). During the high transmission season, highland areas have hotspot cases of clinical malaria, which are uncommon during the low season. These malaria transmission hotspots can be attributed to study zones located near major breeding habitats, which increases human-vector contact and thus exposure to transmission risks. Infections primarily affect highland communities during the wet season, when larval habitats are more common and vector densities are higher (Afrane et al., 2014; Bekolo & Williams, 2019; McCreesh et al., 2018; Touré et al., 2016; Zhou et al., 2015). Clinical malaria cases observed during the rainy season on the highland plateau may explain the well-known highland malaria outbreak (Billingsley et al., 2007; Kipruto et al., 2017; Kristan et al., 2008; Siya et al., 2020). The current study, however, did not look into the effect of topography and seasonality on the immune response to malaria.

In the current study, male in the lakeshore zones were more likely to contract malaria than females. Other studies have found high rates of malaria in males, which supports the current study's findings (Diiro et al., 2016; Ochwedo et al., 2021).Residents' economic activities, such as

night fishing and dusk small-scale businesses, may cause them to remain outside without protective measures, exposing themselves to mosquito bites. Females, on the other hand, were more likely to contract malaria in the hillside and highland plateau zones, most likely as a result of dusk activities such as selling vegetables and cooking outside at night, which exposes them to mosquito bites (Jenkins et al., 2015). Furthermore, females have pre-natal clinic appointments during pregnancy and frequently take their children to seek treatment, which may explain their high hospital seeking behavior and, as a result, their lower clinical malaria incidences when compared to males (Budu et al., 2020; Franckel & Lalou, 2009).

According to the current study findings, malaria incidences were high among school-going children in all topographical zones. Lower bednet usage among school-aged children exposes them to high mosquito bites at night, which may explain why malaria incidences are higher in this age group (Kamau et al., 2017; Minakawa et al., 2021; Ochwedo et al., 2021). The lower infections among children under the age of five compared to school-age children could be attributed to the children being cared for by their parents and sleeping under mosquito nets at night (Abossie et al., 2020; Maketa et al., 2015).

The majority of infections in the current study occur in school-aged children. School-aged children serve as malaria infection reservoirs, ensuring the spread of malaria in the community (Walldorf et al., 2015). Most infections may be linked to asymptomatic individuals acting as infection reservoirs, where they acquire the gametocyte and thus become infective over time. A similar study in Mozambique discovered that self-reported symptomatic malaria is extremely common among children, and that factors facilitating access to health care are associated with

symptomatic malaria diagnosis (Carlucci et al., 2017). Individuals in malaria-endemic areas develop adaptive immunity to the *P. falciparum* parasite, resulting in a decreasing rate of infection with age (Agwu et al., 2009). Children, unlike adults, are unable to make treatment decisions on their own because their parents or guardians determine the treatment pattern (Budu et al., 2020; Franckel & Lalou, 2009).

Individuals suspected of having malaria often start by doing nothing, then self-medicate with drugs from drug stores or traditional medications, and when the condition worsens, they seek treatment at health facilities. Delayed treatment may contribute to the development of malaria complications (Dave-agboola & Raji, 2018). Malaria contributes to higher number of out-patient visits in Kenya's health facilities in western Kenya (Kenya Malaria Control Programme, 2019).. In the current study, more than 80% of residents either self-medicate or do nothing when they have febrile illness, with only less than 20% seeking treatment in a health facility. In the current study, less than 20% of residents were reported to seek malaria treatment in health facilities, with an estimated 80% of febrile cases being underreported, with a proportion of whom could be malaria cases not being recorded in health facilities. A large proportion of the community does not seek treatment at health care facilities due to lack of antimalarial in health facilities, the affordability of malaria diagnosis and distance to health facilities, confidence in the treatment, and socioeconomic status. As a result, approximately 31% of febrile cases self-diagnose and self-treat with drugs obtained from local drug shops located in nearly every shopping center. A Nigerian study found that approximately 88% of residents prefer to manage malaria at home, with only about 12% visiting health facilities (Dave-agboola & Raji, 2018). A major source of concern is the use of antimalarial drugs in the absence of a confirmed test. Despite seeking

treatment from drug stores and traditional medication, inappropriate treatment may have contributed to the observed higher clinical malaria cases in the current study.

Traditional medicine is commonly used to treat fever in African communities, especially during the early stages of illness or when the symptoms are mild (Dave-agboola & Raji, 2018; Gathwira et al., 2010; Heng et al., 2017). The hillside zone, which is mostly hilly and has a lot of herbs and shrub plantation, explains why the majority of the residents in the current study are more likely to seek traditional medicine. Local herbs are more accessible and affordable because they can be obtained from the fields or traditional healers. Traditional healers have a good understanding of malaria symptoms and causes, according to studies in the Democratic Republic of the Congo, Guinea, and Kenya, resulting in consistent knowledge of antimalarial plants (Gathwira et al., 2010; Manya et al., 2020; Traore et al., 2013). Herbal medications have been reported to be involved in parasite clearance (Dave-agboola & Raji, 2018; Gathwira et al., 2010; Heng et al., 2017). The current study, however, did not follow up on the parasite clearance by traditional herbs. The study showed the type of housing wall and the floor type, distance to medication access, and hospital payment method all influenced the clinical malaria treatment. Residents from the lake zone, for example, were more likely to seek treatment in a public hospital and purchase antimalarial drugs from local drug stores. This was greatly influenced by the distance and ease of access. The severity of fever as a result of *P. falciparum* infection drives people to seek treatment (Carlucci et al., 2017) which is heavily influenced by accessibility, availability, and affordability of treatment services (Karyana et al., 2016). The current study residents reported taking analgesics to relieve pain before taking antimalarials, which may explain why there were fewer active fever cases in the study zone.

