

PREVALENCE OF *Wuchereria bancrofti* AND *Plasmodium falciparum* INFECTION IN *ANOPHELES* MOSQUITOES AND HUMAN BLOOD SAMPLED FROM MATAYOS IN BUSIA COUNTY, WESTERN KENYA

CAROLINE WACERA MAINA

**MASTER OF SCIENCE IN
MOLECULAR BIOLOGY AND BIOINFORMATICS**

**JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY**

2026

**Prevalence of *Wuchereria bancrofti* and *Plasmodium falciparum*
Infection in *Anopheles* Mosquitoes and Human Blood Sampled from
Matayos in Busia County, Western Kenya**

Caroline Wacera Maina

**A Thesis Submitted in Partial Fulfilment of the Requirements for
the Degree of Master of Science in Molecular Biology and
Bioinformatics of the Jomo Kenyatta University of Agriculture and
Technology**

2026

DECLARATION

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

Signature.....Date.....

Caroline Wacera Maina

This thesis has been submitted for examination with our approval as the University Supervisors

Signature.....Date.....

Dr. Joel L. Bargul, PhD

JKUAT, Kenya

Signature.....Date.....

Dr. Luna Kamau, PhD

KEMRI, Kenya

DEDICATION

To my parents Eutychus Maina Murage and Irene Wachuka Maina, whose encouragement inspired me to pursue my studies beyond the undergraduate level. To my husband, Eric Mwangi Kimani, whose financial and emotional support was crucial throughout my journey, and to my daughter, Kyla Neema Mwangi, who graciously allowed me time away from her to focus on my studies. I am also grateful to my siblings. Thank you all for your unwavering love and support.

ACKNOWLEDGEMENT

I acknowledge the almighty God for his guidance, provision and gift of good health throughout my studies. I also wish to express my sincere gratitude and recognition to my supervisors, Dr. Luna Kamau, Dr. Joel Bargul and Dr. Nancy Kinyatta for their guidance in designing and implementing this project and input into my publication and thesis. I appreciate your exceptional patience, mentorship and encouragement. I also acknowledge the funding received from the Government of Kenya through the grant No. KEMRI/IRG/002/2017/18 to Luna Kamau.

TABLE OF CONTENTS

DECLARATION.....	ii
DEDICATION.....	iii
ACKNOWLEDGEMENT.....	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF APPENDICES	xi
ABBREVIATIONS AND ACRONYMS.....	xii
ABSTRACT	xiii
CHAPTER ONE	1
INTRODUCTION.....	1
1.1 Background of the Study	1
1.2 Statement of the Problem	5
1.3 Justification	7
1.4 Objectives the Study.....	9
1.4.1 General Objective	9
1.4.2 Specific Objectives	9
1.5 Research Questions	9
CHAPTER TWO	10
LITERATURE REVIEW.....	10
2.1 Global Distribution and Epidemiology of Malaria.....	10

2.2 Global Distribution and Epidemiology of Lymphatic Filariasis	11
2.3 Taxonomy.....	12
2.4 Pathogenesis of Malaria	12
2.5 Pathogenesis of Lymphatic Filariasis.....	14
2.6 Vectors of Malaria and Lymphatic Filariasis	15
2.7 Identification Features of <i>Anopheles funestus</i> and <i>Anopheles gambiae</i> Complexes	16
2.8 Diagnosis	18
2.8.1 Light Microscopy.....	18
2.8.2 Antigen Detection Test.....	19
2.8.3 Antibody Detection.....	20
2.8.4 Molecular Diagnosis	20
2.9 Treatment of <i>Lymphatic Filariasis</i> and Malaria.....	21
2.10 Co-Infections	23
2.10.1 Co-infection of <i>Wuchereria bancrofti</i> and <i>Plasmodium falciparum</i>	23
2.10.2 Co-infection in Mosquito Vectors	23
2.10.3 Co-infection in Human Blood.....	23
2.11 Parasite Interactions and Transmission Implications	24
2.12 Public Health Implications	24
2.13 Summary and Critique of Existing Literature	25
2.13.1 Summary.....	25

2.13.2 Critique of Existing Literature	27
CHAPTER THREE	30
MATERIALS AND METHODS	30
3.1 Study Design	30
3.2 Sampling Site	31
3.3 Collection of Mosquito and Blood Samples.....	32
3.4 Sample Size Determination	32
3.5 Inclusion and Exclusion Criteria	34
3.6 Ethical Consideration	34
3.7 Laboratory Methods	34
3.7.1 DNA Extraction	35
3.7.2 Molecular Identification of Mosquitoes	36
3.7.3 Molecular Detection of <i>Wuchereria bancrofti</i>	37
3.7.4 Molecular Detection of <i>Plasmodium falciparum</i>	38
3.7.5 Sequencing	38
3.8 Data and Phylogenetic Analysis	38
CHAPTER FOUR.....	40
RESULTS	40
4.1 Introduction	40
4.2 Diversity of Species within the Collected <i>An. gambiae</i> and <i>An. funestus</i> Complexes	40
4.3 Infections with <i>W. bancrofti</i> and <i>P. falciparum</i>	41

4.4 Sequencing Analysis	45
CHAPTER FIVE.....	48
DISCUSSION, CONCLUSION AND RECOMMENDATIONS.....	48
5.1 Discussion	48
5.2 Conclusion.....	51
5.3 Recommendations	52
5.4 Study Limitations	53
REFERENCES.....	54
APPENDICES	74

LIST OF TABLES

Table 3:1: PCR Primers for Molecular Identification of Mosquito Species, <i>W. bancrofti</i> , and <i>P. falciparum</i> Parasites	36
Table 4:1: Number of Positive and Negative Infections of Plasmodium and Filariasis from Mosquito Species.....	42
Table 4:2: Infection Rate of Mosquito Species.....	42
Table 4.3: Showing the Sequences from this Study were Identical to Each Other and Shared 97 to 100% Nucleotide (nt) Similarity with Reference <i>W. bancrofti</i> Sequences available on GenBank	46

LIST OF FIGURES

Figure 2.1: The Life Cycle of <i>Plasmodium falciparum</i>	13
Figure 2.2: The Life Cycle of <i>Wuchereria bancrofti</i>	15
Figure 2.3: <i>An. funestus</i> and <i>An. gambiae</i> Mosquito Species ((MalariaGEN, 2021))	17
Figure 3.1: A Map of Kenya showing the Sampling Site in Matayos Constituency, Busibwabo Ward, Busia County	31
Figure 4.1: Gel Electrophoresis of PCR Products of <i>An. funestus</i> Species Complex Mosquito Samples	40
Figure 4.2: Distribution of Mosquito of Species in the <i>An. funestus</i> Complex (a) and (b) Mosquito Species in the <i>An. gambiae</i> Complex (b)	41
Figure 4.3: Number of Negative Infections (a) and Positive Infections (b) from <i>plasmodium</i> and <i>filariasis</i> for Each Mosquito Species	43
Figure 4.4: Gel Electrophoresis of PCR Products for <i>Plasmodium falciparum</i> Detection.	44
Figure 4.5: Gel Image	45
Figure 4.6: Maximum Likelihood Dendrogram Tree of <i>W. bancrofti</i> 18S rRNA Gene.	47

LIST OF APPENDICES

Appendix I: Ethical Approval Letter from SERU	74
--	----

ABBREVIATIONS AND ACRONYMS

PCR	Polymerase Chain Reaction
MDA	Mass Drug Administration
DNA	Deoxyribonucleic Acid
rRNA	ribosomal Ribonucleic Acid
CDC	Center for Disease Control
GPELF	Global Program to Eliminate Lymphatic Filariasis
ADL	Adenolymphangitis
KEMRI	Kenya Medical Research Institute
LF	Lymphatic Filariasis
MF	Microfilariae
WHO	World Health Organization

ABSTRACT

Lymphatic filariasis and malaria are mosquito-transmitted diseases that are simultaneously endemic in several regions of Kenya. Despite control efforts, malaria continues to cause significant morbidity and mortality globally, with an estimated 249 million cases and over 610,000 deaths reported in the year 2025 while lymphatic filariasis is associated with chronic disability including lymphoedema and hydrocele, with approximately 657 million people in 44 countries still requiring preventive chemotherapy. This cross-sectional study evaluated the simultaneous presence of the parasites responsible for lymphatic filariasis and malaria, namely *Wuchereria bancrofti* and *Plasmodium falciparum*, in both human hosts and anopheline mosquito populations from Matayos Constituency in Busia County, western Kenya where new cases of Lymphatic filariasis had been newly reported. 292 Adult *Anopheles* mosquitoes were collected inside the houses of the 92 participants who tested positive by Filarial Test Strip. Field sampling was conducted to obtain mosquitoes belonging to the *Anopheles gambiae* and *Anopheles funestus* species complexes, which were subsequently analyzed by Polymerase Chain Reaction (PCR) to detect infections with *Wuchereria bancrofti* and *Plasmodium falciparum*. A total of 292 female *Anopheles* mosquitoes, together with 92 human blood specimens, were obtained and processed for laboratory analysis. PCR-based species identification was successful for 288 mosquitoes, corresponding to 98.63% of the collected specimens. Among the identified mosquitoes, members of the *An. funestus* complex predominated, accounting for 247 individuals (85.76%), whereas the *An. gambiae* complex contributed 41 specimens (14.24%). No specimens belonging to *An. parensis* or *An. vaneedeni*, both recognized members of the *An. funestus* complex, were detected in the sample population. Molecular screening revealed two mosquito infections with *Wuchereria bancrofti*, one occurring in *An. lesoni* and the other in *An. funestus*. These findings correspond to infection prevalences of 0.46% and 4.17% for the respective species. No evidence of *Plasmodium falciparum* infection was observed in either the mosquito specimens or the human blood samples analyzed. In contrast, *Wuchereria bancrofti* was detected in two blood samples, corresponding to a prevalence of 2.17%. Phylogenetic characterization based on partial 18S rRNA gene sequences of *W. bancrofti* demonstrated a high degree of genetic similarity to isolates previously reported in other studies. Furthermore, neither the mosquito samples nor the human blood specimens showed concurrent infections involving both *P. falciparum* and *W. bancrofti*. Overall, the predominance of *Anopheles funestus* mosquitoes within the study area calls for the implementation of effective vector control strategies to reduce mosquito population and minimize disease transmission. The detection of *Wuchereria bancrofti* in both *Anopheles* mosquitoes and human blood samples in Busia County indicates ongoing transmission of lymphatic filariasis. Therefore, there is a need to strengthen case detection and implement appropriate treatment programs for affected individuals to reduce the disease burden and interrupt transmission. The strong phylogenetic similarity observed between the isolates analyzed in this study and those reported from other endemic areas underscores the importance of ongoing molecular epidemiological monitoring. Continuous surveillance can support the timely identification of novel genetic changes, inform disease elimination strategies, and enhance understanding of the spatial distribution and transmission patterns of *Wuchereria bancrofti* strains across different regions.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Lymphatic filariasis and Malaria are mosquito-borne parasitic diseases that share *Anopheles* mosquitoes as their primary vectors. Consequently, co-infections may occur in areas where both diseases are endemic (World Health Organization, 2024; Snow et al., 2021). Across tropical Africa, transmission of these diseases is facilitated by several common *Anopheles* species, including *An. arabiensis*, *An. funestus*, *An. merus*, *An. melas* and *An. gambiae* (Sinka, 2013). In Kenya, malaria vectors *An. funestus* and *An. gambiae* have been documented in locations where transmission of both *Plasmodium falciparum* and *Wuchereria bancrofti* occurs, indicating the potential for overlapping disease transmission (Bartilol et al., 2024; Njenga et al., 2017). The presence of these vectors has also been reported in Kenya's western and Nyanza provinces near Lake Victoria, as well as in the coastal region (Orondo et al., 2021; Wanjala & Kweka, 2018).

Malaria remains a major parasitic infection affecting populations in subtropical and tropical areas worldwide. It is transmitted through the bites of infected female *Anopheles* mosquitoes and continues to contribute significantly to global mortality, with recent estimates reporting more than 600,000 deaths annually (World Health Organization, 2024). The disease is caused by various species of *Plasmodium* parasites, which include *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* with humans being their intermediate hosts (Lai et al., 2025). *P. falciparum* is the most common causal agent of malaria in Africa, the Caribbean, South-East Asia and South America, while *P. vivax* infections are commonly reported in America, Asia, and North Africa (World Health Organization, 2024). In 1998, the Roll Back Malaria (RBM) partnership was launched with the aim of reducing the impact and transmission of malaria worldwide (Roll Back Malaria Partnership, 2023). Despite these efforts, malaria remained a public health concern, with over 3 billion people exposed to the risk of infection across 97 countries by 2015. During the same period, the disease continued to cause hundreds of thousands of deaths and more than 200 million cases each year

(WHO, 2015). The WHO adopted the Global Technical Strategy for Malaria 2016–2030, together with the Action and Investment to defeat Malaria 2016–2030 framework, to help in the fight against malaria globally (WHO, 2021).

In Kenya, malaria is endemic to the coastal, Nyanza and Western regions where Busia has consistently reported among the highest prevalence rates (Kenya National Malaria Programme, 2020). Transmission is higher in these regions compared to low transmission areas such as Nairobi, Machakos, Kitui, Embu, Marigat as well as Ngurumani (President’s Malaria Initiative, 2022). In the past years, there has been the threat of failing malaria treatment leading to a change of first-line drugs. The first change was in 1998 where Sulfadoxine-pyrimethamine (SP) replaced Chloroquine (CQ), while the second change was in 2004 when Artemisinin therapy was named as the first-line treatment against malaria (Ministry of Health Kenya, 2023). However, SP is recommended for intermittent preventive treatment in pregnancy only in endemic regions.

Lymphatic filariasis, on the other hand, is one of the neglected tropical vector-borne diseases that cause swelling of limbs (lymphoedema), testis (hydrocoele) and elephantiasis (World Health Organization, 2024). This disabling swelling is the consequence of damage caused by thread-like nematodes to the lymphatic system. The lymphatic system is part of the immune system which moves fluids through the body while removing waste, bacteria, and viruses via lymph nodes (Standring, 2020). Damage to the lymphatic system causes fluid accumulation in tissues resulting in disabling swellings. The thread-like nematodes responsible are *Brugia malayi*, *Brugia timori* and *Wuchereria bancrofti* transmitted by mosquitoes from the genus *Anopheles* and *Culex*. (WHO, 2024).

In 2000, the WHO launched the Global Programme to Eliminate Lymphatic Filariasis (GPELF) as a strategy aimed at reducing the transmission of lymphatic filariasis in endemic areas. The programme focused on mass drug administration (MDA), involving the distribution of medicines such as albendazole, ivermectin, and diethylcarbamazine to at-risk populations (World Health Organization, 2023). In addition to reducing transmission, the programme seeks to improve the quality of life

of affected individuals by promoting morbidity management and preventing disability that comes with lymphatic filariasis (WHO, 2024).

In Kenya, lymphatic filariasis (LF) is endemic to the coastal region since ecological conditions favor transmission (World Health Organization, 2023). The LF elimination programme was first launched in Kilifi County where the entire population was treated with diethylcarbamazine citrate and albendazole. By 2011, the programme had expanded to Tana River and Lamu counties (Njenga et al., 2017). The programme has not been implemented in Busia County since no infections had been reported there at that time.

There are several methods used to diagnose malaria and filariasis including antibody detection (Mackenzie et al., 2022), microscopy, antigen detection (Rodgers et al., 2021) and molecular diagnostic methods. Antigen and antibody detection methods rely on detection of parasite-derived proteins in blood during infection (World Health Organization, 2023; Farrar et al., 2023).

Both diseases lymphatic filariasis and Malaria are transmitted by *Anopheles* mosquitoes, creating the potential for mixed infections in both the vector and human populations in endemic regions. In tropical Africa, key vectors include *An. gambiae*, *An. funestus*, *An. arabiensis*, and others. In Kenya, these primary vectors have been documented in coastal regions and western areas near Lake Victoria, including the Nyanza and Western provinces where Busia County is located.

Critically, the background states that the LF elimination programme has not been implemented in Busia County because no infections had been reported there at that time. Studies suggested that *Anopheles* mosquitoes co-infected with *W. bancrofti* and *Plasmodium* may exhibit altered parasite loads. For instance, *W. bancrofti*-infected *An. gambiae* have been found to carry higher numbers of *Plasmodium* sporozoites, and vice-versa, possibly due to microfilariae disrupting the mosquito midgut and facilitating parasite migration. Conversely, other research indicates that co-infection might reduce mosquito survival, potentially altering transmission dynamics. This complex interaction suggests that controlling one disease could inadvertently impact the transmission of the other in co-endemic areas.

Despite the ongoing malaria control efforts, the core problem is that Busia County is still suffering from persistently high malaria transmission. Concurrently, there is a lack of knowledge regarding the presence and prevalence of lymphatic filariasis in the region, as no LF cases had been historically reported, and consequently, no LF elimination programme such as mass drug administration has been deployed. This presents a critical gap: the status of potential co-infections of *W. bancrofti* and *Plasmodium* in both the *Anopheles* mosquito vectors and the human population of Busia County is unknown. Given the documented interactions between these parasites in vectors elsewhere, understanding whether both pathogens are circulating in Busia is a prerequisite for designing effective, integrated vector management and disease control strategies. The study is justified by the need to gather baseline data on mixed infections before any intensified filariasis control such as mass drug administration is considered for the area, as such interventions could have unforeseen consequences on malaria transmission dynamics.