The rapid emergence and spread of the COVID-19 has resulted in massive global disruptions that are affecting people's lives and well-being. The devastation caused by the pandemic could be greatly exacerbated if the response jeopardizes the provision of life-saving malaria services (WHO, 2020). The COVID-19-related challenges have contributed to an increase in antimalarial and RDT stockout rates, resulting in a drop in test-and-treat policy adherence (U.S. President's Malaria Initiative, 2022). Reduced funding for vector interventions, combined with competing health care challenges like the ongoing COVID-19, may result in a rollback of malaria control gains, resulting in increased morbidity and mortality from malaria (Ajayi et al., 2020; Heuschen et al., 2021; Weiss et al., 2021). Furthermore, fear and stigma were generated as a result of the COVID-19 situation. According to the current study, ultrasensitive malaria RDT diagnosis had a higher specificity (99%) and a good sensitivity (66%) in detecting malaria febrile cases. The study's findings confirm the high sensitivity of the ultrasensitive malaria RDT when compared to RT-PCR, as previously reported (Acquah et al., 2021; Danwang et al., 2021; Reichert et al., 2020). To reduce the complication of malaria cases, the government should invest in supportive supervision of CHVs as well as the provision of more sensitive RDTs and antimalarial to strengthen community malaria case management.

The effectiveness of malaria intervention strategies is determined by whether people with *Plasmodium* infection receive affordable prompt care at the health facility. The WHO initiative Test, Treat, Track promotes the testing of all suspected cases of malaria, the treatment of all confirmed cases, and the tracking of the disease through an accurate and timely surveillance system (WHO, 2012). The current study found a high rate of clinical malaria positivity, as well as misdiagnosis and inappropriate treatment at the health facility. With the high sensitivity of

malaria RDT, hospitals with poor microscopy should augment their diagnostic capability with RDTs to reduce the high proportion of misdiagnosed cases. The current study therefore recommend strengthening of supportive supervision, monitoring, and evaluation of technicians performing the diagnosis in health facilities in order to fully implement effective malaria case management.

5.4. Limitations of the Study

The study looked at how the dry and wet seasons influenced submicroscopic infection, entomological indices of malaria, and clinical malaria incidences. However, the dry season was shorter than usual, and the rainy season was not as heavy as expected, potentially reducing the magnitude of the seasonal effect. Throughout the study period, all visits took place during the day. Since samples were collected from residents found during visitation, some household members may have been overlooked during the study period, particularly those who leave in the morning early hours and return at night late hours, as a result, frequent visits were made during the study. Some interview questions required recall, and some residents were unable to recall the time frame in great detail; thus, feedback on the results may have overestimated or underestimated the febrile history and treatment pattern. However, in this study, the CHVs visited the residents' homes on a regular basis to verify their responses. Travel history was not taken into account and there could have been imported cases of malaria despite the influence of topography on clinical malaria.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1. Summary

The study's findings revealed that topographic and seasonality are significantly associated with submicroscopic *Plasmodium* infection. The likelihood of submicroscopic infection was higher in the lakeshore zone during both the wet and dry seasons (AOR: 2.71, 95% CI=1.85-3.95; $p<0.0001$) and hillside zone (AOR: 1.74, 95% CI=1.17–2.61, $p=0.007$) than in the highland plateau zones. Furthermore, topography and seasonality were found to be significant predictors of adult *Anopheles* vector abundance, larval densities, and habitat types. The majority of larval habitats were found during the wet season and along the lakeshore zone. The animal hoof prints with the highest larval densities in the lakeshore zone were the most productive breeding habitats in the study. Clinical malaria cases were twice as common in lakeshore zone (OR: 2.02, 95% CI=1.62-2.50, $p<0.0001$) and 1.4 times more common on the hillside (OR: 1.42, 95% CI=1.13-1.79, $p=0.002$) than on the highland plateau. Residents were 1.49 times more at risk of clinical malaria during wet seasons (OR: 1.49, 95% CI=1.24-1.80, $p<0.0001$) than during the dry season. The treatment patterns of residents with clinical malaria differed significantly in lakeshore zone ($p<0.0001$) and hillside zones ($p<0.0001$), but not in the highland plateau zone ($p=0.431$).

6.2. Conclusions

i. The prevalence of sub-microscopic infection was influenced by topography and seasonality, with lakeshore zone and wet seasons having the highest infection carriage.

ii. The availability of larval habitats, larval density, and adult mosquito relative abundance were all influenced by topography and seasonality. The lakeshore and hillside zones had higher *Anopheles* larval densities than the highland plateau, and they were higher during the wet season than the dry season. *Anopheles* vector abundance was higher in the lakeshore zone compared to the highland plateau, but there was no difference in the hillside zone. The abundance of adult Anopheles vectors was greater during the wet season than during the dry season.

iii. The clinical malaria cases were greatly influenced by topography and seasonality. Clinical malaria cases were common in the lakeshore and hillside zones than on the highland plateau zones. During the wet season, residents were more likely to contract clinical malaria than during the dry season.