The study was designed to address this problem by implementing objectives focusing on vector species identification, detection of mixed infections in mosquitoes, and genetic analysis of any parasites found. The background highlights that important malaria vectors, including *Anopheles gambiae* and *Anopheles funestus*, exist as species complexes composed of closely related species that are difficult to distinguish based on morphology alone. Despite their similarities, these species may exhibit substantial differences in their behavioral characteristics, ecological preferences, and ability to transmit malaria parasites. For instance, members of the *Anopheles gambiae* complex, *Anopheles gambiae sensu stricto*, *Anopheles arabiensis* and *Anopheles melas* each demonstrates different varying capacities and ecological adaptations that favor malaria transmission.

The aim of this study was to determine the occurrence of *Anopheles funestus* and *Anopheles gambiae* using molecular method (PCR) to identify the specific sibling species present in Matayos. This is crucial because knowing which vector species are present determines the most effective control measures such as indoor residual spraying vs. larval source management and helps predict transmission potential. The study used *Anopheles* mosquitoes collected from the study site "Matayos" and tested

them for the presence of infection with both *Wuchereria bancrofti* and *Plasmodium falciparum*. This objective directly addresses the problem statement by providing the first empirical data on the extent of dual-pathogen carriage in the local mosquito population. The findings revealed whether the ecological conditions in Busia allow for frequent mixed infections, which, as the background suggests, could influence transmission dynamics of both diseases. A high proportion of co-infected mosquitoes supported the hypothesis that integrated control is necessary.

Lastly, the study performed sequencing of the parasite DNA. These sequences were then used to construct a phylogenetic tree, which is a diagram showing the evolutionary relationships between different *W. bancrofti* isolates. By comparing the *W. bancrofti* sequences from Matayos to those from other regions (the Kenyan coast, or other African countries), the study aimed to determine the evolutionary origin and relatedness of the local strain. Understanding the parasite's genetic diversity and evolutionary history provides insights into its transmission patterns, potential responses to drugs, and help track the spread of the parasite, which is valuable information for elimination programme.

The study moved beyond simple disease surveillance by: (i) identifying the precise vector species present, (ii) measuring the rate of mixed *P. falciparum* and *W. bancrofti* infections in those vectors for the first time in Busia County, and (iii) performing a genetic fingerprint of any *W. bancrofti* found to understand its origins and evolution. Therefore, the ultimate goal was to generate evidence that will guide integrated disease control strategies in a region where the filariasis status is unknown but malaria is endemic.

1.2 Statement of the Problem

Malaria and lymphatic filariasis are transmitted by *Anopheles* mosquito vectors and are co-endemic in Kenya (Njenga et al., 2017; Sinka, 2013). Malaria causes significant morbidity and mortality with an estimated 249 million cases and over 600,000 deaths reported in 2023 (World Health Organization, 2024), while lymphatic filariasis is

associated with chronic disability including lymphoedema and hydrocele, with approximately 657 million people in 44 countries still requiring preventive chemotherapy (World Health Organization, 2023). Filariasis may also result in acute adenolymphangitis (ADL), an inflammatory condition of the lymph vessels and nodes characterized by recurrent fever, chills and headache (Babu & Nutman, 2014).

The 2024 World Malaria Report reported that the malaria burden remained highest in countries such, the Democratic Republic of Congo, Nigeria, Mozambique and Uganda in sub-Saharan Africa. The African Region continues to experience the greatest impact of the disease, contributing to more than 90% of malaria-related cases and fatalities globally reflecting persistent transmission despite ongoing interventions (World Health Organization, 2024).

On the other hand, WHO estimates that in 2023, 657 million people across 44 countries required preventive chemotherapy for lymphatic filariasis, while over 850 million people no longer required treatment due to successful elimination efforts in several countries (World Health Organization, 2023). In addition, approximately 40 million people were reported to be living with chronic manifestations of Lymphatic filariasis, including 25 million men living with hydrocele and millions affected by lymphoedema (Ndeffo-Mbah & Galvani, 2017).

Lymphatic filariasis causes physical disability due to chronic swelling of the limbs (lymphoedema), which significantly affects mobility and daily activities such as walking, standing, household chores and toileting. Affected individuals also experience social stigma, which can lead to reduced educational attainment and loss of employment opportunities due to reduced productivity and discrimination (Zhao & Shen, 2025).

Studies on co-infection of *Anopheles* mosquitoes with *Plasmodium falciparum* and *Wuchereria bancrofti* have reported that *An. gambiae* infected with *Wuchereria bancrofti* may harbor a higher number of *Plasmodium* sporozoites (Muturi et al., 2006). Similar observations have been reported in other countries such Papua New Guinea, where *Anopheles punctulatus* mosquitoes carrying *Plasmodium* infections were observed to contain greater quantities of *Wuchereria bancrofti* larvae than

mosquitoes without the infection (Wilairatana et al., 2022). This association may be attributed to the disruption of physiological processes within the mosquito midgut caused by migrating microfilariae, facilitating parasite penetration into the haemocoel (Wilairatana et al., 2022).

Further studies suggest that co-infection of mosquitoes with filarial and malarial parasites may reduce mosquito survival and transmission efficiency, leading to the hypothesis that control of one disease in co-endemic areas could influence transmission dynamics of the other (Coulibaly, 2016). Reduced survival of co-infected mosquitoes may alter vector population structure and transmission potential (Amimo, 2023; Muturi et al., 2006).

Given continued malaria transmission in Busia County and the persistent risk of lymphatic filariasis in endemic regions of Kenya (World Health Organization, 2024), it is important to investigate mixed infections of mosquitoes with *Wuchereria bancrofti* and *Plasmodium* species prior to intensified filariasis control interventions. The findings will support evidence for integrated vector management strategies targeting both diseases simultaneously.

1.3 Justification

Based on estimates provided by the WHO and CDC, malaria remains a significant public health challenge in Kenya, with an estimated 3–5 million clinical cases reported each year and over 10,000 associated deaths. The greatest impact of the disease is observed among communities living in the western regions of the country, where malaria transmission and burden remain particularly high. (WHO, 2024, CDC 2023). A study conducted in western Kenya by Bashir et al. (2019) reported Busia County as having the highest malaria prevalence at 37%, while Bungoma County recorded the lowest prevalence at 18%. In contrast, an estimated 3.6 million people remain at risk of lymphatic filariasis in the coastal endemic regions of Kenya, with cross-sectional surveys reporting an overall prevalence of approximately 1%–2% in endemic coastal counties (Njenga et al., 2017; World Health Organization, 2023).

Higher focal prevalence has previously been reported in Ndau Island, Lamu County, whereas Kwale and Kilifi counties continue to demonstrate low but persistent transmission below 2% (Njenga et al., 2017). Despite the presence of abundant competent mosquito vectors in Busia County, limited information is available regarding the distribution, prevalence, and transmission patterns of lymphatic filariasis within the region. Therefore, this study was designed to investigate the potential occurrence of concurrent transmission of malaria and lymphatic filariasis through *Anopheles* mosquito vectors in Busia County. The study was driven by the observation of the major vectors of *Plasmodium falciparum* and *Wuchereria bancrofti*, namely *Anopheles gambiae* and *Anopheles funestus* (Sinka et al., 2012; Muturi et al., 2006), which may support simultaneous transmission of both parasites. In addition, suspected filarial cases have occasionally been referred to diagnostic laboratories, including KEMRI, through routine surveillance systems, suggesting the possible presence of undetected transmission. Surveillance and health facility records remain critical in identifying disease hotspots and informing elimination programmes (World Health Organization, 2023).

Previous experimental and field studies have shown that mosquitoes infected with *Wuchereria bancrofti* may under certain conditions harbor higher *Plasmodium falciparum* sporozoite loads compared to uninfected mosquitoes (Muturi et al., 2006). However, vector co-infection with both parasites has also been associated with reduced mosquito survival, which may subsequently influence disease transmission dynamics (Wilairatana et al., 2022; Coulibaly, 2016). It has further been hypothesized that interventions targeting either malaria or lymphatic filariasis in co-endemic regions may indirectly affect the transmission patterns of the other disease through changes in vector population structure, parasite development, and mosquito survival rates. There is therefore a need to investigate vector co-infection and co-transmission patterns in Busia County to support evidence-based integrated vector management strategies.

The findings from this study are expected to provide important baseline data on the occurrence of malaria and lymphatic filariasis co-transmission in Busia County, thereby contributing to improved surveillance and targeted vector control interventions. The study may also strengthen integrated disease prevention

programmes by informing public health policies aimed at reducing transmission of both diseases simultaneously. Ultimately, the outcomes of this study are expected to benefit communities in Busia County through improved disease monitoring, enhanced vector control strategies, reduced disease burden, and better allocation of public health resources, thereby contributing to improved health and quality of life among populations living in endemic areas.

1.4 Objectives of the Study

1.4.1 General Objective

To determine the prevalence of *Wuchereria bancrofti* and *Plasmodium falciparum* infections in *Anopheles* mosquitoes and human blood sampled from Busia County, Western Kenya and to characterize the parasites using phylogenetic analysis.

1.4.2 Specific Objectives

- i. To determine the diversity of species within the collected *An. gambiae* and *An. funestus* complexes.
- ii. To determine whether *Anopheles* mosquitoes and blood samples from the study area are co-infected with *Plasmodium falciparum* and *Wuchereria bancrofti*.
- iii. To characterize *Plasmodium falciparum* and *Wuchereria bancrofti* parasites by Phylogenetic analysis

1.5 Research Questions

- i. How diverse are the species of field-collected *An. gambiae* and *An. funestus* complexes?
- ii. What proportion of *Anopheles* mosquitoes and human blood samples are co-infected with *Wuchereria bancrofti* and *Plasmodium falciparum*?
- iii. What are the phylogenetic relationships of *Wuchereria bancrofti* and *Plasmodium falciparum* isolates compared to previously reported sequences?

CHAPTER TWO

LITERATURE REVIEW

2.1 Global Distribution and Epidemiology of Malaria

The World Malaria Report (2025) estimated 282 million malaria cases in 2024 globally, reflecting a continued rise compared to previous years. This represents an increase of about 9 million cases from 2023, with transmission remaining highest in endemic regions (World Health Organization, 2025). The continuing rise in malaria burden is largely attributed to countries in Africa such as the Democratic Republic of Congo, Mozambique, Uganda, Nigeria, and Ethiopia, in which the highest number of malaria infections and deaths have been reported. Collectively two-thirds of the global malaria burden have been reported in these countries (World Health Organization, 2025). In comparison, the WHO South-East Asia Region contributes a relatively smaller fraction of global malaria cases, although transmission remains a concern in several areas.

India remains the dominant contributor, while countries such as Sri Lanka and Bhutan continue to maintain malaria-free status following WHO certification (World Health Organization, 2025). In the Eastern Mediterranean Region, Sudan, Yemen, and Pakistan continue to report the highest malaria burden, while countries such as Saudi Arabia and the Islamic Republic of Iran have maintained zero indigenous malaria transmission for several consecutive years (World Health Organization, 2025). In the WHO Region of the Americas, Brazil, Colombia, and Venezuela account for most malaria cases, while countries such as Paraguay, El Salvador, and Argentina have been certified malaria-free (World Health Organization, 2025).

The report further indicated that global malaria deaths were estimated at approximately 610,000 in 2024, representing a slight increase compared to 2023 (World Health Organization, 2025). Despite this burden, cumulative global malaria control efforts since 2000 have averted approximately 2.2 billion cases and over 12 million deaths, largely through vector control, improved diagnosis, and treatment interventions (World Health Organization, 2025).

2.2 Global Distribution and Epidemiology of Lymphatic Filariasis

Lymphatic filariasis (LF) transmission is influenced by environmental factors, increasing with temperature, rainfall, and vegetation cover, and decreasing with altitude (World Health Organization, 2023). Non-endemic areas are defined as those with infection prevalence below 1% (Cromwell et al., 2020; World Health Organization, 2023). LF remains endemic in many tropical and subtropical regions despite elimination in countries such as China, Japan, and South Korea. In Asia and the Americas, transmission is still predicted or sustained in countries including Sri Lanka, India (eastern regions), Australia's northern coast, New Guinea, Brazil, Guyana, Central America, and the Caribbean (Asare et al., 2024).

Following the establishment of the Global Programme to Eliminate Lymphatic Filariasis in 2000, considerable advancements have been achieved in efforts aimed at controlling and eliminating the disease. Initially, 81 countries were endemic, with over 886 million people at risk and more than 120 million infected globally (World Health Organization, 2023). Between 2002 and 2012, large-scale preventive chemotherapy using albendazole and diethylcarbamazine reached billions of treatments, preventing or curing millions of cases (Ottesen et al., 2008). Recent reports suggest that about 657 million individuals in 44 countries are still in need of interventions such as mass drug administration (MDA) while over 850 million no longer need treatment due to successful control efforts. However, around 40 million people continue to suffer from chronic conditions such as lymphoedema and hydrocele (World Health Organization, 2023; Ndeffo-Mbah & Galvani, 2017).

Progress in elimination has been substantial, with over 20 countries validated as having eliminated LF transmission in their communities including Togo, Malawi, Egypt, Sri Lanka, Thailand, and Vanuatu (World Health Organization, 2024). Despite this, LF remains endemic in 43 countries, mainly in Africa, where more than 30 countries including Kenya, Nigeria, Madagascar, Ethiopia, and Tanzania still require MDA. Nigeria remains the highest-burden country, followed by Madagascar and the Democratic Republic of Congo (Sinha et al., 2023; World Health Organization, 2024). LF remains a major public health challenge particularly in Africa, where sustained

transmission continues to require ongoing preventive chemotherapy and integrated vector control strategies.

2.3 Taxonomy

Filarial worms belong to the phylum Nematoda, class *Chromadorea*, order *Rhabditida*, suborder *Spirurina*, and family *Onchocercidae*, which includes a wide range of parasitic nematodes responsible for human diseases such as lymphatic filariasis and onchocerciasis (Wang, 2021; WHO, 2022). The family *Onchocercidae* comprises over 60 genera of filarial nematodes that are transmitted through insect vectors and are responsible for significant global morbidity (De Souza & Bockarie, 2025). Lymphatic filariasis is caused by three nematode species, *Brugia malayi*, *Brugia timori* and *Wuchereria bancrofti* but *W. bancrofti* accounts for approximately 90% of global infections (WHO, 2022; Stolk et al., 2015).

Plasmodium parasites belong to the phylum *Apicomplexa*, class *Aconoidasida*, order *Haemosporida*, and family *Plasmodiidae*, representing protozoan parasites responsible for malaria in humans (WHO, 2023; CDC, 2024). Over 100 species of *Plasmodium* have been documented worldwide; however, the principal species known to infect humans are *P. ovale*, *P. vivax*, *Plasmodium falciparum*, *P. knowlesi* and *P. malariae* (Lai et al., 2025; Haldar et al., 2018). However, *P. falciparum* causes the majority of malaria-associated deaths globally, especially in Africa (WHO, 2023; Lopez & Koepfli, 2021).

2.4 Pathogenesis of Malaria

Infection with malaria begins when female *Anopheles* mosquitoes inject the sporozoites of *Plasmodium* parasites into the host's bloodstream (Figure 2.1). These sporozoites then undergo asexual replication in the liver after travelling to the liver forming merozoites. The merozoites are then released into the bloodstream (Cowman et al., 2016). These merozoites then invade the red blood cells, where they develop into trophozoites and eventually schizonts, leading to rupture of the infected cells and release of parasites that infect additional erythrocytes (World Health Organization,

2023). A proportion of the merozoites develop into sexual forms called gametocytes, which remain circulating within the host's bloodstream. (Cowman et al., 2016).

During a blood meal the circulating gametocytes are taken up by mosquitoes and transported to the midgut. In the mosquito gut, female and male gametes fuse to form zygotes, which develop into motile ookinetes and subsequently into oocysts (World Health Organization, 2023). The oocysts formed on the midgut wall eventually rupture, releasing sporozoites into the haemocoel of the mosquito. These sporozoites migrate to the salivary glands, where they become infective to a new human host during subsequent blood feeding. Once introduced into the human bloodstream, the parasite life cycle is reinitiated (Cowman et al., 2016).