6.3. Recommendations

6.3.1. Recommendations from current study

i. In the Lake Victoria region of western Kenya, topographic features of the local landscape and rainfall seasonality are important predictors of submicroscopic malaria infection. To maintain progress toward parasite reservoir elimination, strategies for identifying malaria transmission determinants within a heterogeneous landscape should be developed to guide the formulation of target site specific interventions across different eco-epidemiological settings. Furthermore, Diagnostic tests more sensitive than blood smear microscopy will allow for monitoring and targeting geographic sites where additional vector interventions are needed to reduce malaria transmission.

ii. To allow for the updating of information on malaria transmission intensities, relevant entomological indicators such as vector species diversity, vector ecology, and bionomics must be included in entomological surveillance databases, particularly in heterogeneous landscapes. Understanding vector ecology and bionomics, as well as reducing their availability, will be critical for malaria control and elimination via the implementation of targeted environmental management interventions across heterogeneous landscape.

iii. Seasonal malaria chemoprevention and during the peak transmission season tailored towards topographical zones will present opportunities for reducing clinical episodes and potentially eliminating malaria. To track the impact of malaria control interventions across heterogeneous landscapes, clinical malaria incidences reporting at the community level should be strengthened by providing supportive supervision of community health volunteers.

6.3.2. Recommendations for future studies

i. The influence of topography on immunogenic correlates of *Plasmodium* infection suppression, which could be contributing to the heterogeneous distribution of low density *Plasmodium* infection, should be studied.

ii. When designing an adaptive vector control intervention, the impact of seasonality and topography on malaria entomological indices should be considered. The impact of topographical variation on low density *Plasmodium* infection ability to infect mosquitos and thus sustain gametocyte carriage in lowland and highland areas should be studied.

iii. The effect of topography and seasonality on the immune response to malaria, as well as malaria hotspots that contribute to outbreaks in low transmission settings, should be the focus of future research. Furthermore, future research should concentrate on predictive models to aid in the coordination of efforts to keep *Plasmodium* infection rates low and, potentially, eradicate malaria.

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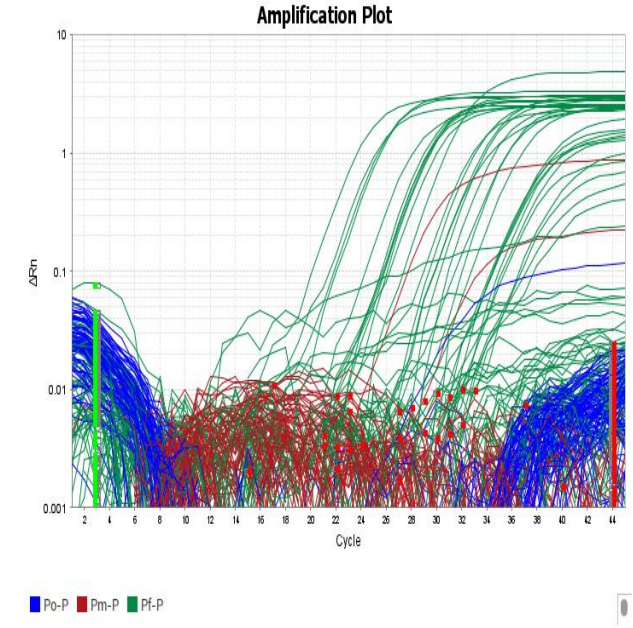
APPENDICES

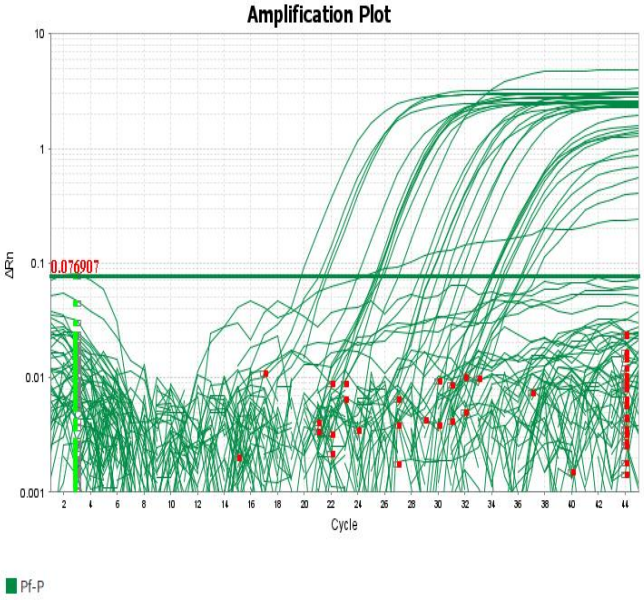
Appendix I Demographic Questionnaire on Open Data Kit

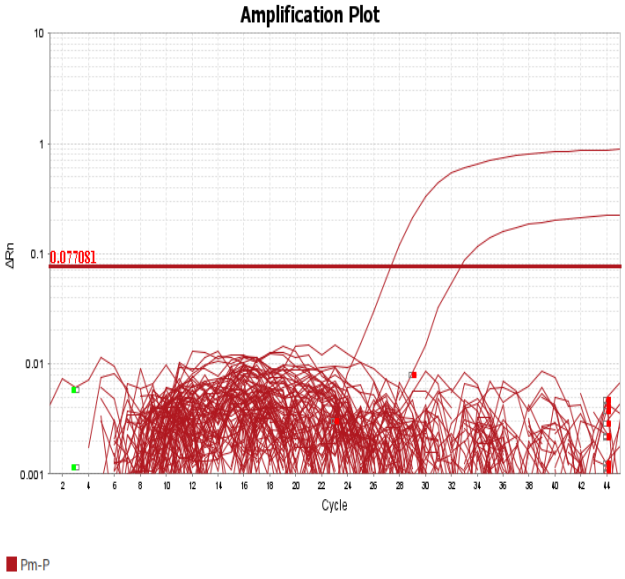
Survey	Options
1. Survey Date	
2. Study Site	Kisumu County
2a. Sub-County of household location	Nyakach Sub-County
2b. Location of household	
2d. Ecological Zone of cluster	LK – Lakeshore, MD – Hillside (Middle), NB – Highland Plateau (Nyabondo)
3. Household Serial Number	
3a. Household No.	
Geo-location	
4. House GPS Readings	
► Latitude	
► Longitude	
► Elevation	
5. BedNet Use	
a. Mosquito Prevention	0. Do Nothing, 1. Use screened windows/ doors, 2. Use bednet, 3. Regular use of commercial sprays, 4. Regular use of coils, 5. Burn herbs/cow dung, 6. Fan, 7. Use other commercial repellents, 8. Smoking, 9. Government IRS (Indoor Residual Spraying), 99. Others
b. Number of Bednet Owned	
c. Bednet Type	0. N/A, 1. Regular untreated net, 2. LLIN, Long Lasting Insecticidal Net, 5. PBO Net, 99. Others
d. Bednet Usage	0. N/A (No Bednet), 1. Every Night, 2. 5 ~ 6 Nights, 3. 2 ~ 4 Nights, 4. 0 or 1 Night
6. Health Facility Accessibility	
6. Primary Seeking Treatment Method	1. Do nothing, wait for a couple of days, 2. Self-medication with (traditional) herbs, 3. See the traditional healer, 4. Buy and use medicine from chemist/pharmacy/shop, 5. Treat in a public hospital/health center/facility, 6. Treat in a private clinic, 99. Others
House Structures	
7. Roof Material	0. NA / No Roof, 1. Corrugated Iron Sheet, 6. Makuti (Thatch), 5. Grass (Other than Thatching), 2. Tiles, 3. Concrete, 4. Asbestos Sheets, 7. Tin, 8. Mud/Dung, 99. Others
8. Floor type	1. Cement &. Tiles, 2. Wood, 3. Dirt/Mud/Earth, 99. Others
9. Wall Material	1. Stone/ Brick/Block, 2. Mud/Wood, 3. Mud/Cement, 4. Corrugated Iron Sheets, 99. Others
Resident	
R0. Is Resident NEW added (in this house)?	Yes/No
Resident Information	
R1. Study Code for Resident	
R2a. First Name	
R2b. Last Name	
R2c. Does resident absent (but still living in this household)?	Yes/No
Age & Gender	
R3a. Date of Birth for Resident	
R3b. Age	
R4. Sex	Male/Female
Demographics	

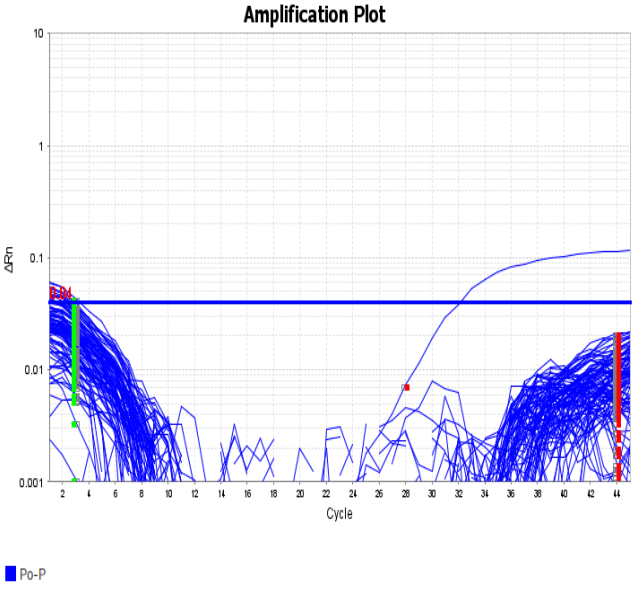
R8. Occupation	1. Agricultural worker, 2. Trader, 3. Office worker/Teacher, 4. Student, 5. Non-school Child (Age < 15), 6. Guardsman/Soldier/Peace officer, 7. Herdsman, 8. Fisherman, 9. Mining, 10. Factory/Construction worker, 11. Unemployed, 12. Salesperson, Shop Clerk, Server, 13. Own Business/Shopkeeper, 99. Others
R9. Education	1. Illiteracy Adult (Age>15)/Never attend School, 2. Preschool (before school age), 3. Primary school, 4. Middle (lower Secondary) school, 5. High (upper Secondary) school, 6. College or University, 7. Graduate school or higher, 99. Others
Visit Status	
R11. Any Malaria Symptoms?	X. None, A. Fever, B. Chills/Shivering, C. Malaise, D. Fatigue, E. Muscle pain, F. Joint pain, G. Headache, H. Irritability, I. Nausea, K. Vomiting, M. Diarrhea, O. Abdomen pains, P. Loss of appetite, R. Breathing difficulty, S. Dizziness, T. Coughing, U. Stomachache, Z. Others
Visit Memo	
R13a. Temperature (°C)	

Appendix II *Plasmodium* species RT-PCR Amplification plots









Po-P *Plasmodium ovale*-Probe
Pm-P *Plasmodium malariae*-Probe
Pf-P *Plasmodium falciparum*-Probe
The line number is a value set above the baseline/background. Above it, a smooth curve is considered a positive signal.