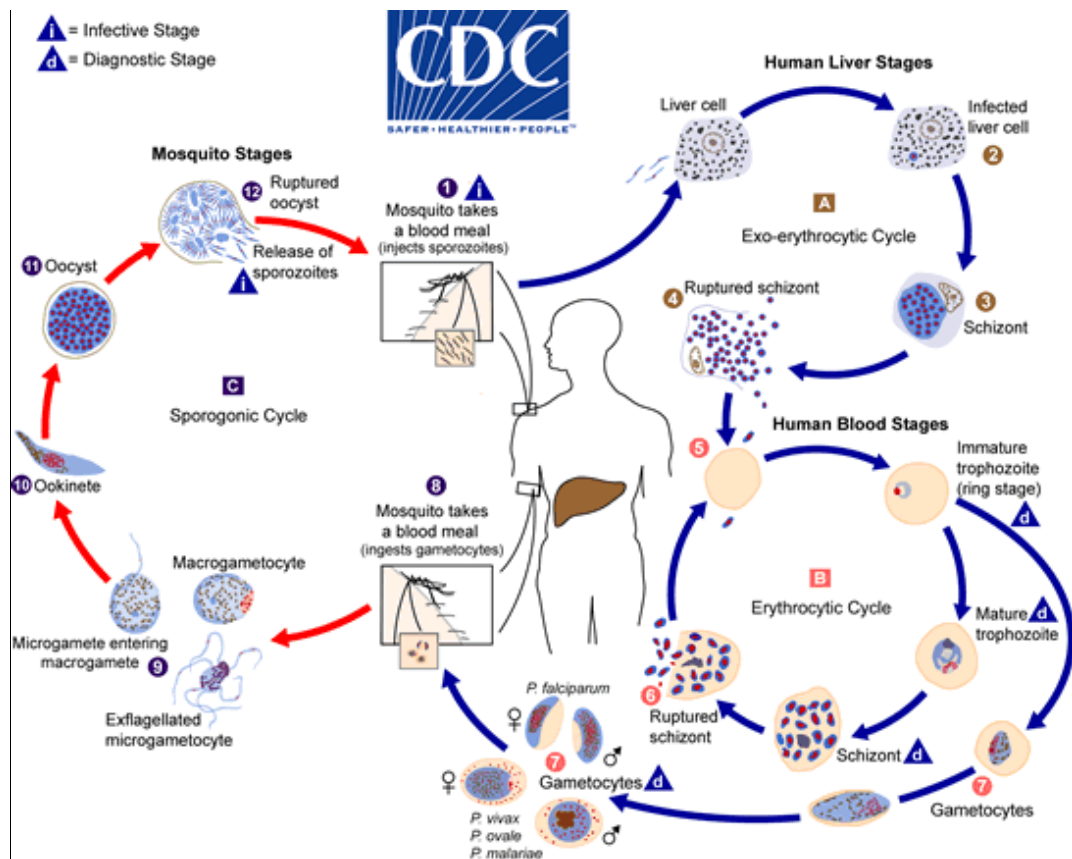


Figure 2.1: The Life Cycle of *Plasmodium falciparum*

Source: CDC 2019

The clinical symptoms experienced during malaria infection, such as fever, arise primarily from the cyclical rupture of infected red blood cells and the subsequent release of parasites and cellular debris into the bloodstream (White et al., 2014). This process triggers inflammatory responses that contribute to disease manifestation. Other symptoms include chills, severe anaemia, cough, headache, muscle pain, vomiting, abdominal discomfort, seizures, and respiratory distress, particularly in severe cases of *Plasmodium falciparum* infection (World Health Organization, 2023).

2.5 Pathogenesis of Lymphatic Filariasis

Mosquitoes that transmit filarial worms, as well as *Plasmodium* parasites and some arboviruses, acquire microfilariae when taking a blood meal from an infected individual (World Health Organization, 2023). The microfilariae then lose their sheaths and develop into first- and second-stage larvae before maturing into third-stage infective larvae within the thoracic muscles of the mosquito, after which they migrate through the haemocoel to the proboscis (Goel & Goel, 2016). During a blood meal, the infective larvae on the mosquito proboscis, are released onto the skin of a human host. The larvae then enter the body through the bite site and migrate to the lymphatic vessels, where they mature into adult worms. These adult worms produce sheathed microfilariae that circulate in the bloodstream. The microfilariae are again ingested when another mosquito feeds on an infected individual and later shed their sheaths and develop into infective larvae within approximately one to two weeks completing the transmission cycle (World Health Organization, 2023; Centers for Disease Control and Prevention, 2024).

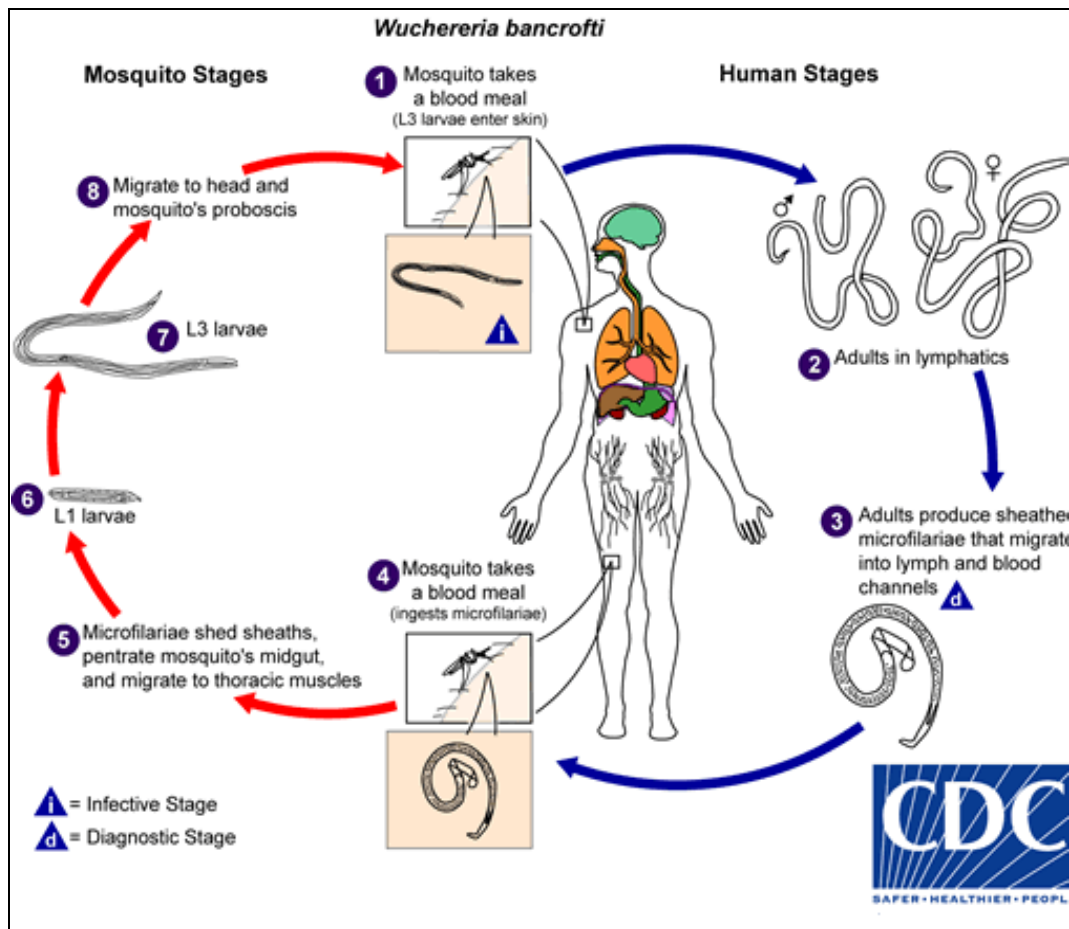


Figure 2.2: The Life Cycle of *Wuchereria bancrofti*

Source: CDC 2019

2.6 Vectors of Malaria and Lymphatic filariasis

Malaria is not transmitted by all mosquito species but is instead exclusively spread by specific species of female *Anopheles* mosquitoes, which serve as the biological vectors responsible for parasite transmission (World Health Organization, 2024). Transmission occurs when an infected female mosquito inoculates *Plasmodium* parasites into a human host during blood feeding. In Africa, the main malaria vectors include *Anopheles funestus*, *Anopheles arabiensis*, as well as members of the *Anopheles gambiae* complex, all of which are highly efficient in transmitting malaria due to their strong preference for human blood and close association with human settlements (WHO, 2024; Coetzee et al., 2020). These vector species differ in their

ecology, biting behavior, and breeding habitats, which significantly influences malaria transmission dynamics across different ecological zones.

In contrast, lymphatic filariasis (LF) is transmitted by a wider range of mosquito genera, reflecting a more complex vector ecology. The disease is caused by filarial parasites such as *Wuchereria bancrofti*, and transmission can be mediated by mosquitoes belonging to the genera *Anopheles*, *Aedes*, *Culex*, and *Mansonia*, depending on geographical and environmental conditions (WHO, 2023). The wide diversity of vector species plays a key role in the worldwide spread and continued transmission of *lymphatic filariasis* in both rural and urban environments. In Africa, *Anopheles funestus* and *Anopheles gambiae* remain the primary vectors of lymphatic filariasis, especially in rural regions where environmental conditions support their breeding and where malaria is also highly endemic (WHO, 2023; Cano et al., 2014). The shared transmission of malaria and lymphatic filariasis by these mosquito species illustrates the complex epidemiology of mosquito-borne infections in the region and emphasizes the need for integrated vector control approaches.

2.7 Identification Features of *Anopheles funestus* and *Anopheles gambiae* Complexes

In Africa, the *Anopheles funestus* and the *Anopheles gambiae* complex group are the two major malaria vectors and are also involved in the transmission of *Wuchereria bancrofti*, the parasite responsible for *lymphatic filariasis*. Although both groups contain highly efficient vectors, they differ significantly in morphology, ecology, and behaviour, which influences their role in disease transmission dynamics (Sinka, 2013).

The *Anopheles gambiae* complex members are morphologically indistinguishable and include sibling species such as *An. coluzzii*, *An. gambiae sensu stricto* and *An. arabiensis*. These species are generally small, slender mosquitoes with lightly scaled bodies and distinctly spotted wings. A key morphological feature used in field identification is the palpal banding pattern in females, where alternating dark and pale bands are present, along with a relatively uniform wing spotting pattern. However, despite these external features, species-level identification within the complex is not

reliable using morphology alone, as members are cryptic and require molecular techniques such as PCR for accurate differentiation.

In contrast, the *Anopheles funestus* group, particularly *An. funestus sensu stricto*, is generally larger, darker, and more robust in appearance. The wings of *An. funestus* show more uniform pale spots along the veins, and the body is covered with darker scales compared to *An. gambiae*. Females also exhibit long, slender palps with distinct but less contrasting banding patterns than those of the *An. gambiae* complex. These morphological differences, although subtle, allow for preliminary separation of the two groups under stereomicroscopic examination (Kyalo et al., 2017).



Figure 2.3: *An. funestus* and *An. gambiae* Mosquito Species ((MalariaGEN, 2021))

Ecologically, the two groups occupy different larval habitats, which further supports their differentiation. The *Anopheles gambiae* complex typically breeds in temporary, sunlit water bodies such as rain pools, puddles, and hoof prints. These habitats are often short-lived and appear following rainfall events, contributing to the rapid population expansion of *An. gambiae* during wet seasons. Conversely, *An. funestus* prefers permanent or semi-permanent water bodies with emergent vegetation, such as swamps, irrigation canals, and stream margins. This ecological preference allows *An. funestus* populations to persist during dry seasons, maintaining year-round transmission potential (St. Laurent et al., 2021).

Behaviorally, both groups are highly anthropophilic and endophilic, meaning they preferentially feed on humans and often enter houses to blood-feed at night. However, *An. gambiae* tends to exhibit more opportunistic breeding and rapid population fluctuations in response to rainfall, whereas *An. funestus* demonstrates more stable

population dynamics due to its reliance on permanent aquatic habitats (Beloconi et al., 2023). These differences directly influence seasonal malaria transmission patterns and also affect the transmission dynamics of *Wuchereria bancrofti*, which requires longer mosquito survival times for parasite development.

From a disease transmission perspective, both mosquito groups are capable vectors of *Plasmodium falciparum* and *Wuchereria bancrofti*, but their efficiency differs. *An. gambiae* is considered one of the most efficient malaria vectors globally due to its strong human preference and high vector competence. *Anopheles funestus* is also a key vector in many parts of Africa, largely due to its long lifespan and its capacity to maintain transmission even during dry periods (WHO, 2023). This complementary ecological function means that both vector groups are essential in sustaining the endemic transmission of *Plasmodium falciparum* and *Wuchereria bancrofti* in co-endemic regions such as western Kenya.

Given their cryptic nature, molecular identification remains essential for distinguishing sibling species within each group. While morphological characteristics provide a useful preliminary classification, PCR-based assays targeting ribosomal DNA and mitochondrial markers are required for definitive identification and for accurate entomological surveillance in epidemiological studies.

2.8 Diagnosis

Several methods that have been used historically to detect malaria and lymphatic filariasis which include the following;

2.8.1 Light Microscopy

The microscopy is recommended by the WHO as the standard method for the diagnosis of malaria and it involves the microscopic examination of stained blood films using Giemsa or other Romanowsky stains (World Health Organization, 2023; Agarwal et al., 2020). Blood samples are typically obtained through finger prick or earlobe collection, as parasite density is often higher in capillary blood (World Health Organization, 2023). Blood films are prepared following standard procedures,

including the preparation of thick and thin smears, staining, and examination under a 100× oil immersion objective for parasite detection and species identification (World Health Organization, 2023).

Microscopy is also a standard method for the diagnosis of lymphatic filariasis and involves detection of circulating microfilariae in peripheral blood (World Health Organization, 2023). Because many filarial parasites exhibit nocturnal periodicity, blood samples are preferably collected at night to increase the likelihood of detection, and thick smears are commonly prepared for examination (Goel & Goel, 2016). The smears are stained using Giemsa or similar stains and examined microscopically for the presence of microfilariae. This method is most effective during the early stages of infection when microfilariae are present in peripheral circulation, whereas in advanced disease stages such as lymphoedema, microfilariae may be absent or reduced in the blood (World Health Organization, 2023; Goel & Goel, 2016).

2.8.2 Antigen Detection Test

This method relies on the detection of antigens released into the host bloodstream during infection with malaria or *lymphatic filariasis*. For malaria diagnosis, parasite-derived antigens such as aldolase, *Plasmodium* lactate dehydrogenase (pLDH) and histidine-rich protein 2 (HRP2) produced during erythrocytic stages are detected using rapid diagnostic tests (RDTs) (World Health Organization, 2023; Agarwal et al., 2020). An example of such an RDT is the BinaxNOW malaria test.

In addition, other methods detect circulating filarial antigens released by adult worms in the human bloodstream and are performed using ELISA or rapid card-based assays (World Health Organization, 2023; Mackenzie et al., 2022). These methods have advantages over microscopy in that blood samples obtained by finger prick can be collected at any time of day, unlike microscopy for microfilariae which often requires night sampling due to periodicity. However, antigen detection is most useful when adult worms are alive, particularly during the early stages of infection (Mackenzie et al., 2022). Examples of such tests include the Og4C3 ELISA and immunochromatographic card tests (ICT), including the more recent filarial test strip (FTS) (World Health Organization, 2023). However, most of these antigen detection

assays are specific for *Wuchereria bancrofti*, with limited applicability for detecting *Brugia malayi* infections (Mackenzie et al., 2022).

2.8.3 Antibody Detection

This technique is designed to identify antibodies generated in response to parasitic infections. In the diagnosis of malaria, the indirect fluorescent antibody (IFA) assay has also been used, however, it is not routinely applied because it primarily detects past exposure rather than active infection (World Health Organization, 2023; Bousema et al., 2014). In this method, antigens derived from *Plasmodium* species form complexes with antibodies present in the patient's serum. Following this, a fluorescein-labelled anti-human antibody is added to bind to the antigen–antibody complexes, which are then visualized under a fluorescence microscope. A sample is considered positive when characteristic apple-green fluorescence is observed (Bousema et al., 2014).

Recombinant filarial antigens are used in the diagnosis of lymphatic filariasis, to detect anti-filarial antibodies, and unlike antigen detection methods, these tests can indicate both past and current exposure (World Health Organization, 2023; Mackenzie et al., 2022). An example is the *Brugia* Rapid test, which utilizes recombinant antigens of *Brugia malayi* to detect IgG4 antibodies against filarial parasites, although it demonstrates reduced sensitivity for *Wuchereria bancrofti* infections (Mackenzie et al., 2022).

2.8.4 Molecular Diagnosis

Molecular diagnosis involves detection of parasite DNA. The method relies on specific primers for the amplification of parasite DNA using Polymerase Chain Reaction (PCR). Commercial kits like the Qiagen kit are used to extract DNA after which it is amplified and detected through real-time PCR assays which significantly improve sensitivity and allow precise quantification of parasite load (Lazrek et al., 2023; e et al., 2025). Real-time PCR has become a widely accepted molecular tool due to its high sensitivity and ability to detect low-density infections that are often missed by conventional diagnostic methods (He et al., 2024; WHO, 2023). However, because

molecular diagnostic laboratories remain limited in many routine healthcare settings, particularly in low-resource countries, this method is often used as a confirmatory test following initial diagnosis using rapid diagnostic tests (RDTs) or microscopy (CDC, 2024; WHO, 2023).

This molecular approach has been further refined to improve the sensitivity and specificity of detecting parasitic infections, including filarial nematodes, using PCR-based techniques. It relies on the identification of highly repeated DNA sequences within the parasite genome, which serve as reliable molecular targets for amplification (Elaagip & Higazi, 2024; Frontiers in Parasitology, 2024). Commonly targeted genetic markers include multicopy ribosomal RNA gene regions, which are highly conserved and widely used in modern diagnostic assays due to their diagnostic reliability (Nyenke, 2024; Mbanefo & Kumar, 2020). Once these conserved sequences are identified, specific primers are designed and synthesized to target them in PCR assays, enabling rapid and highly specific detection of filarial nematodes and significantly reducing diagnostic turnaround time compared to traditional microscopy (Mahale et al., 2023; PLOS NTD, 2024).

2.9 Treatment of *Lymphatic Filariasis* and Malaria

Treatment of malaria especially in endemic areas is always guided by national protocols that outline the management of severe malaria, malaria in pregnancy and uncomplicated cases. Artemisinin-based combination therapies (ACTs) is currently recommended by WHO as the first-line treatment for uncomplicated *Plasmodium falciparum* malaria due to their high efficacy and ability to delay resistance development (WHO, 2023; CDC, 2024). ACTs remain the most effective antimalarial regimen because they combine two drugs with different mechanisms of action, thereby improving parasite clearance and reducing the likelihood of drug resistance emergence (White & Chotivanich, 2024; Lai et al., 2025).

Infections caused by *Plasmodium vivax* are also treated using ACTs, while chloroquine may still be used in areas where resistance is not documented (WHO, 2023). Tafenoquine or primaquine is used the treatment and elimination of liver hypnozoites of *P. vivax*, thereby preventing relapse infections (Taylor et al., 2015; WHO, 2023).

Severe malaria cases are treated with injectable artesunate followed by a full 3-day course of ACT once the patient can tolerate oral medication, as this approach significantly reduces mortality compared to older therapies (WHO, 2023; Sinclair et al., 2012).

The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was established by WHO with the goal to support the elimination of lymphatic filariasis (LF) by reducing the transmission and development of disability in affected populations. The programme is primarily implemented through preventive chemotherapy delivered via mass drug administration (MDA), (WHO, 2022). Drugs effective against filarial nematodes include ivermectin, albendazole, diethylcarbamazine (DEC), and in some settings, combination regimens depending on co-endemicity with other parasites (Stolk et al., 2015; WHO, 2022). GPELF recommends annual MDA with albendazole (400 mg) combined with diethylcarbamazine citrate (6 mg/kg) or ivermectin in endemic populations aged two years and above to reduce microfilarial levels and interrupt transmission (WHO, 2022; De Souza & Bockarie, 2025).

Acute adenolymphangitis (ADL), caused by secondary bacterial and fungal infections of lymphoedematous limbs, is managed through symptomatic and supportive care. Treatment includes analgesics for pain relief and antibiotics to control bacterial infection, alongside antifungal agents when indicated (WHO, 2023; Negussie et al., 2018). ADL episodes typically present with painful swelling of lymph nodes, fever, chills, headache, nausea, and vomiting, lasting several days and contributing to disability in affected individuals. Management focuses on improving hygiene, including washing affected limbs with soap and water, preventing entry lesions, and reducing recurrence (WHO, 2023).

In chronic cases of lymphatic filariasis, surgical intervention such as hydrocelectomy is performed to manage hydrocele, a condition characterized by abnormal fluid accumulation in the scrotum leading to swelling and discomfort. Surgical management remains the most effective treatment for advanced hydrocele cases and significantly improves quality of life in affected patients (WHO, 2022; Capuano & Capuano, 2012).

2.10 Co-Infections

2.10.1 Co-Infection of *Wuchereria bancrofti* and *Plasmodium falciparum*

In co-endemic areas, especially in sub-Saharan Africa, co-infection with *Wuchereria bancrofti* and *Plasmodium falciparum* remains an important public health concern. Recent studies continue to confirm that both parasites share common *Anopheles* mosquito vectors, enabling simultaneous transmission within the same ecological settings (Abraham et al., 2024; Muturi et al., 2006).

2.10.2 Co-Infection in Mosquito Vectors

Recent molecular xenomonitoring studies demonstrate that *Anopheles gambiae s.l.* can harbor both *P. falciparum* and *W. bancrofti* simultaneously, confirming the biological feasibility of co-infection in mosquito vectors (Abraham et al., 2024). Field-based evidence from East Africa has shown that a proportion of wild-caught mosquitoes contain both malaria sporozoites and filarial larvae, although co-infected mosquitoes remain relatively rare due to reduced mosquito survival following multiple infections (Muturi et al., 2006; Abraham et al., 2024). A 2024 Kenyan coastal study further confirmed that *Anopheles gambiae* populations exhibited concurrent infections with both parasites, with co-infection rates ranging from low but measurable proportions in field populations (Muturi et al., 2006). These findings indicate that a single mosquito bite has the potential to transmit both malaria and lymphatic filariasis, although transmission efficiency is influenced by parasite load and vector survival dynamics.

2.10.3 Co-Infection in Human Blood

Human co-infection occurs in populations exposed to high mosquito biting rates in endemic regions. Recent field studies in Kenya and other parts of East Africa report that individuals can simultaneously harbor *P. falciparum* parasites in red blood cells and *W. bancrofti* microfilariae in peripheral blood (Muturi et al., 2006; Abraham et al., 2024). Although co-infection is documented, prevalence levels vary widely depending on transmission intensity, vector control coverage, and mass drug administration programs targeting lymphatic filariasis. Studies suggest that while co-infection is

biologically common, its detection in cross-sectional surveys is often underestimated due to the periodic nature of microfilarial circulation and submicroscopic malaria infections (Abraham et al., 2024).

2.11 Parasite Interactions and Transmission Implications

Evidence suggests that interactions between *W. bancrofti* and *P. falciparum* within both the human host and mosquito vector may influence transmission dynamics. In mosquitoes, dual infection may reduce survivorship, potentially limiting onward transmission despite co-ingestion of both parasites (Muturi et al., 2006). This indicates a possible biological constraint on co-transmission efficiency. In humans, co-infection may also influence disease dynamics through immune modulation. Helminth infections such as lymphatic filariasis are known to induce regulatory immune responses that may alter host susceptibility to malaria infection (World Health Organization, 2023). However, recent field evidence suggests that the direction and magnitude of this effect remain inconsistent across endemic settings, with some studies reporting increased malaria susceptibility and others showing no significant effect (Muturi et al., 2006).

2.12 Public Health Implications

Recent literature emphasizes that co-endemicity of malaria and lymphatic filariasis necessitates integrated surveillance and control strategies. Studies recommend combining vector control interventions (such as the use of insecticide-treated nets) to reduce transmission of these parasites simultaneously (World Health Organization, 2023; Abraham et al., 2024). Molecular xenomonitoring of mosquito populations is increasingly being used to detect co-infections and evaluate intervention impact in real time (Abraham et al., 2024). Despite progress, key gaps remain in understanding the epidemiological significance of co-infection, particularly regarding how parasite interactions within mosquitoes' influence transmission efficiency and how human immune responses are modulated during simultaneous infection.

2.13 Summary and Critique of Existing Literature

2.13.1 Summary

Two vector-borne diseases, malaria and lymphatic filariasis, are important public health problems in tropical and subtropical countries, with a large and extensive body of literature on the global distribution, epidemiology, pathogenesis and diagnosis of both. The review is a compilation of what is known from the peer reviewed studies, data from global elimination programme, and WHO reports within the last 20 years.

In the year 2025, World Malaria Report reported that there were about 282 million cases of malaria and at least 610,000 deaths globally in the year 2024. 90% of these cases were reported in African countries of Mozambique, Democratic Republic of Congo, Nigeria, Uganda and Ethiopia contributing to two-thirds of the reported cases and deaths. Since 2000 when the programme to eliminate Lymphatic filariasis was established, substantial progress has also been reported. When the programme began, over 120 million individuals had been reported to be infected with the disease in 81 countries. Recent estimates have reported a reduction in the number of people infected and requiring mass drug administration to 657 million people in 44 countries while about 850 million people no longer needing treatment. In addition, at least 20 countries have been validated as having eliminated lymphatic filariasis as a public health problem.

Wuchereria bancrofti is believed to cause around 90% of LF infections worldwide while *Plasmodium falciparum* is the most virulent species of malaria. They both have some important characteristics: they are spread by female *Anopheles* mosquitoes, particularly the *Anopheles gambiae* complex and *An. funestus* group in Africa. The life cycles of both are known, and the malaria parasites replicate in the liver before invading erythrocytes, while filarial worms grow in the lymphatic vessels and release microfilariae which show nocturnal periodicity.

One of the many strengths of the literature reviewed is that species of vectors have been characterized in detail. Morphologically indistinguishable, the *Anopheles gambiae* complex consists of sibling species, *An. arabiensis*, *An. coluzzii*, and *An.*

gambiae s.s., which can only be definitively distinguished using molecular methods. They are ephemeral (temporary) and inshore (sunlit) species. *An. funestus*, on the other hand, is associated with permanent or semi-permanent water bodies where there is emergent vegetation, allowing the parasites to be transmitted throughout the year. Anthropophilic and endophagic, with greater longevity and dry season persistence for *An. funestus*. There are four literature-diagnostic categories for both diseases. The WHO recommended standard is light microscopy, which is sensitive depending on the parasite load and for LF, the timing of sampling is best in the dark. There are some assays which have the advantage of any-time sampling, such as antigen detection tests (including rapid diagnostic tests (RDTs) for malaria HRP2, pLDH, aldolase) and LF tests (Og4C3 ELISA, immunochromatographic card tests, Filarial Test Strip). Antibody detection techniques are useful for the diagnosis of past exposure but not for diagnosis of acute infection. Molecular diagnosis by PCR on 18S rRNA and repeated DNA is most sensitive and specific, but is only available in specialist labs.

Artemisinin-based combination therapies (ACTs) are WHO recommended first-line drugs for uncomplicated *P. falciparum* malaria. GPELF recommends an annual mass drug administration of albendazole with either diethylcarbamazine or ivermectin for LF. Chronic LF treatment involves hydrocelectomy surgery for advanced cases of hydrocele and lymphoedema management via hygienic procedures.

In the mosquito vector and in humans, co-infection of *W. bancrofti* and *P. falciparum* are discussed in the literature. Molecular Xeno monitoring studies conducted in East Africa, such as the studies carried out on the Kenyan coast (2024), have shown the compatibility of *Anopheles gambiae* in harboring both parasites at the same time, albeit with low co-infection percentages. Although two parasites are ingested simultaneously, evidence indicates the dual infection lowers the mosquito's survivorship, which may limit the spread of other parasites. Helminth-induced regulatory immune responses can influence the susceptibility to malaria in humans, with some studies showing an increased susceptibility in certain endemic areas but other studies finding a decreased susceptibility.

2.13.2 Critique of Existing Literature

The review of the literature available on the problem of malaria and LF co-infection has yielded a strong background of knowledge, however there are some methodological, conceptual and contextual limitations to be critically examined.

Even though the global reports from world health organization are authoritative, these reports are a limitation in providing the understanding to the local level. Epidemiological estimates (e.g., 282million malaria cases, 657million people to receive LF preventive chemotherapy) combine very different transmission situations. These figures are not very useful for predicting or comparing for Matayos, which is in Busia County. Though western Kenya is known as a co-endemic region, there is no sub-national stratification of the literature for the country of Kenya. There are no prevalence data available for Matayos constituency, and this methodology makes an assumption of 50% prevalence, but this study addresses that gap. It is correct to state, as has been done in the literature, that the species of the *An. gambiae* complex are morphologically indistinguishable and PCR is needed for species identification. However, this review does not critically assess the performance traits of various molecular markers in the field. The 18S rRNA gene has been extensively used for *P. falciparum* detection, while there are no reports of other limitations like copy number variation, or the fact that it is detecting residual DNA rather than active infection. The review does not consider the potential of cross reactivity or the possibility of antigen persistence following successful treatment, which is important in assessing mass drug administration programs, for antigen detection tests, and only briefly mentions that they are applicable to *W. bancrofti* but not to *Brugia* species.

Mosquito co-infection studies (Muturi et al., 2006; Abraham et al., 2024) are reported to show that *Plasmodium* sporozoite levels are higher in *An. gambiae* infected with *W. bancrofti*. The direction of the causality, however, and confounding factors have not been critically examined in the literature. The role of filarial infection in facilitating the development of the malaria parasite is not known, nor is it known whether both infections are merely a reflection of greater exposure of mosquitoes to infected human blood. The mechanism suggested (physical disruption of midgut by migrating

microfilaria) is stated as fact, but is based on a few experimental studies, many of which were carried out in the laboratory, which may not reflect field transmission dynamics. References in the literature include those ranging from 2006 to 2025, though there are some important references that are dated. The 2002-2012 data on treatment coverage are taken from Ottesen et al., 2008 which is now more than 10 years old. Systematic reviews in the literature would benefit from more recent reviews, as GPELF has had several country validations for elimination since 2015. Furthermore, the continental-level geographical coverage is highly biased towards sub-Saharan Africa, and there is a lack of attention to intra-country heterogeneity. Kenya is cited as being in need of MDA for LF, but no data are available for the county of interest, Busia, which is the study site in this study.

According to the literature, both *An. gambiae* and *An. funestus* breed in temporary water bodies, the latter in permanent water bodies associated with vegetation. It doesn't consider, however, how changes in land use, agricultural activities or climate variability within Busia County specifically may affect these habitat distributions. There is also no discussion of insecticide resistance which is a major determinant of the effectiveness of vector control in Western Kenya. As both diseases are targeted, insecticide resistance patterns have direct implications for understanding the transmission persistence. There is conflicting information in the literature about the outcomes of co-infection, with one mechanism arguing that co-infection would facilitate transmission via disruption of the midgut, and another that it would limit transmission via the reduced survival of mosquitoes. These conflicting results are not summarized nor are testable hypotheses suggested to account for the discrepancy. In addition, the discussion on human immune modulation is rather superficial, with respect to mentioning that helminth infection causes regulatory response but does not specify the cytokine profiles (e.g., IL10, TGF β), T-cell subsets involved, or whether these are different in the various stages of filarial infection. The literature suggests that using insecticide treated nets and mass drug administration together is recommended for an integrated approach; however, it does not critically analyze the operational challenges around taking up mass drug administration in populations where insecticide treated nets were also introduced, or logistical feasibility of running simultaneous campaigns, or potential drug interactions. No cost-effectiveness, sampling frequency

and thresholds for interventions are discussed for the recommendation to implement molecular Xeno monitoring.

The literature available provides background information on the epidemiology and epidemiology of malaria and LF, vector biology, and diagnostic techniques, but there are still gaps in the literature regarding Matayos, Busia County. They are the lack of local prevalence data, need for more critical evaluation of the co-infection mechanisms in the field, outdated reference values of key data from the elimination programme, insufficient awareness of insecticide resistance, and limited guidance on integrated control.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

The mosquito and blood samples used in this study were collected from Busia County in Matayos constituency, Busibwabo ward under the project **mapping emerging infectious diseases in selected sites in Kenya within the context of climate change**. This was a cross-sectional study assessing the prevalence of *Wuchereria bancrofti* and *Plasmodium falciparum* infections in *Anopheles* mosquitoes and human blood samples collected from Matayos, Busia County, Western Kenya. The sampling design used in the selection of samples for this study was based on purposive sampling where only *Anopheles* mosquitoes collected in the main project were selected to be used in the study because malaria parasites are only transmitted by these mosquitoes. Blood samples that were used are those that were collected from consenting participants who had tested positive for filariasis infection by the Filarial Test Strip.

3.2 Sampling Site

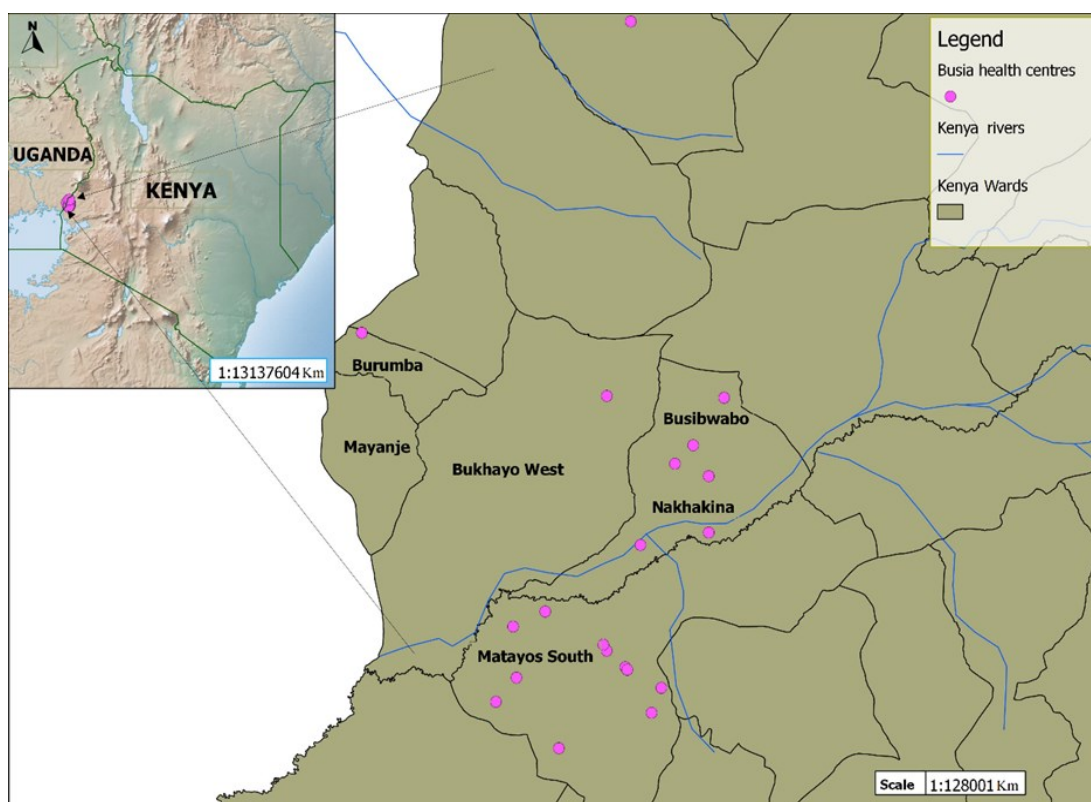


Figure 3.1: A Map of Kenya showing the Sampling Site in Matayos Constituency, Busibwabo Ward, Busia County

Source: Generated using the QGIS Software

The samples used in this study were collected in Matayos constituency, Busibwabo ward, in Busia County where cases of filarial infection had been reported in June 2019 and March 2020 and stored at KEMRI. Busia County borders Lake Victoria to the South and Busia District, Uganda to the West which makes it susceptible to the diseases with the lake providing a suitable breeding site the *Anopheles* mosquito vectors of filarial nematodes as well as *Plasmodium falciparum*. In addition, Uganda is not only an LF-endemic area but according to WHO (2018), 5% of the total deaths due to malaria reported in the WHO African region occurred in this country, while in 2022, 5% of the malaria cases also occurred in Uganda. Therefore, there is the possibility of transmission of both malaria and filariasis in the bordering region of Busia County. Busia is also a malaria endemic region (Bashir *et al.*, 2019). All

mosquitoes collected from this study were preserved dry in silica gel and transported to the the Centre for Biotechnology Research and Development at the Kenya Medical Research Institute where laboratory analyses were carried out.