Appendix III Questionnaire on the Larval Habitat Survey on the Open Data Kit

Larval Habitat Survey	**Options**
1. Survey Date	
2. Study Site	Nyakach Sub-County- Kisumu County
2d. Ecological Zone of cluster	LK – Lakeshore, MD – Hillside (Middle), NB – Highland Plateau (Nyabondo)
2e. Cluster of habitat location	
3. Habitat Serial Number	
3a. Habitat No.	
Geo-location	
4. House GPS Readings	
► Latitude	
► Longitude	
► Elevation	
Environment	
5. Habitat Type	A. Drainage ditch, B. River edge/Reservoir shoreline, C. Swamp/Marshes, D. Rice puddle, E. Animal footprint, F. Tire track/Road paddle, G. Fish pond, I. Man-made pond, K. Natural pond/Rain pool, M. Rock pool, N. Water container, O. Irrigation concrete lining (canal/structure), R. Spring or Brick pit, Z. Others
6. Landuse Type (Surrounding Environment)	1. Forest, 2. Shrubland, 3. Grassland/Pasture, 4. Wetland/Swamp, 5. Cultivated land, 6. Urban and built-up, 7. Road, 8. Barren land/Bare Rock, 9. Water, 99. Others
7. Vegetation Coverage %	
8. Shade Coverage %	
9. Substrate Type	1. Gravel/Pebble, 2. Sand, 3. Mud/Dirt, 4. Concrete (Artificial Lining), 5. Plastics 99. Others, 0. N/A (Not available)
10. Distance to Nearby House	1. Less than 100 meters, 2. 100 ~ 200 meters, 3. Over 200meters
If Landuse is Cultivated Land or Cropland	
6b. Crop Type	0. N/A, Not Cropland, 1. Rice, 2. Beans, 3. Maize, 4. Sorghum, 5. Millet, 6. Sugarcane, 7. Cassava, 8. Cotton, 9. Potato, 15. Sugarcane, 16. Any Vegetable, 99. Others
Habitat Measure	
10a. Length (m)	
10b. Width (m)	
10c. Depth (m)	
11. Number of Dips	
12. Larvae Counts: An. gambiae	
12a. An. gambiae: L1/L2	
12b. An. gambiae: L3/L4	
12c. An. gambiae: Pupa	
13. Larvae Counts: An. funestus	
13a. An. funestus: L1/L2	
13b. An. funestus: L3/L4	
13c. An. funestus: Pupa	
14. Larvae Counts: An. pharoensis	
14a. An. pharoensis: L1/L2	
14b. An. pharoensis: L3/L4	
14c. An. pharoensis: Pupa	
15. Larvae Counts: An. coustani	
15a. An. coustani: L1/L2	
15b. An. coustani: L3/L4	
15c. An. coustani: Pupa	
Larvae Counts: Others	
16. Aedes	
17. Culex	
18. Other Species	
Survey Note or Annotation	
Survey Note	
Survey Staff	

Appendix IV Questionnaire on the Adult Vector Survey on the Open Data Kit

Adult Vector Survey	Options
1. Survey Date	
2. Study Site	Nyakach Sub-County- Kisumu County
Study Site	
2d. Ecological Zone of cluster	LK – Lakeshore, MD – Hillside (Middle), NB – Highland Plateau (Nyabondo)
2e. Cluster of household location	
3. Household Serial Number	
3a. Household No.	
Geo-location	
4. House GPS Readings	
► Latitude	
► Longitude	
► Elevation	
5. Household Population	
Age <5:	
Age 5 - 15:	
Age >15	
House Structures	
6. Roof type	01. Corrugated Iron Sheet, 5. Grass, 2. Tiles, 3. Concrete, 7. Tin, 8. Mud/Dung, 99. Others
7. Wall Material	1. Stone/ Brick/Block, 2. Mud/Wood, 3. Mud/Cement, 4. Corrugated Iron Sheets, 99. Others
8. Wall Material	1. Stone, 2. Brick/Block, 3. Mud/Wood, 4. Mud/Cement, 6. Corrugated Iron Sheets, 7
9. Screen Window	Yes/No
10. Open Vent Space	Yes/No
House Environment	
11. Landuse Type	1. Forest/Shrubland, 2. Grassland/Pasture, 3. Wetland/Swamp, 4. Cultivated/Cropland, 6. 99. Others
12. Distance to Mosquito Habitat	1. Less than 100 meters, 2. 100 ~ 200 meters, 3. Over 200meters,
Trapping Information	
13. Trapping (Sampling) Method	1. PSC, Pyrethrum spray catch, 4. CDCLT light trap without attractant,
14. Trapping Location	1. Indoor - living room,
15. Hours of Collection	1. Overnight from dusk till dawn, 2. Morning from dawn till noon, 3. Afternoon noon to dusk
16. Weather Condition	1. Clear/Fair Sky, 2. Cloudy, 3. Overcast, 4. Rainy, 5. Shower Storm/Thunderstorm, 99. Others
17. Mosquito Prevention	0. Do Nothing, 1. Use window screen/screen door, 2. Use bednet, 3. Regular use commercial spray, 4. Regular use coils, 5. Burn herbs/cow dung, 6. Fan, 9. IRS. 99. Others
18. Bednet Own by Type	
18a. Regular Net	
18b. LLIN	
18c. PBO type Bednet	
18fd Other Bednet	
19. How many Mosquito?	
Mosquito Counts by Species	
a. Species	*An. gambiae*, *An. funestus*, *An. pharoensis*, *An. Coustani*, *An.* Other species, Aedes, Culex, Others
b. Male Total	
c. Female Total	
c1. Empty	
c2. Blood-Fed	
c3. Half-Gravid	
c4. Gravid	
Survey Note or Annotation	
20c. Survey Note	
20d. Survey Staff	