3.3 Collection of Mosquito and Blood Samples

Before consent was sought from the study participants, the purpose and importance of the study was first explained after which each participant was asked to sign a consent form. Blood samples from children were only collected after informed consent was sought and obtained from their parents and guardians and assent by the children while samples from adults that is those above 18 years was taken after informed consent.

Blood samples were collected from consenting participants who had tested positive for filariasis infection by the FTS (Filarial Test Strip). First, approximately 75 µl of blood was obtained from finger pricks to test for the presence of circulating filarial antigen using the Filarial Test Strip (FTS). An additional 2ml of blood was taken through the venipuncture of the median cubital vein from both adults 18 years and above and children 5-17 years who tested positive after the FTS.

This study used a purposive sampling study design where only *Anopheles* mosquitoes were selected because malaria parasites are only transmitted by these mosquitoes. Anopheline mosquitoes were identified using standard morphological character keys to distinguish them from the other species (Gillies and Meillon, 1968). Adult mosquitoes were collected inside the identified houses of those who tested positive for Filarial Test strip as well as outdoor. CDC light traps were used for the indoor collection mounted within 1 metre of the bed at 6 pm as well as the outdoor collection by hanging the traps near the houses and under trees. The light traps were retrieved the following day at 6 am.

3.4 Sample Size Determination

To determine the number of people from Matayos constituency to be tested for lymphatic filariasis, a prevalence of 50% was assumed since the prevalence of

filariasis was unknown in this area. The sample size was determined using the Fischer's method as follows;

$$N = \frac{Z^2 PQ}{d^2}$$

$$d^2$$

Where;

N = The minimum sample size

Z = the standard score at 1.96

P = the proportion of the target population estimated to be infected 0.5

Q = 1-P (1-0.5) = 0.5

D = the minimum expected error= (100-95)/100= 0.05.

$$N = \frac{(1.96)^2 \times 0.5 \times 0.5}{(0.05)^2}$$

$$(0.05)^2$$

$$N = 385$$

Using the formula, a total of 385 participants were screened for filariasis in Matayos Constituency as part of the broader project *Mapping emerging infectious diseases in selected sites in Kenya within the context of climate change*. 92 tested positive for *Wuchereria bancrofti* and this was the number of samples that was used in this study for detection of *Plasmodium falciparum* and *Wuchereria bancrofti* via PCR.

Mosquitoes were also collected from the houses of participants who tested positive after a filarial test strip. A total of 1304 mosquitoes were collected from all houses sampled, 292 of which were identified as *Anopheles* mosquitoes based on morphological characteristics. Therefore, for this study a total of 292 *Anopheles* mosquitoes were used.

3.5 Inclusion and Exclusion Criteria

Human participants were recruited from the study area based on predefined eligibility criteria. Individuals who provided written informed consent to participate in the study were eligible for inclusion. For participants below 18 years of age, written parental or guardian consent was obtained, and assent was sought from children aged 5-17 years. Participants who tested positive for Lymphatic filariasis by the Filarial Test Strip (FTS) provided blood samples for further molecular analysis.

Individuals who declined to provide informed consent or assent, were severely ill at the time of recruitment or were unable to provide a blood sample were excluded from the study. Participants who tested negative for circulating filarial antigen using the FTS were also excluded from blood sample collection and subsequent laboratory analyses.

For the entomological component, only adult female *Anopheles* mosquitoes collected from the study area were included in the study because they are the primary vectors responsible for the transmission of both *Plasmodium falciparum* and *Wuchereria bancrofti* in Kenya. Mosquitoes belonging to genera other than *Anopheles*, such as *Culex* and *Aedes*, were excluded from the study. This ensured that both the human and mosquito samples analyzed were relevant to the study objectives of investigating the co-transmission of lymphatic filariasis and malaria in Busia County.

3.6 Ethical Consideration

Before implementation, this study was reviewed and approved by the Scientific and Ethics Review Unit (SERU) at KEMRI, Approval no. SERU/3561.

3.7 Laboratory Methods

The mosquito vectors collected from Matayos constituency were used. *Anopheles* mosquitoes were first identified by morphological keys (Gillies and Coetzee 1987). After the morphological identification, the presence of the parasites *P. falciparum* and *W. bancrofti* was tested in the *Anopheles* mosquitoes by PCR with primers targeting the gene encoding the *Plasmodium* 18S Ribosomal rRNAs (Rougemont *et al.*, 2004)

and the long DNA repeat (LDR) GenBank accession number AY297458 respectively (Erttmann *et al.*, 1987).

3.7.1 DNA Extraction

The DNA from the 92 blood samples was isolated using the procedure of Lahiri and Nurnberger (Lahiri *et al.*, 1992). Briefly, 300 µl of NaOH plus 1% triton was added into sterile Eppendorf tubes containing 200 µl of the blood sample and vortexed gently. After vortexing, the mixture was warmed at 65°C for 30 minutes in a thermomixer then the pH adjusted to 8 with HCl 1:4 (dilution) or 1M NaOH. The mixture was then spun in a centrifuge at 4°C, 14000 rpm for 5 minutes and the supernatant transferred into clean Eppendorf tubes. Once in the clean Eppendorf tubes, the samples were heated again at 100°C for 5 minutes in a thermomixer and later cooled quickly on ice and incubated overnight at -80°C. The next day, 500 µl of absolute ethanol was added to the mixture and spun at 4°C, 14000 rpm for 20 minutes. The supernatant was then poured out and the pellet washed thrice with 70% alcohol, dried and suspended in 50 µl elution buffer. The samples were stored in a fridge at -20°C for use when needed.

DNA was extracted from the 292 mosquitoes following the alcohol precipitation method of Collins *et al.*, (Collins *et al.*, 1987). Briefly, heads and thoraces of mosquitoes were individually placed in 1.5ml tubes and ground in 50 µl of grinding buffer. Potassium acetate was then added, the contents of the tube mixed thoroughly, and the tubes cooled on ice water or crushed ice for 30 minutes. The samples were then centrifuged at 10,000 rpm for 10 minutes and the supernatant transferred into clean tubes. 200 µl cold 95% Ethanol was added, and the sample stored in the freezer overnight at -20°C. The following day, the mixture was centrifuged, and DNA eluted through addition of 200 µl 70% ethanol, centrifugation and then pouring off, followed by addition of 200 µl 95% ethanol, centrifugation and again pouring off. The DNA pellet was re-suspended using 100 µl of PCR water after drying overnight at room temperature and stored at -20°C ready for use in PCR.

Table 3:1. PCR Primers for Molecular Identification of Mosquito Species, *W. bancrofti*, and *P. falciparum* Parasites

Primer name	Primer sequence	Target gene	Target organism	Amplicon size	Primer source
UN	GTGTGCCCCCTTCCTCGATG T		Universal primer for <i>Anopheles gambiae</i> complex		
GAM	CTGGTTTGGTCGGCACGTT T	rDNA intergenic spacer region	<i>An. gambiae</i> s.s.	390	[39]
MER	TGACCAACCCACTCCCTTG A		<i>An. merus</i>	464	
ARA	AAGTGTCTTCTCCATCCT A		<i>An. arabiensis</i>	315	
QUA	CAGACCAAGATGGTTAGTA T		<i>An. quadrianulatus</i>	153	
UV	TGT GAA CTG CAG GAC ACA T		Universal primer for <i>An. funestus</i> complex		
FUN	GCA TCG ATG GGT TAA TCA TG,	Internal transcribed spacer 2 region of nuclear rDNA	<i>An. funestus</i> s.s.	500	[22]
VAN	TGT CGA CTT GGT AGC CGA AC		<i>An. vaneedeni</i>	550	
RIV	CAA GCC GTT CGA CCC TGA TT		<i>An. rivulorum</i>	450	
OAR	TGC GGT CCC AAG CTA GGT TC		<i>An. parensis</i>	250	
LEES	TAC ACG GGC GCC ATG TAG TT		<i>An. lesoni</i>	150	
NV1	CGTGATGGCATCAAAGTAG CG	18S rRNA gene	<i>W. bancrofti</i>	188	[47]
NV2	CCCTCACTTACCATAAGAC AAC				
rFAL1	TTAAACTGGTTTGGGAAAA CCAAATATATT	18S rRNA gene	<i>P. falciparum</i>	205	[37]
rFAL2	ACACAATGAACTCAATCAT GACTACCCGTC				

3.7.2 Molecular Identification of Mosquitoes

Extracted DNA was used to identify members of the *An. funestus* s.l. and *An. gambiae* s.l. complexes by PCR using established protocols (Kabbale et al., Koekemoer et al., 2002; 2016 Scott et al., 1993;). The *An. gambiae* complex was identified using the primers QUA, GAM, ARA, MER and UN (Table 3.1). The primers used to identify the *An. funestus* complex were UV, FUN, VAN, RIV, PAR, LEE (Table 3.1). The PCR reaction was performed in 30 µl volumes including 6µl HOT FIREPol® Blend Master

Mix (Solis BioDyne, Tartu, Estonia), 1.5 µl of 10 µM forward and reverse primers and 25 ng of template DNA and topped up to reaction volume using nuclease free water.

The PCR reaction was run in a 96-well Gene Amp® PCR system with the conditions consisting of a single initial denaturation step at 94°C for 12 minutes followed by 35 cycling steps of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds and extension at 72°C for 30 seconds and final extension at 72°C for 7 minutes. PCR products were resolved in ethidium bromide-stained 2% agarose gel electrophoresis and visualized under ultraviolet (UV) light using a gel documentation system (EZ Imager, Bio Red, CA). Known positive samples were used as controls for each mosquito species, including *Anopheles gambiae sensu stricto* and *Anopheles arabiensis* from the *Anopheles gambiae* complex, and *Anopheles lesoni* and *Anopheles funestus sensu stricto* from the *Anopheles funestus* complex while PCR water was used as the negative control. In addition, to minimize contamination during PCR analysis, PCR preparation was conducted in a laminar flow hood under sterile conditions using filter tips and dedicated reagents. Separate areas were maintained for DNA extraction, PCR setup, and post-amplification analysis.

3.7.3 Molecular Detection of *Wuchereria bancrofti*

Species-specific primers NV1 forward primer and the reverse primer NV2 (Table 3.1)

were used to detect and amplify a non-coding DNA sequence target of *W. bancrofti* which is 188 bp long from the DNA extracted earlier from the blood and mosquitoes. The PCR reaction mix contained 12 µl of 10× buffer Bioline (with MgCl₂ and dNTPs), 5 pmol/µl of NV1 and NV2 primers each, 5 µl DNA template and PCR water to top up the reaction volume to 25 µl. The PCR reaction was run in a PCR system with the conditions consisting of a single initial denaturation step at 95°C for 5 minutes, 40 cycling steps of denaturation at 94°C for 30 seconds, annealing at 54°C for 45 seconds and extension at 72°C for 30 seconds and a final extension step of 72°C for 10 minutes. PCR products were resolved in ethidium bromide-stained 2% agarose gel electrophoresis and visualized under ultraviolet (UV) light. PCR water was used as the negative control while known positive samples containing *Wuchereria bancrofti* DNA

were included as positive controls during PCR amplification to confirm successful detection and amplification.

3.7.4 Molecular Detection of *Plasmodium falciparum*

The DNA extracted from the mosquitoes and blood samples were also used to check for the presence of *P. falciparum* using PCR with forward primer rFAL1 and reverse primer rFAL2 (Table 3.1) to detect and amplify the 18S rRNA sequence target of *P. falciparum*, approximately 100 base pair long. The PCR reaction mix contained PCR buffer, dNTPs, Taq polymerase, 18Sf and 18Sr primers each, DNA template and water to top up the reaction volume to 30 µl. The PCR reaction was run in a PCR system machine with the conditions consisting of a single initial denaturation step at 94°C for 3 minutes, 30 cycling steps of denaturation at 94°C for 1 minute, annealing at 55°C for 2 minutes and extension at 72°C for 2 minutes and a final extension step of 72°C for 10 minutes. PCR products were then detected by agarose gel electrophoresis on 2% ethidium bromide-stained agarose gel and visualized under ultraviolet (UV) light. Known positive samples containing *Plasmodium falciparum* DNA were used as positive controls during PCR amplification to verify successful detection of the parasite while PCR water was used as the negative control.

3.7.5 Sequencing

Four PCR-positive amplicons for *Wuchereria bancrofti*, comprising two from human blood samples and two from mosquito samples, were purified using the ISOLATE II PCR and Gel Kit (Bioline, UK) according to the manufacturer's protocol and subsequently submitted to Macrogen Inc. (The Netherlands) for Sanger sequencing.

3.8 Data and Phylogenetic Analysis

Data on co-infections were recorded in laboratory books and entered into Microsoft Excel. Data on mosquito infections with *Wuchereria bancrofti* and *Plasmodium* species were analyzed using percentage and frequency distribution methods, with cross-tabulation employed to estimate infection rates and graphical representations of infection across different mosquito species were produced using SPSS statistical software version 28. The nucleotide sequences from this study were edited and aligned

alongside reference sequences from GenBank in the database using the MAFFT plugin in Geneious Prime software. The PhyML v. 3.0 plugin in Geneious software was used to compute nucleotide similarities and to estimate phylogenetic relationships using the maximum likelihood (ML) method with 1000 bootstrap replications. Dendrogram trees were constructed using Figtree v1.4.4.

CHAPTER FOUR

RESULTS

4.1 Introduction

This section presents the study descriptive statistics, sequential analysis, maximum likelihood dendrogram tree of *W. bancrofti* 18S rRNA gene and discussion.

4.2 Diversity of Species within the Collected *An. gambiae* and *An. funestus* Complexes

For *Anopheles funestus* group species identification using PCR, electrophoresis gels were visualized under ultraviolet light, where specific band sizes were used for identification: 450 bp corresponded to *An. rivulorum*, 250 bp to *An. parensis*, 150 bp to *An. lesoni*, 550 bp to *An. vaneedeni*, and 500 bp to *An. funestus* s.s. (Figure 4.1).

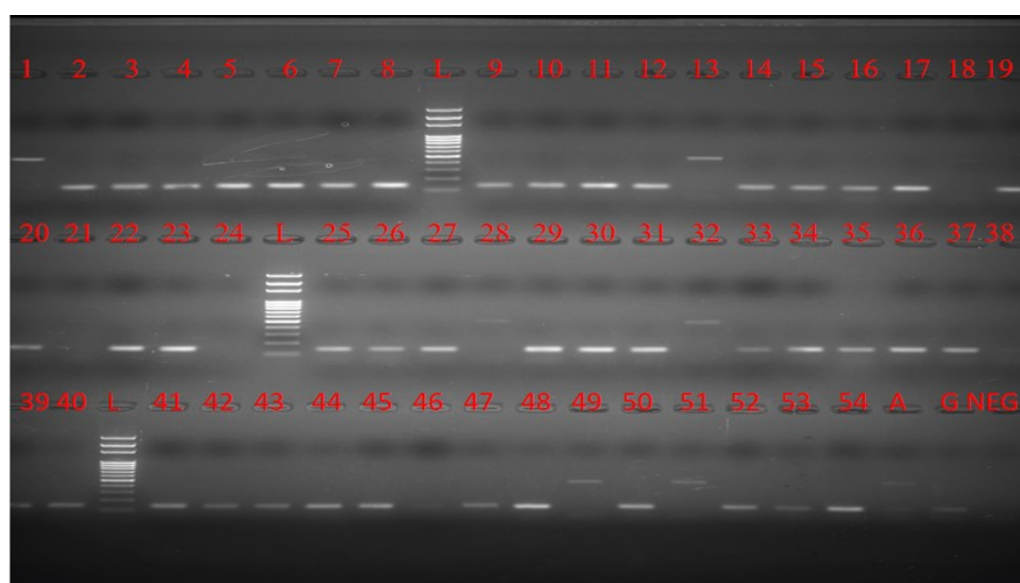


Figure 4.1: Gel Electrophoresis of PCR Products of *An. funestus* Species Complex Mosquito Samples

Wells indicated as L indicate 100 base pair (bp) DNA ladder/marker. Wells 1, 13, 28, 32, 49, 51 and A: *An. rivulorum* showing 450bp band. Well 18, 21, 24, 28, 38, 46 bands and NEG did not amplify for *An. funestus*. The rest of the wells from 2 to 54 and G: *An. lesoni* showing 150 bp band. A was used as a positive control for *An. rivulorum* while G was used as a positive control for *An. lesoni*

A total of 1304 mosquitoes were collected from all houses sampled, 292 of which were identified as *Anopheles* mosquitoes based on morphological characteristics. Out of 292 mosquitoes analyzed, 288 (98.63%) were successfully identified using PCR. No *Plasmodium falciparum* infections were detected, while only two mosquitoes were positive for *Wuchereria bancrofti*, giving an infection rate of 0.68%. The majority of identified specimens belonged to the *Anopheles funestus* complex, which accounted for 85.76% (247/288), whereas those from the *An. gambiae* complex constituted 14.24% (41/288). From the *An. funestus* complex, the majority were from *An. lesoni* (87.04%, 215/247), then *An. funestus* at (9.72%, 24/247) and *An. rivulorum* (3.24%, 8/247). However, *An. parensis* or *An. vaneedeni* were not identified. Among the *An. gambiae* complex, *An. gambiae* s.s. represented 87.80% (36/41), while *An. arabiensis* accounted for 12.20% (5/41).

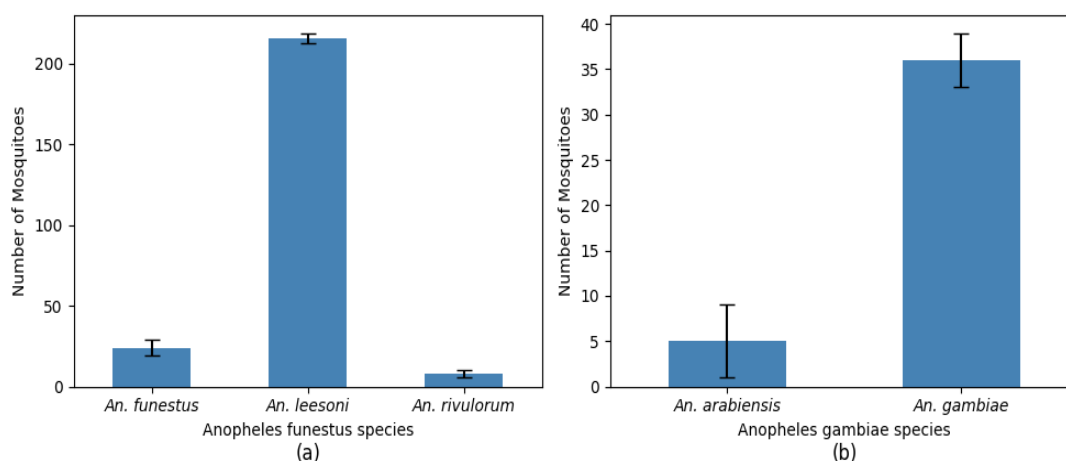


Figure 4.2 Distribution of Mosquito of Species in the *An. funestus* Complex (a) and (b) Mosquito Species in the *An. gambiae* Complex (b)

4.3 Infections with *W. bancrofti* and *P. falciparum*

Two *Wuchereria bancrofti* infections were identified in mosquitoes, with one detected in *An. lesoni* (1/215; 0.46% infection rate) and one in *An. funestus* (1/24; 4.17% infection rate). Among the 92 human blood samples analyzed, no *Plasmodium falciparum* infections were observed, while two samples were positive for *W. bancrofti*, giving an infection rate of 2.17%.

Table 4:1: Number of Positive and Negative Infections of *Plasmodium* and Filariasis from Mosquito Species

Species	Type of infection			
	Filariasis		<i>Plasmodium</i>	
	Negative	Positive	Negative	Positive
<i>An. arabiensis</i>	5	0	5	0
<i>An. funestus</i>	23	1	24	0
<i>An. gambiae</i>	36	0	36	0
<i>An. lesoni</i>	214	1	215	0
<i>An. rivulorum</i>	8	0	8	0

The infection rates for *Plasmodium* and filarial across five *Anopheles* mosquito species show that there was no detection of *Plasmodium* infection in any of the samples, which indicates that none of the mosquito species were carrying malaria *Plasmodium falciparum* parasites during sampling. Two species tested positive for *wuchereria bancrofti*, *An. funestus* (4.17%) and *An. lesoni* (0.46%) while *An. arabiensis*, *An. gambiae*, and *An. rivulorum* had no filarial infections. These results indicate that *An. funestus* and *An. lesoni* plays a role in transmitting lymphatic filariasis, while none of the species are currently contributing to malaria transmission. Therefore, the data highlight species-specific differences in vector competence for filariasis, where *An. funestus* came out as the most relevant potential vector.

Table 4:2: Infection Rate of Mosquito Species

Species	Infection Rate Estimates		95%Confidence Interval
	<i>Plasmodium</i>	Filariasis	
<i>An. arabiensis</i>	0.00	0.00	(0.00-0.00)
<i>An. funestus</i>	0.00	4.17	(0.00-12.16)
<i>An. gambiae</i>	0.00	0.00	(0.00-0.00)
<i>An. lesoni</i>	0.00	0.46	(0.00-1.37)
<i>An. rivulorum</i>	0.00	0.00	(0.00-0.00)
<i>not amplified</i>	0.00	0.00	(0.00-0.00)

The Figure 4.3 Describes distribution of the number of negative infections indicated as figure (a) and the number of positive infections indicated as figure (b) from *plasmodium* and *filariasis* for each of the mosquito species. The figure shows that there was only one positive *filariasis* infection attributed to mosquito species *An. fenestus*

and one positive *filariasis* infection attributed to mosquito species *An. lesoni*. The number of negative *Plasmodium* and *filariasis* infections for all the cases were many with *An. lesoni* having the largest number.

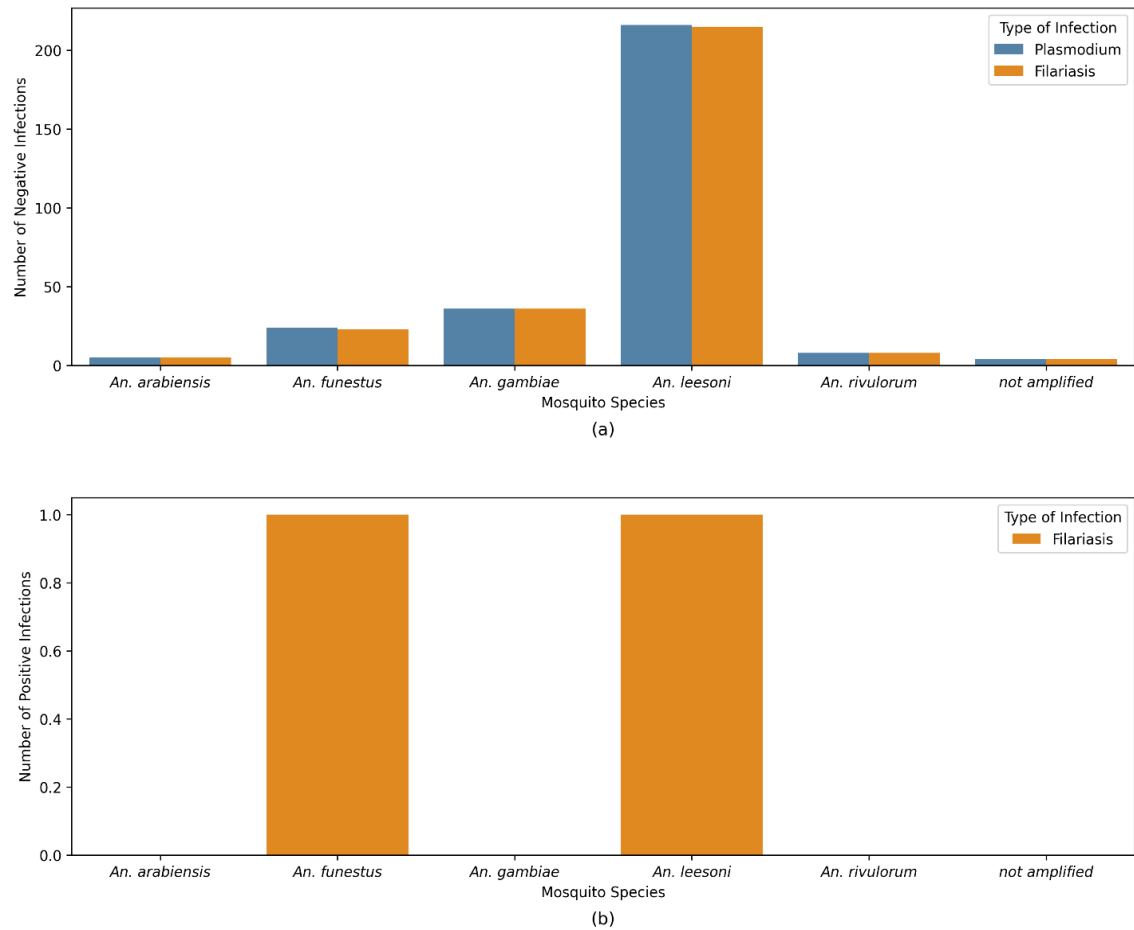


Figure 4.3: Number of Negative Infections (a) and Positive Infections (b) from *plasmodium* and *filariasis* for Each Mosquito Species

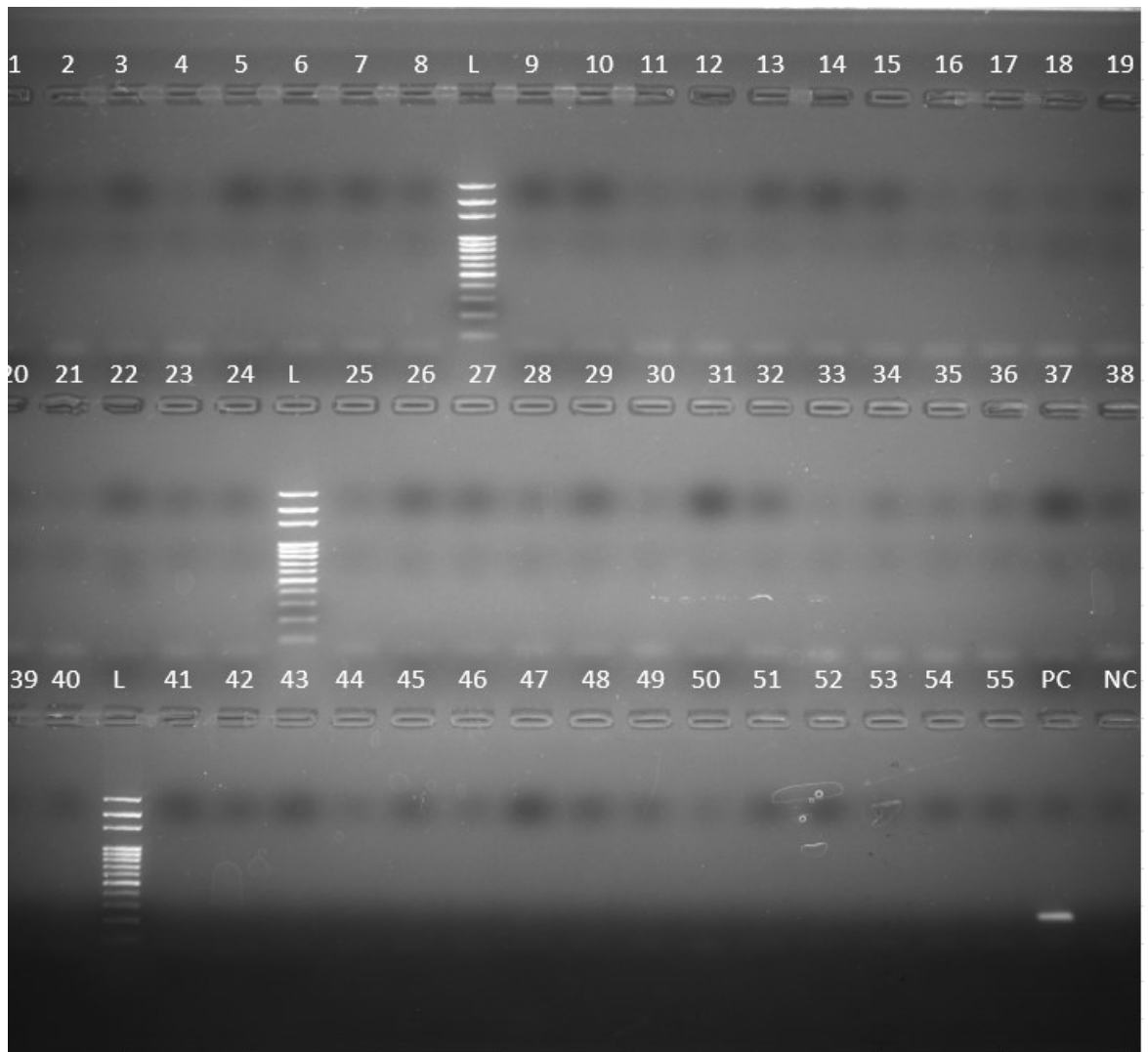


Figure 4.4: Gel Electrophoresis of PCR Products for *Plasmodium falciparum* Detection

The gel electrophoresis band is PCR amplified DNA that was used to detect the presence of *Plasmodium* DNA in the mosquitoes' samples. The band in the lane PC shows that the target *Plasmodium* gene was amplified (positive sample), and no bands in lane indicate that target *Plasmodium* gene was not amplified (negative samples). "L" lanes are DNA ladders, which are used as molecular size markers. The positive control was used to verify the validity, specificity and reliability of the PCR amplification results obtained. The well labelled NC was the negative control (PCR Water was used as the negative control).

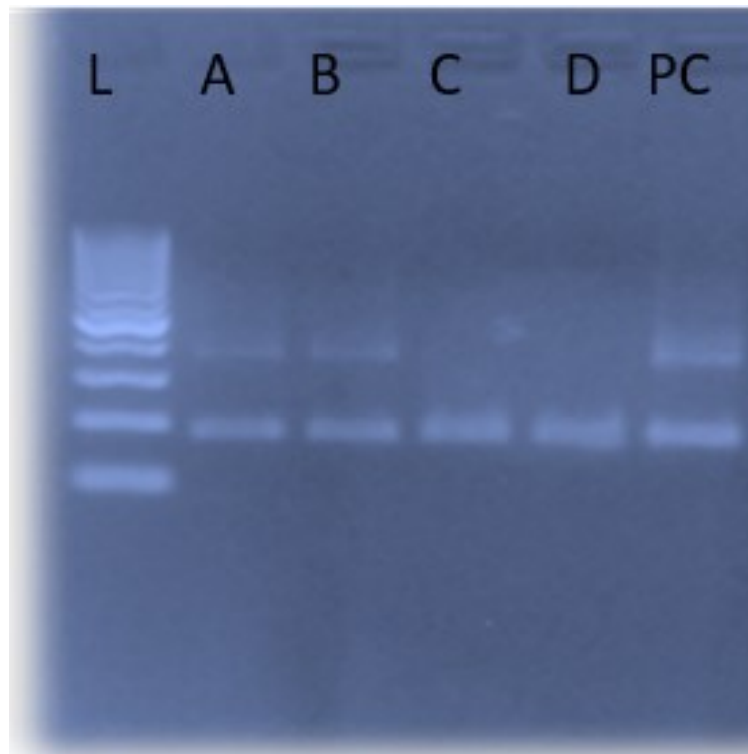


Figure 4.5: Gel Image

Well indicated as L indicate 100 base pair (bp) DNA ladder/marker. PC indicates the positive control while the wells A, B, C and D indicate the positive amplicons, two detected from the mosquitoes and two detected from the blood samples.

4.4 Sequencing Analysis

The four positive amplicons obtained from this study were sequenced and submitted to the GenBank and provided with GenBank accession numbers; PQ429114, PQ429115, PQ429116 and PQ429117.

Sequencing analysis of the 18S rRNA gene of *W. bancrofti* obtained from this study were identical to each other and shared 97 to 100% nucleotide (nt) similarity with reference *W. bancrofti* sequences available on GenBank nr database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (4.3). BLAST analysis of the 188 bp 18S rRNA gene sequences from the four samples BM2_NV, BM3_NV, BM9_NV, and BM16_NV confirmed their identity as *Wuchereria bancrofti*. The sequences were identical indicating a genetically homogeneous parasite population circulating in Matayos. BLAST search against GenBank non-redundant database returned a high

sequence homology (97.90-99.15%) to multiple *W. bancrofti* reference sequences. The four samples showed strong matches E-values ranging from 1e-49 to 3e-58; 97.90 - 99.15% identity to the nuclear scaffold attachment region sequence Accession No. AY297458.1. Samples BM3_NV, BM9_NV, and BM16_NV also matched the *W. bancrofti* Ssp 1 repeat DNA sequence L20344.1 with 98.61-99.15% identity and highly significant E-values (1e-48 to 2e-60). Sample BM9_NV showed a lower but notable match 86.90% identity, E-value 2e-17 to the *W. bancrofti* mitochondrial DNA complete genome (AP017705.1) (Table 4.3). There were no other nematode sequences that showed significant similarity, therefore confirming the specificity of the PCR amplification. Phylogenetic analysis of the 18S rRNA gene (188 bp) revealed that all *Wuchereria bancrofti* sequences generated in this study clustered closely with reference sequences from Israel, the Cook Islands, and Kenya.

Table 4.3: Showing the Sequences from this Study were Identical to Each Other and Shared 97 to 100% Nucleotide (nt) Similarity with Reference *W. bancrofti* Sequences available on GenBank

Sample ID	Gene description	E-value	% Identity	Accession No.
BM2_NV	<i>W. bancrofti</i> nuclear scaffold / matrix attachment region	3e-58	97.90	AY297458.1
BM3_NV	<i>W. bancrofti</i> nuclear scaffold / matrix attachment region	2e-61	98.61	AY297458.1
	<i>W. bancrofti</i> Ssp 1 repeat DNA sequence	2e-60	98.61	L20344.1
BM9_NV	<i>W. bancrofti</i> nuclear scaffold/matrix attachment region	1e-57	98.54	AY297458.1
	<i>W. bancrofti</i> Ssp 1 repeat DNA sequence	4e-57	98.55	L20344.1
	<i>W. bancrofti</i> mitochondrial DNA complete genome	2e-17	86.90	AP017705.1
BM16_NV	<i>W. bancrofti</i> nuclear scaffold/matrix attachment region	1e-49	99.15	AY297458.1
	<i>W. bancrofti</i> Ssp 1 repeat DNA sequence	1e-48	99.15	L20344.1
	<i>W. bancrofti</i> mitochondrial DNA complete genome			



Figure 4.6: Maximum Likelihood Dendrogram Tree of *W. bancrofti* 18S rRNA Gene.