Appendix V Clinical malaria Fever questionnaire

Active case detection of malaria Report Form Instructions

#	Study Code	Age	Sex	Report Date	Temp °C	RDT	Treatment?	Occupation?	Education?	ITN?
1	**LK01001-01**	**74**	**F**	**21-05-2020**	**38.4**	**Pos**	**5**	**1**	**1**	**N**
2	**LK01011-04**	**14**	**M**	**24-05-2020**	**37.9**	**Neg**	**1**	**6**	**2**	**Y**
3										
4										
5										

Report Date: Can be local date format while field survey
Temp °C: resident's body temperature in **°C**
RDT: What are the RDT results? **NA** -- Not Done/Not Available, **Neg** -- Negative, **Pos --** Positive, implying Malaria *Pf* positive
Treatment: Treatment seeking method. What kind of treatment method this resident will use?

1. Do nothing, wait for a couple of days
2. Self-medication with (traditional) herbs
3. Sought treatment with the traditional healer
4. Buy and use medicine from chemist/pharmacy/shop
5. Sought treatment in a public health facility
6. Sought treatment in a private clinic
99. Others

Occupation: Income generating activity. What kind of occupation is the resident?

1. Farmer
2. Small scale business
3. Office worker
4. Unemployed
5. Student
6. Non-school child
99. Others

Education: Highest education level. What level of education is the resident?

1. Never attended school
2. Pre-school age
3**.** Primary
4. Secondary
5. College & above

LLIN: Has this residents slept under LLIN previous night? Fill **Y** for '**Yes**' or **N** for '**No**'

Comment:

Appendix VI Passive case detection of malaria questionnaire

PCD Survey (REDCap)	Options
Survey Date	
Study Site	Nyakach, Kisumu County |
1. Clinic/Health Facility	NKH-Lakeshore | NK_NMB, - Hillside | NK_NBH, Highland plateau
2a. First Name	
b. Last Name	
3. DSS/MBS Study Code	
4b. Age	
5c. Sex	M, Male | F, Female | U, Unknown
5. Occupation	1, 1. Agricultural worker | 2, 2. Trader | 3, 3. Office worker/Teacher | 4, 4. Student | 5, 5. Non-school child | 6, 6. Guardsman/Soldier/Peace officer | 7, 7. Herdsman | 8, 8. Fisherman | 9, 9. Mining | 10, 10. Factory/Construction worker | 11, 11. Unemployed | 12, 12. Salesperson, Shop Clerk, Server | 13, 13. Own Business/Shopkeeper | 99, 99. Others
6. Education	1, 1. Illiteracy/Never attend School | 2, 2. Preschool (before school age) | 3, 3. Primary education | 4, Secondary. 5. College or University, 99. Others
7. Mosquito Prevention	0, 0. Do Nothing | 1, 1. Use screened windows/ doors | 2, 2. Use bednet | 3, 3. Regular use of commercial sprays | 4, 4. Regular use of coils | 5, 5. Burn herbs/cow dung | 6, 6. Fan | 7, 7. Use other commercial repellents | 8, 8. Smoking | 9, 9. Government IRS (Indoor Residual Spraying) | 10, 10. Draining stagnant water | 11, 11. Clean up house environment | 99, 99. Others
b. Bednet Usage	0, 0. N/A, No Bednet | 1, 1. Every Night | 2, 2. 5 ~ 6 Nights | 3, 3. 2 ~ 4 Nights | 4, 4. 0 or 1 Night
8a. Temperature (°C)	
b. Fever Day	
9a. Symptoms	X, X. None | A, A. Fever | B, B. Chills/Shivering | C, C. Malaise | D, D. Fatigue | E, E. Muscle pain | F, F. Joint pain | G, G. Headache | H, H. Irritability | I, I. Nausea | K, K. Vomiting | M, M. Diarrhea | O, O. Abdomen pains | P, P. Loss of appetite | R, R. Breathing difficulty | S, S. Dizziness | T, T. Coughing | U, U. Stomachache | Z, Z. Others
b. Severe Symptoms	0, 0. None | 1, 1. Impaired consciousness | 2, 2. Prostration | 3, 3. Multiple convulsions | 4, 4. Respiratory distress (Metabolic acidosis) | 5, 5. Circulatory collapse | 6, 6. Jaundice | 7, 7. Hemoglobinuria | 8, 8. Abnormal bleeding | 9, 9. Pulmonary edema (radiological) | 10, 10. Death | 99, 99. Others
10. Prescription	0, 0. N/A or No prescription | 1, 1. Coartem - Artemether-Lumefantrine (AT) tablet | 2, 2. Other ACT (Artesunate IV or Artemether IM) | 3, 3. Chloroquine | 4, 4. Sulfadoxine-pyrimethamine, SP (Fansidar) | 5, 5. Quinine (oral (QSO4) or IV) | 6, 6. Primaquine | 7, 7. Don't know or writing not legible | 99, 99. Others
11. Rapid Diagnostic Test (RDT) Result	X, X. N/A, Not Done | N, Negative | P, Positive | U, Undetermined
12. Microscopic Diagnosis Result	X, X. N/A, Not Done | N, Negative | P, Positive | U, Undetermined