The information provided include GenBank accession numbers, species identifications and countries of origin. Sequences obtained from this study are bolded

The sequence of *Myripristis murdjan* was used as an outgroup because it is evolutionarily distant from the filarial nematodes analysed in this study. Its inclusion provided a baseline for rooting the phylogenetic tree and allowed the evolutionary divergence among the *Wuchereria bancrofti* isolates from Busia and reference filarial sequences from different geographical regions to be inferred. The clear separation of the outgroup from the ingroup taxa supports the reliability of the phylogenetic reconstruction.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This study was designed to generate baseline information to support integrated control strategies for lymphatic filariasis and malaria in the study site. The results showed a higher number of mosquitoes from the *Anopheles funestus* group, indicating its possible ecological dominance. This finding aligns with other studies which report that *An. funestus* prefers to breed in semi-permanent and permanent stagnant water bodies that persist year-round, particularly in vegetated swamps, river margins, and other stable aquatic habitats. (Kahamba et al., 2022). The presence of Lake Victoria and the Sio River in the locality provides stable aquatic habitats that support the continuous proliferation of these vectors.

In contrast, *Anopheles gambiae sensu stricto* typically breeds in temporary and transient aquatic habitats such as rain pools, puddles, hoof prints, agricultural fields, and other sunlit water collections that are frequently associated with human activity and seasonal rainfall patterns. Recent studies have also documented shifts in malaria vector composition in several African regions, with *Anopheles arabiensis* increasingly replacing *An. gambiae sensu stricto* as the dominant vector, partly due to selective pressure associated with the widespread use of long-lasting insecticidal nets (LLINs) and indoor vector control interventions (Bayoh et al., 2010; Sougoufara et al., 2016). High LLIN coverage and utilization in Busia County have similarly been reported following mass distribution campaigns aimed at achieving universal household coverage (Bertozzi-Villa et al., 2021, World Health Organization, 2023).

Behavioral adaptations of malaria vector species play an important role in transmission dynamics and the mosquito behavior also influences the effectiveness of vector control interventions. *Anopheles arabiensis* tends to feed and rest outdoors as it is largely exophilic and exophagic. In addition, the species shows flexible host preferences such as feeding on livestock reducing its contact with control measures such as indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) (Mwanga et al.,

2019; WHO, 2024). In contrast, *Anopheles funestus* is highly anthropophilic and endophilic, with a strong preference for feeding on human hosts and resting indoors, characteristics that enhance its efficiency in malaria transmission (Kaindoa et al., 2017).

The low observed infection rates in both human blood and in the mosquito samples in the study site may partly be explained by the presence of the relatively higher abundance of *Anopheles funestus* compared to members of the *An. gambiae*. However, *An. gambiae* sensu lato still remains one of the most efficient vectors of lymphatic filariasis and *Plasmodium falciparum* because of its strong anthropophilic feeding behavior and high vector competence, the contribution of *An. funestus* to transmission varies depending on ecological and environmental conditions (Ogola et al., 2018; Kahamba et al., 2022). Furthermore, reduced abundance of *An. gambiae* during peak transmission periods may contribute to lower vector infectivity rates and reduced transmission intensity.

The absence of *Plasmodium falciparum* in mosquito samples from Busia County may reflect reduced transmission intensity rather than true absence of the parasite. High LLIN coverage and other vector control interventions likely reduce human–vector contact and lower infection rates in mosquito populations (World Health Organization, 2023). In addition, malaria transmission is often spatially and temporally focal, and low sporozoite prevalence can make detection difficult in cross-sectional sampling due to the small proportion of infectious mosquitoes at any given time (McCann et al., 2014; Russell et al., 2010).

The reduction in malaria infections observed in Busia County may also be associated with intensified malaria control strategies implemented around and after 2019 by the Government of Kenya and its partners. These interventions included indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLINs), prompt diagnosis and treatment of malaria cases, and strengthened community awareness programmes. Studies conducted in western Kenya around 2019 reported that integrated vector control interventions, particularly the combined use of LLINs and IRS, contributed significantly to reductions in malaria transmission, vector density, and malaria

prevalence in endemic regions such as Busia County (Bashir et al., 2019, Dulacha et al. 2022). Therefore, the absence or reduced occurrence of malaria infections in the study area may be attributed to the effectiveness of these ongoing malaria prevention and control measures.

The sustained use of LLINs has also been shown to significantly reduce human-vector contact and suppress transmission of *Wuchereria bancrofti*, the causative agent of lymphatic filariasis and malaria (WHO, 2023; WHO, 2024). As a result, reduced blood-feeding opportunities may also decrease the likelihood of mosquitoes acquiring simultaneous infections with both malaria parasites and filarial worms. The absence of co-infection in mosquito samples in the current study is consistent with previous findings that report the rarity of concomitant infections in *Anopheles* mosquitoes, particularly in natural field populations (Wilairatana et al., 2022).

Previous studies conducted in Kenya and other endemic regions have similarly reported low levels of malaria and lymphatic filariasis co-transmission, suggesting that simultaneous infection and transmission within natural mosquito populations may be relatively uncommon (Coulibaly et al., 2021; Kotepui et al., 2023). This phenomenon may be attributed to ecological and biological constraints that limit the concurrent development and survival of both parasites within the same mosquito vector.

One possible explanation for the low occurrence of co-infection is the relatively short lifespan of mosquito vectors such as *Anopheles gambiae*, which may die before both malaria parasites and filarial worms complete their extrinsic developmental cycles within the mosquito host. This reduces the likelihood of simultaneous transmission of both pathogens (Slater et al., 2013; Kotepui et al., 2023). In addition, recent studies suggest that vector immune responses, physiological constraints, and potential competition between parasites within the mosquito may further limit the successful development of co-infecting organisms (Slater et al., 2013, Muturi et al., 2008).

In conclusion, the results from this study suggest that mosquitoes from the *An. funestus* complex plays an important role in the transmission of *Wuchereria bancrofti* in the study site Matayos, Busia County. This aligns with recent molecular studies conducted in East Africa that have detected filarial infections across members of the *An. funestus*

complex (Bartilol et al., 2024; WHO, 2023). However, it is important to note that molecular detection methods used in this study did not distinguish between infective and non-infective stages of the parasite, which may influence interpretation of transmission potential.

Phylogenetic analysis also collaborates with earlier results where the sequences of *W. bancrofti* from this study branched with other sequences reported from Kenya (MK471348) and Israel among others. (Kinyatta *et al.*, 2019). The results suggest that the parasites from this study are highly similar to other *W. Bancrofti*, but different from other filarial worms such as *D. immitis* and *B. timori* while considering the partial 18S rRNA gene sequences.

5.2 Conclusion

1. Analysis of species diversity within the *Anopheles funestus* and *Anopheles gambiae* complexes indicated that a majority of the collected mosquitoes belonged to the *An. funestus* complex at 85.76% (247/288), with the *An. gambiae* complex accounting for 14.24% (41/288). Within the *An. funestus* complex, *An. lesoni* was the most abundant species at 87.04% (215/247) with *An. funestus* at 9.72% (24/247) and *An. rivulorum* at 3.24% (8/247). However, the *An. gambiae* complex showed a lower diversity with the highest number belonging to the *An. gambiae* at 87.80% (36/41) and *An. arabiensis* at 12.20% (5/41). Overall, both vector complexes were present, although the *An. funestus* complex exhibited higher species diversity. The absence of other documented sibling species may reflect ecological or geographical factors limiting their occurrence in the study area.
2. The analysis revealed no evidence of mixed infections with *Wuchereria bancrofti* and *Plasmodium falciparum* among the anopheline mosquitoes examined. All blood samples tested negative for *P. falciparum*, indicating the absence of detectable malaria parasite infection in the mosquito population during the sampling period. In contrast, filarial infections were detected in a small proportion of mosquitoes, specifically in *An. funestus* (1/24; 4.17%) and *An. lesoni* (1/215; 0.46%). However, none of the infected mosquitoes

harbored *P. falciparum* concurrently. Furthermore, no specimens of *An. arabiensis*, *An. gambiae*, or *An. rivulorum* were positive for either mixed infections or co-carriage of the two pathogens. These findings indicate that there was no evidence of concurrent transmission of lymphatic filariasis and malaria parasites within the sampled anopheline mosquito population.

3. The sequence analysis confirmed that the detected parasites were *Wuchereria bancrofti* and showed a high degree of genetic similarity to known reference sequences in GenBank. BLAST results revealed sequence identities ranging from 97.90% to 99.15%, with highly significant E-values between 1e-49 and 3e-58. All four samples closely matched the *W. bancrofti* nuclear scaffold attachment region sequence (Accession No. AY297458.1), while three samples (BM3_NV, BM9_NV, and BM16_NV) also showed strong similarity (98.61%–99.15% identity) to the *W. bancrofti* Ssp I repeat DNA sequence (Accession No. L20344.1), with E-values ranging from 1e-48 to 2e-60. These findings indicate that the *W. bancrofti* isolates detected in this study are genetically closely related to previously reported *W. bancrofti* strains and show no evidence of substantial genetic divergence. This indicates that the parasites circulating in the study area are part of a broadly distributed genetic lineage and that the genetic markers employed are appropriate for detecting and characterizing local *Wuchereria bancrofti* populations.

5.3 Recommendations

1. The presence of a high proportion of *Anopheles funestus* in the study area calls for the implementation of effective vector control strategies to reduce mosquito population and minimize disease transmission.
2. The detection of *Wuchereria bancrofti* in both *Anopheles* mosquitoes and human blood samples in Busia County indicates ongoing transmission of lymphatic filariasis. Therefore, there is a need to strengthen case detection and implement appropriate treatment programmes for affected individuals to reduce the disease burden and interrupt transmission.
3. The close phylogenetic relationship between the isolates from this study and reference isolates from other endemic regions highlights the need for continued

molecular epidemiological surveillance. Continued molecular surveillance of *Wuchereria bancrofti* should be maintained to monitor any emerging genetic variations in parasite populations over time. Regular genetic characterization of circulating strains will help ensure that the molecular markers currently used for detection remain effective and will provide valuable information to support lymphatic filariasis surveillance and elimination programs.

5.4 Study Limitations

1. A key limitation of this study was the absence of night-time blood sampling for microfilariae detection.
2. Only a limited number of human samples and mosquitoes were analyzed, which could have led to the low level of infections.
3. The study used purposive sampling based on clinical case reports, which may have led to an overestimation or underestimation of the true prevalence, as the sample was not representative of the general population.
4. The quality of the DNA was not determined

REFERENCES

- Abraham, P. R., Ramalingam, B., Mohapatra, P., Krishnamoorthy, K., Hoti, S. L., & Kumar, A. (2024). Detection of *Wuchereria bancrofti* infection in mosquitoes in areas co-endemic with *Brugia malayi* in Balasore district, Odisha, India. *Scientific Reports*, 14, 16780. <https://doi.org/10.1038/s41598-024-67188-2>
- Agarwal, R., Choi, L., Johnson, S., & Takwoingi, Y. (2020). Rapid diagnostic tests for *Plasmodium vivax* malaria in endemic countries. *Cochrane Database of Systematic Reviews*, (11).
- Agrawal, V. K., & Sashindran, V. K. (2011). Lymphatic Filariasis in India: Problems, Challenges, and New Initiatives. *The medical journal, Armed Forces India*, 62(4), 359–362. doi:10.1016/S0377-1237(06)80109-7
- Aliota MT, Chen C-C, Dagoro H, Fuchs JF, Christensen BM (2011). Filarial Worms Reduce *Plasmodium* Infectivity in Mosquitoes. *PLoS Neglected Tropical Diseases* 5(2): e963. doi:10.1371/journal.pntd.0000963.
- Amimo, F. A. (2023). Malaria transmission dynamics in East Africa. In *Malaria-Transmission, Diagnosis and Treatment*. IntechOpen.
- Asare, K. K., Adu-Gyamfi, C. O., Afful, P., Abotsi, G. K., Benyem, G., & Wilmot, D. (2024). Lymphatic Filariasis (LF) Elimination: What Is the Current Situation? In *Mosquito-Borne Tropical Diseases*. IntechOpen.
- Babu, S., & Nutman, T. B. (2014). Immunology of lymphatic filariasis. *Parasite immunology*, 36(8), 338–346. <https://doi.org/10.1111/pim.12081>
- Bartilol, B., Babu, L., Garama, K., Karisa, J., Kamau, A., Mwandawiro, C., ... & Rono, M. (2024). Xenomonitoring of Lymphatic filariasis and risk factors for transmission on the Kenyan coast. *Medrxiv*, 2024-01.

- Bartilol, B., et al. (2024). *Molecular xenomonitoring reveals Anopheles funestus and An. rivulorum as primary vectors of lymphatic filariasis in coastal Kenya*. Parasites & Vectors. <https://doi.org/10.1186/s13071-024-06513-0>
- Bashir, I.M., Nyakoe, N. & van der Sande, M (2019). Targeting remaining pockets of malaria transmission in Kenya to hasten progress towards national elimination goals: an assessment of prevalence and risk factors in children from the Lake endemic region. *Malaria Journal*. **18**, 233 <https://doi.org/10.1186/s12936-019-2876-x>
- Bayoh MN, Mathias DK, Odiere MR, Mutuku FM, Kamau L, Gimnig JE, *et al* (2010). *Anopheles gambiae*: historical population decline associated with regional distribution of insecticide-treated bed nets in western Nyanza province, Kenya. *Malaria Journal*. 9:62.
- Beloconi, A., Nyawanda, B. O., Bigogo, G., Khagayi, S., Obor, D., Danquah, I., ... & Vounatsou, P. (2023). Malaria, climate variability, and interventions: modelling transmission dynamics. *Scientific Reports*, 13(1), 7367.
- Bertoizzi-Villa, A., Bever, C. A., Koenker, H., Weiss, D. J., Vargas-Ruiz, C., Nandi, A. K., Gibson, H. S., Harris, J., Battle, K. E., Rumisha, S. F., Keddie, S., Amratia, P., Arambepola, R., Cameron, E., Chestnutt, E. G., Collins, E. L., Millar, J., Mishra, S., Rozier, J., ... Bhatt, S. (2021). *Maps and metrics of insecticide-treated net access, use, and nets-per-capita in Africa from 2000–2020*. *Nature Communications*, 12, 3589. <https://doi.org/10.1038/s41467-021-23707-7>
- Bockarie M. J., Pedersen E. M., White G. B., Michael E. (2009). Role of vector control in the global program to eliminate lymphatic filariasis. *Annual Review of Entomology*. 54, 469–487 [10.1146/annurev.ento.54.110807.090626](https://doi.org/10.1146/annurev.ento.54.110807.090626)
- Bousema, T., Okell, L., Felger, I., & Drakeley, C. (2014). Asymptomatic malaria infections: Detectability, transmissibility, and public health relevance. *Nature Reviews Microbiology*, 12(12), 833–840

- Cano, J., Rebollo, M. P., Golding, N., Pullan, R. L., Crellen, T., Soler, A., ... Brooker, S. J. (2014). The global distribution and transmission limits of lymphatic filariasis: past and present. *Parasites & vectors*, 7, 466. doi:10.1186/s13071-014-0466-x.
- Cano, J., Rebollo, M. P., Golding, N., & Bockarie, M. (2021). Lymphatic filariasis elimination: Progress, challenges and prospects. *Infectious Diseases of Poverty*, 10, 1–14.
- Capuano, G. P., & Capuano, C. (2012). Surgical management of morbidity due to lymphatic filariasis: the usefulness of a standardized international clinical classification of hydroceles. *Trop Biomed*, 29(1), 24-38.
- Centers for Disease Control and Prevention (CDC). (n.d.). *Malaria*. DPDx—Laboratory Identification of Parasitic Diseases of Public Health Concern. Retrieved May 15, 2026, from <https://www.cdc.gov/dpdx/malaria/index.html>
- Centers for Disease Control and Prevention (CDC). (n.d.). Lymphatic filariasis. DPDx—Laboratory Identification of Parasitic Diseases of Public Health Concern. Retrieved May 15, 2026, from <https://www.cdc.gov/dpdx/lymphaticfilariasis/index.html>
- Centers for Disease Control and Prevention. (2024). *Malaria parasites and transmission*. <https://www.cdc.gov/malaria/>
- Centers for Disease Control and Prevention. (2024). *Malaria treatment guidelines*. <https://www.cdc.gov/malaria/>
- Centers for Disease Control and Prevention. (2024). *Malaria diagnostic testing and laboratory methods*. <https://www.cdc.gov/malaria/>
- Centers for Disease Control and Prevention. (2024). *Lymphatic filariasis*. <https://www.cdc.gov/parasites/lymphaticfilariasis>

- Chandy, A., Thakur, A. S., Singh, M. P., & Manigauha, A. (2011). A review of neglected tropical diseases: filariasis. *Asian Pacific journal of tropical medicine*, 4(7), 581–586. [https://doi.org/10.1016/S1995-7645\(11\)60150-8](https://doi.org/10.1016/S1995-7645(11)60150-8).
- Clark, T., Foster, L., Bryman, A., & Sloan, L. (2021). *Bryman's social research methods*. Oxford University Press.
- Coetzee, M. (2020) Key to the Females of Afrotropical Anopheles Mosquitoes (Diptera: Culicidae). *Malaria Journal*, 19, 1-20. <https://doi.org/10.1186/s12936-020-3144-9>
- Collins, F.H., Mendez, M.A., Rasmussen, P.C., Mahaffey, H.J., Besansky, N.J., and Fennerty, V.A (1987). Ribosomal RNA gene probe differentiates member species of the *Anopheles gambiae* complex. *American Journal of Tropical Medicine and Hygiene*, 37: 37D 41.
- Coulibaly, Y. I. (2016). *Vector competence and filariasis transmission in Mali*. The University of Liverpool (United Kingdom).
- Coulibaly, S., Sawadogo, S. P., Hien, A. S., Nikièma, A. S., Sangaré, I., Rabila, B., Koala, L., Bougouma, C., Bougma, R. W., Ouedraogo, G. A., & Dabiré, R. K. (2021). Malaria and lymphatic filariasis co-transmission in endemic health districts in Burkina Faso. *Advances in Entomology*, 9(4), 221–233. <https://doi.org/10.4236/ae.2021.94014>
- Cowman, A. F., Healer, J., Marapana, D., & Marsh, K. (2016). Malaria: Biology and disease. *Cell*, 167(3), 610–624. <https://doi.org/10.1016/j.cell.2016.07.055>
- Cromwell, E. A., Schmidt, C. A., Kwong, K. T., Pigott, D. M., Mupfasoni, D., Biswas, G., ... & Negoi, I. (2020). The global distribution of lymphatic filariasis, 2000–18: a geospatial analysis. *The Lancet Global Health*, 8(9), e1186-e1194.
- Derua YA, Alifrangis M, Magesa SM, Kisinza WN, Simonsen PE (2015). Sibling species of the *Anopheles funestus* group, and their infection with malaria and lymphatic filarial parasites, in archived and newly collected specimens from

northeastern Tanzania. *Malaria Journal*, 14:104. Published 2015 Mar 6. doi:10.1186/s12936-015-0616-4.