Appendix VII Ethical approval letter

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

REF: MSU/DRPI/MUERC/00992/21 **Date**: 7th January, 2022

TO: Otambo Wilfred Ouma
PHD/SC/000113/2018
Department of Zoology
School of Physical and Biological Sciences
Maseno University
P.O. Box, Private Bag, Maseno, Kenya

Dear Sir,

RE: Influence of Landscape Heterogeneity on Malaria Burden in Nyakach Sub-County, Western Kenya

This is to inform you that **Maseno University Ethics Review Committee (MUERC)** has reviewed and approved your above research proposal. Your application approval number is MUERC/00992/21.The approval period is 7th January, 2022 – 6th January, 2023.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by Maseno University Ethics Review Committee (MUERC).
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to Maseno University Ethics Review Committee (MUERC) within 24 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to Maseno University Ethics Review Committee (MUERC) within 24 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to Maseno University Ethics Review Committee (MUERC).

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

MASENO UNIVERSITY ETHICS REVIEW COMMITTEE 07 JAN 2022 P.O. BOX PRIVATE BAG MASENO

Prof. Philip O. Owuor, PhD, FAAS, FKNAS
Chairman, MUERC

Appendix VIII Authorization from County Director of Health, Kisumu

COUNTY GOVERNMENT OF KISUMU

Telegrams: "PRO.(MED)"
Tel: 254-057-2020105
Fax: 254-057-2023176
E-mail: kisumucdh@gmail.com
When replying please quote:

County Director of Health,
Kisumu.
P.O. Box 721-40100,
KISUMU.

DEPARTMENT OF HEALTH

RE: GN 133 VOL IX (415)

Date: 10th April 2019

To

SCMOH – Nyakach Subcounty

Facility in-charges – Nyakach Subcounty

Dear Sir,

RE: STUDY APPROVAL – Changing Epidemiology, Transmission and Pathogenesis of *Plasmodium falciparum* and *P. vivax* Malaria

The above study is being conducted in Homabay by the International Center of Excellence for Malaria Research (ICEMR). ICEMR wishes to expand their study to Nyakach subcounty. It is hereby noted that ethical approval for the project has been granted by Maseno University.

The purpose of this letter is to inform you that the study has been approved by this office for implementation with immediate effect. By copy of this letter, the principal investigator is requested to provide you with periodic reports on the progress of the study.

Dr. Onyango D.
County Director of health
Kisumu County.

COUNTY DIRECTOR OF HEALTH KISUMU

Appendix IX Authorization from Sub-County Commissioner, Nyakach

THE PRESIDENCY

MINISTRY OF INTERIOR & COORDINATION OF NATIONAL GOVERNMENT

TELEGRAMS "DISTRICTER"

Telephone
When replying please quote
Our Ref: **NYK/PH/13/1(200)**

DEPUTY COUNTY COMMISSIONER
NYAKACH SUB COUNTY
P.O BOX 115
PAP-ONDITI
Date: 9th May, 2019

ALL CHIEFS- NYAKACH SUB- COUNTY
ALL A/ CHIEFS- NYAKACH SUB-COUNTY

RE: STUDY APPROVAL- Changing Epidemiology, Transmission and Pathogenesis of Plasmodium falciparum and P. Vivax Malaria.

The above subject refers;

The above study is being conducted in Homabay and Nyakach in Kisumu County by the International Center of Excellence for Malaria Research (ICEMR). The ethical approval for the project has been granted by Maseno University.

The purpose of this letter is to inform you that the study has been approved by this office for implementation. All Chiefs and Assistant Chiefs are requested to accord the team all necessary assistance needed.

Thank you.

DEPUTY COUNTY COMMISSIONER
NYAKACH SUB-COUNTY
P.O. Box 115 - 40111
PAP-ONDITI

C.W. SANGURA
DEPUTY COUNTY COMMISSIONER
NYAKACH SUB-COUNTY

Copy to: ACC –LOWER NYAKACH

ACC- WEST NYAKACH

ACC- NORTH NYAKACH

ACC- UPPER NYAKACH

Appendix X Consent to participate in the study

Title of Study: Influence of landscape heterogeneity on malaria burden in Nyakach Sub-County, Western Kenya

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

Name of Volunteer: _____

Age of Volunteer: _____

Introduction

Otambo Wilfred Ouma is a postgraduate student at Maseno University, School of Physical and Biological Sciences, Zoology Department with the supervisors; Dr. Patrick Onyango of Zoology Department and Prof. Collins Ouma of Biomedical Department both of Maseno University, and Dr. John Githure of International Centre of Excellence for Malaria Research. I am requesting you to be part of the study as a sample donor.

Purpose of the study

The goal of this research is to look into the impact of landscape heterogeneity on malaria burden in Nyakach Sub-County, Western Kenya.

Requirements

Approximately 250ul of blood will be collected by finger prick in heparin microtainers. 100ul of the withdrawn blood from participants will be used to prepare dried blood spots and 150ul will be used to prepare thick and thin smears. All participants will have a unique identifier that links them to their laboratory results, demography and location. Samples will be analyzed by microscopy and qPCR at the ICEMR laboratory. Unauthorized test for the study will not be carried out on the blood samples.

Benefits

You will not receive financial benefit from your participation, however, If you have a fever or become ill, you will be directed to the nearest clinic for treatment.

Discomforts and risks:

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

Confidentiality

To the extent permitted by law, information about you will be kept strictly confidential. Your identity will be coded but not linked to any published results.

Freedom to withdraw:

Your participation in this study is entirely voluntary, and you may withdraw at any time without prejudice or effect on your future health care.

Questions and complains

Questions and complains to the study will be addressed to Otambo Wilfred Ouma through the contact +254728583180 or an email at oumaotambo@gmail.com. In case of any questions with regards to your right you may contact Maseno University Ethics review secretariat on 057

351221 or mail them at P.O.BOX Private Bag, Maseno or email at muercsecretariant@maseno.ac.ke.

Consent statement:

I have read the above statements and agree to participate in this experiment under the terms outlined above. I understand that if any questions or concerns regarding this project I can contact the investigator Otambo Wilfred or the Maseno University Ethics Review secretariat.

Participant signature: _____ Date: _____

OR

Parent/legal guardian's signature: _____ Date: _____

Principal investigator: _____ Date: _____

Informed consent in Luo

Yie donjo e nonro

Wii nonro: en gik magegi mopogre e piny mawadakie makonyo malaria hinyo dhano ei Gweng' mar Nyakach manie yoo podho chieng ma Kenya.

Obokeni ibo konigo kasto kila ng'ato modonjo e noro noketo koke.

Ning ngama odonjo e noro: _____

Hik ng'ama odonjo e nonro: _____

Chakrouk

Otambo Wilfred Ouma en japuonjre e Mbalariany mar Maseno. Watiyo kanyakla gi Daktari Patrick Onyango kod Profesa Collins Ouma mag Mbalariany ma Maseno, gi Daktari John Githure matiyo e nonro mar Malaria research. Akwayi mondo ibedie achiel kuom joma bobetie enonrowani

Tiend nonro

Tiend nonroni en mondo wanon ni en gik magegi mopogre e piny mawadakie makonyo malaria hinyo dhano ei Gweng' mar Nyakach manie yoo podho chieng ma Kenya.

Chenro

Remo marom gi 250ul ibokau kuomi e lweti tieketo e tube matin. Bange, 100ul e remo maneosegul ibolos go remo othuo ei kalatas gi 150ul ibo tek e glass to iloso mondo eng'igpo kute makaelo malaria. Kila nga'to ibomii number mibotigo kuom fuenye kar tiyo gi nying ma nyiso kuma giaye kain dichuo kata nyako. Remo mogul ibo ter e malaria research kuma ibo ng'ii kute makelo malaria. Onge pim maok ondik e nonroni mibiro timni.

Chiwo mar nonro

Onge chiwo mibiro mi joma odonjo e nonro, mak mana ki tuo, ibokoni kaka onego idhi e clinic machiengni kodi mondo iyud thieth.

Hinyruok manyalo betie

Chuyo luedo manyalo kelo rem matin. Ibotii gi sandan maler matin to bermoknyal hinyo dhano kum ng'ato ka ng'ato. Kaka ibotime okanober gi rem maduong kata okang', nokel hinyruok gi kute mamoko.

Pando weche

Weche mora mora michiwo ibo pand b rit gi chik. Ibro pand nyingi ka janonro kindiko nying magalala mondo jononro kik ng'eyi kata wecheni okanegoli kigo book.

Hero mar weyo nonro

Bet mari e nonro k en hechi to inyalo weyo bet e noro samora mor maok ni bokethoni kaka ithiedhi e hospital.

Penjo gi yuagrok

Penjo mora mora gi yuagruok ei nonroni inyalo neno Otambo Wilfred Ouma e namba simu 0728583180 kata e mbui oumaotambo@gmail.com. Penjo kata yuak malure gi ratiro mari inyalo gocho ni jochiu ratiro ma Mbalariany ma Maseno e namba simu 057351221 kata indik e sanduk mar posta Private Bag, Maseno kata e mbui muercsecretariant@maseno.ac.ke.

Ogiri miti mar donjo e nonro

Asesomo gik moko duto mondik to ayie donjo e nondro madhi timre kalure gi chike mantie. Ang'eyo ni kapenjo kata ywak mora mora manyalo betie anyalo chopo ne Wilfred Otambo e number simu kata joma chiwo oboke mar timo nonro e Mbalariany ma Maseno

Sei mari:_____ **Tarik: _____**

KATA

Sei mar janyuol/Jarit Moyangi_____ **Tarik: _____**

Wuon Nonro: _____ **Tarik: _____**