De Souza, D. K., & Bockarie, M. J. (2025). Current perspectives in the epidemiology and control of lymphatic filariasis. *Clinical Microbiology Reviews*, 38(2), e00126-23.

Dulacha, D., Were, V., Oyugi, E., Kiptui, R., Owiny, M., Boru, W., Gura, Z., & Perry, R. T. (2022). Reduction in malaria burden following the introduction of indoor residual spraying in areas protected by long-lasting insecticidal nets in Western Kenya, 2016–2018. *PLOS ONE*, 17(4), e0266736. <https://doi.org/10.1371/journal.pone.0266736>

Elaagip, A., & Higazi, T. (2024). Advances in the Diagnosis of Filarial Nematodes. *Recent Advancements in the Diagnosis of Human Disease*, 118-140.

El-Bahnasawy, M. M., Fadil, E. E., & Morsy, T. A. (2013). Mosquito vectors of infectious diseases: are they neglected health disaster in Egypt? *Journal of the Egyptian Society of Parasitology*, 43(2), 373–386. <https://doi.org/10.12816/0006393>

Farrar, J., Hotez, P. J., Junghanss, T., Kang, G., Lalloo, D., White, N. J., & Garcia, P. J. (Eds.). (2023). *Manson's Tropical Diseases E-Book*. Elsevier health sciences.

Fischer, P., Boakye, D., & Hamburger, J. (2003). Polymerase chain reaction-based detection of lymphatic filariasis. *Medical microbiology and immunology*, 192(1), 3–7. <https://doi.org/10.1007/s00430-002-0152-z>

Futami K, Dida GO, Sonye GO, Lutiali PA, Mwanja MS, Wagalla S, *et al* (2014) Impacts of insecticide treated bed nets on *Anopheles gambiae* s.l. Populations in Mbita district and Suba district, western Kenya. *Parasites and Vectors*, 7(1):63.

- Ghisi M, Kaminsky R, Mäser P. (2007). Phenotyping and genotyping of *Haemonchus contortus* isolates reveals a new putative candidate mutation for benzimidazole resistance in nematodes. *Veterinary Parasitology*. Mar 31;144(3-4):313-20. Epub 2006 Nov 13. PubMed PMID: 17101226.
- Gilles and D. A. Warrell (ed.), *Essential malariology*, 3rd ed. P Edward Arnold, London, United Kingdom.
- Gillies, M.T. and Coetzee, M. (1987) A supplement to the Anophelinae of Africa South of the Sahara (Afro-Tropical region). Vol. 55, South African Institute for Medical Research, Johannesburg.
- Gillies, M.T., & De Meillon, B. (1968). The Anophelinae of Africa, south of the Sahara (Ethiopian Zoogeographical Region). *Publications of the South African Institute for Medical Research*, 54, 1–343.
- Goel, T. C., & Goel, A. (2016). *Lymphatic filariasis* (No. 8510). Singapore: Springer Singapore.
- Gordon, C. A., Jones, M. K., & McManus, D. P. (2018). The History of Bancroftian Lymphatic Filariasis in Australasia and Oceania: Is There a Threat of Re-Occurrence in Mainland Australia. *Tropical medicine and infectious disease*, 3(2), 58. doi:10.3390/tropicalmed3020058
- Gunathilaka, N (2017). Illustrated key to the adult female *Anopheles* (Diptera: Culicidae) mosquitoes of Sri Lanka. *Applied Entomology and Zoology*, **52**, 69–77. <https://doi.org/10.1007/s13355-016-0455-y>
- Haldar, K., Bhattacharjee, S., & Safeukui, I. (2018). Drug resistance in Plasmodium. *Nature Reviews Microbiology*, 16(3), 156-170.
- He, W., Sendor, R., Potlapalli, V. R., Kashamuka, M. M., Tshetu, A. K., Phanzu, F., ... & Parr, J. B. (2024). Development of new real-time PCR assays for detection and species differentiation of Plasmodium ovale. *PLoS neglected tropical diseases*, 18(9), e0011759.

- Hertz MI, Nana-Djeunga H, Kamgno J, Jelil Njouendou A, Chawa Chunda V, *et al.* (2018) Identification and characterization of *Loa loa* antigens responsible for cross-reactivity with rapid diagnostic tests for lymphatic filariasis. *PLOS Neglected Tropical Diseases* 12(11): e0006963. <https://doi.org/10.1371/journal.pntd.0006963>
- Holmes KK, Bertozzi S, Bloom BR, et al., editors. *Major Infectious Diseases*. 3rd edition. Washington (DC): The International Bank for Reconstruction and Development / The World Bank; 2017 Nov 3. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK525192/> doi: 10.1596/978-1-4648-0524-0
- Howes, R. E., Battle, K. E., Mendis, K. N., Smith, D. L., Cibulskis, R. E., Baird, J. K., & Hay, S. I. (2016). Global Epidemiology of *Plasmodium vivax*. *The American journal of tropical medicine and hygiene*, 95(6 Suppl), 15–34. doi:10.4269/ajtmh.16-0141
- IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2014). Malaria and some polyomaviruses (SV40, BK, JC, and Markel cell viruses). *IARC monographs on the evaluation of carcinogenic risks to humans*, 104, 9–350.
- Kabbale, F. G., Akol, A. M., Kaddu, J. B., & Onapa, A. W (2013). Biting patterns and seasonality of *Anopheles gambiae* sensu lato and *Anopheles funestus* mosquitoes in Kamuli District, Uganda. *Parasites & vectors*, 6, 340, <https://doi.org/10.1186/1756-3305-6-340>
- Kabbale, F. G., Akol, A. M., Kaddu, J. B., Matovu, E., Kazibwe, A., Yadouleton, A., & Onapa, A. W. (2016). Molecular identification of *Anopheles gambiae* sensu stricto Giles (formerly *Anopheles gambiae* Savannah Form) in Kamuli district, Uganda. *African Journal of Biotechnology*, 15(39), 2124–2131.
- Kahamba, N. F., Finda, M., Ngowo, H. S., et al. (2022). Using ecological observations to improve malaria control in areas where *Anopheles funestus* is the dominant

vector. *Malaria Journal*, 21, 158. <https://doi.org/10.1186/s12936-022-04198-3>

Kaindoa, E. W., Matowo, N. S., Ngowo, H. S., Mkandawile, G., Mmbando, A., Finda, M., & Okumu, F. O. (2017). Interventions that effectively target *Anopheles funestus* mosquitoes could significantly improve control of persistent malaria transmission in south-eastern Tanzania. *PLoS ONE*, 12(5), e0177807. <https://doi.org/10.1371/journal.pone.0177807>

Kasili S, Oyieke F, Wamae AK, Bisen C, Mbogo C (2009). Seasonal changes of infectivity rates of Bancroftian filariasis vectors in coast province, Kenya. *Journal of Vector Borne Diseases*, 46(3):219-224.

Kenya. Ministry of Health. Malaria Control Programme (1994). Epidemiology of malaria in Kenya. *African journal of medical practice*, 1(1), 5–6.

Kessler C, Manta V. (1990). Specificity of restriction endonucleases and DNA modification methyltransferases a review (Edition 3). *Gene*. Aug 16;92(1-2):1-248. Review. PubMed PMID: 2172084.

Killeen GF (2014). Characterizing, controlling and eliminating residual malaria transmission. *Malaria Journal*, 13(1):330.

Kinyatta, N., Wambua, L., Mugasa, C., Kamau, L., Mutahi, W., Wachira, D., Langat, S., Ichugu, C., Waigi, E., Githae, R., Luswet, J., & Kagai, J. (2019). Molecular characterization and phylogenetic analysis of *Wuchereria bancrofti* in human blood samples from Malindi and Tana River Delta, endemic regions in Kenya. *bioRxiv*. <https://doi.org/10.1101/648220>

Kitau J, Oxborough RM, Tungu PK, Matowo J, Malima RC, Magesa SM, *et al* (2012). Species shifts in the *Anopheles gambiae* complex: do LLINs successfully control *Anopheles arabiensis*? *PLoS One*, 7(3), e31481.

Koekemoer LL, Kamau L, Hunt RH, Coetzee M. 2002. A cocktail polymerase chain reaction (PCR) assay to identify members of the *Anopheles funestus* (Diptera:

Culicidae) group. *American Journal of Tropical Medicine and Hygiene*, 66(6):804-811.

Kotepui, M., Kotepui, K. U., De Jesus Milanez, G., & Masangkay, F. R. (2023). Prevalence, probability, and characteristics of malaria and filariasis co-infections: A systematic review and meta-analysis. *PLoS Neglected Tropical Diseases*, 17(1), e0010857. <https://doi.org/10.1371/journal.pntd.0010857>

Kramyoo, M., Boonpeng, K., Janchoo, C., Siri wattanakul, K., Phankhod, K., Vongthanom, N., ... & Srisutham, S. (2025). High-sensitivity detection of asymptomatic malaria in high-risk Thai populations using droplet digital PCR. *Acta Tropica*, 107831.

Kubasu, S. S (1997). "The vectors of malaria and filariasis in Kilifi and Kwale Districts of Kenya." *Kenyatta University*.

Kumaraswami V, (2000) The clinical manifestations of lymphatic filariasis. Lymphatic Filariasis. Edited by: Nutman TB. London: Imperial College Press, 103-126.

Kumaraswami V, (2002) The clinical manifestations of lymphatic filariasis. Lymphatic Filariasis. Edited by: Nutman TB. London: Imperial College Press, 103-126.

Kyalo, D., Amratia, P., Mundia, C. W., Mbogo, C. M., Coetzee, M., & Snow, R. W. (2017). south of the Sahara: 1898-2016 [version 1; peer review: 3.

Lahiri D. K., Bye S., Nurnberger J. I., Hodes M. E. and Crisp M (1992). A non-organic and non-enzymatic extraction method gives higher yields of genomic DNA from whole-blood samples than do nine other methods tested. *Journal of Biochemical and Biophysical Methods* 25, 193–205.

Lai, Y., Zhang, H., & Chen, X. (2025). Emerging trends and new developments in global research on artemisinin and its derivatives. *Heliyon*, 11(1).

- Lazrek, Y., et al. (2023). Molecular detection of human *Plasmodium* species using multiplex real-time PCR. *Scientific Reports*, 13, 11388. <https://doi.org/10.1038/s41598-023-38621-9>
- Lopez, L., & Koepfli, C. (2021). Systematic review of *Plasmodium falciparum* and *Plasmodium vivax* polyclonal infections: Impact of prevalence, study population characteristics, and laboratory procedures. *Plos one*, 16(6), e0249382.
- Louangrath and Paul (2014). Sample Size Determination for Non-Finite Population (December 1). Southeast-Asian Journal of Sciences Vol. 3, No. 2 (2014) pp. 141-152. Available at <http://dx.doi.org/10.2139/ssrn.2710455>
- Lupenza, E. T., Gasarasi, D. B., & Minzi, O. M. (2022). Lymphatic filariasis elimination status: *Wuchereria bancrofti* infections in human populations and factors contributing to continued transmission after seven rounds of mass drug administration in Masasi District, Tanzania. *PloS one*, 17(1), e0262693. <https://doi.org/10.1371/journal.pone.0262693>
- Mackenzie, C. D., Souza, A., & Geary, T. G. (2022). Diagnosis and assessment of human filarial infections: current status and challenges. *Human and Animal Filariases: Landscape, Challenges, and Control*, 97-124.
- Mahale, P., et al. (2023). Design and validation of multiplex PCR for *Plasmodium* detection. *Journal of Vector Borne Diseases*, 60(2), 200–206
- MalariaGEN. (2021). *Anopheles gambiae* genomic surveillance project. <https://www.malariagen.net/project/anopheles-gambiae-genomic-surveillance-project/>
- Manguin, S., Bangs, M. J., Pothikasikorn, J., & Chareonviriyaphap, T. (2010). Review on global co-transmission of human *Plasmodium* species and *Wuchereria bancrofti* by *Anopheles* mosquitoes. *Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases*, 10(2), 159–177. <https://doi.org/10.1016/j.meegid.11.014>

- Mawson A. R. (2013). The pathogenesis of malaria: a new perspective. *Pathogens and global health*, 107(3), 122–129. doi:10.1179/2047773213Y.00000000084
- Mbanefo, A., & Kumar, N. (2020). Evaluation of malaria diagnostic methods as a key for successful control and elimination programs. *Tropical medicine and infectious disease*, 5(2), 102.
- McCann, R. S., Ochomo, E., Bayoh, M. N., Vulule, J. M., Hamel, M. J., Gimnig, J. E., Hawley, W. A., & Walker, E. D. (2014). Reemergence of *Anopheles funestus* as a vector of malaria after long-term insecticidal net use in western Kenya. *Emerging Infectious Diseases*, 20(10), 1730–1735. <https://doi.org/10.3201/eid2010.140642>
- McNulty SN, Mitreva M, Weil GJ, Fischer PU. (2013). Inter and intra-specific diversity of parasites that cause lymphatic filariasis. *Infection, Genetics, and Evolution.*;14:137–146.
- Ministry of Health (2014). National guidelines for the diagnosis, treatment, and prevention of malaria in Kenya. Kenya: Nairobi.
- Ministry of Health Kenya. (2023). *Kenya national malaria treatment guidelines*. Government of Kenya.
- Mutanu KN, Lillian W, Wilkinson M, Claire M, Luna K, et al. (2020) Molecular Characterization and Phylogenetic Analysis of *Wuchereria bancrofti* in Human Blood Samples from Malindi and Tana River Delta, Endemic Regions in Kenya. *Journal of Biomedical Research and Review* Vol: 3, Issu: 1 (01-10).
- Mutuku FM, King CH, Mungai P, Mbogo C, Mwangangi J, Muchiri EM, et al (2011). Impact of insecticide-treated bed nets on malaria transmission indices on the south coast of Kenya. *Malaria Journal*, 10:356.
- Muturi EJ, Mbogo CM, Ng'ang'a ZW, et al (2006). Relationship between malaria and filariasis transmission indices in an endemic area along the Kenyan Coast. *Journal of Vector Borne Diseases*, 43(2):77-83.

- Muturi, E. J., Mbogo, C. M., Mwangangi, J. M., Ng'ang'a, Z. W., Kabiru, E. W., Mwandawiro, C., & Beier, J. C. (2006). Concomitant infections of *Plasmodium falciparum* and *Wuchereria bancrofti* on the Kenyan coast. *Filaria journal*, 5, 8. doi:10.1186/1475-2883-5-8
- Muturi, E. J., Jacob, B. G., Kim, C.-H., Mbogo, C. M., & Novak, R. J. (2008). *Are coinfections of malaria and filariasis of any epidemiological significance?* *Parasitology Research*, 102(2), 175–181. <https://doi.org/10.1007/s00436-007-0748-x>
- Mwanga, E. P., Mapua, S. A., Siria, D. J., Ngowo, H. S., Nangacha, F., Mgando, J., Baldini, F., Jiménez, M. G., Ferguson, H. M., Wynne, K., Selvaraj, P., Babayan, S. A., & Okumu, F. O. (2019). Using mid-infrared spectroscopy and supervised machine-learning to identify vertebrate blood meals in the malaria vector, *Anopheles arabiensis*. *Malaria Journal*, 18, 187. <https://doi.org/10.1186/s12936-019-2822-y>
- Negussie, H., Molla, M., Ngari, M., Berkley, J. A., Kivaya, E., Njuguna, P., ... & Davey, G. (2018). Lymphoedema management to prevent acute dermatolymphangioadenitis in podoconiosis in northern Ethiopia (GoLBeT): a pragmatic randomised controlled trial. *The Lancet Global health*, 6(7), e795-e803.
- Ndeffo-Mbah, M. L., & Galvani, A. P. (2017). Global elimination of lymphatic filariasis. *The Lancet Infectious Diseases*, 17(4), 358-359.
- Njenga, S. M., Kanyi, H. M., Mutungi, F. M., Okoyo, C., Matendechero, H. S., Pullan, R. L., ... & Won, K. Y. (2017). Assessment of lymphatic filariasis prior to re-starting mass drug administration campaigns in coastal Kenya. *Parasites & vectors*, 10(1), 99. <https://doi.org/10.1186/s13071-017-2044-5>
- Nyenke, C. U. (2024). Emerging techniques in malaria diagnosis. *Sreenivasan A, Deepa K, Chtitambare K, Khan I (eds.)*.

- Ogola, E. O., Fillinger, U., Ondiba, I. M., Villinger, J., Masiga, D. K., Torto, B., & Tchouassi, D. P. (2018). *Insights into malaria transmission among Anopheles funestus mosquitoes, Kenya. Parasites & Vectors, 11*, 577. <https://doi.org/10.1186/s13071-018-3171-3>
- Okara, R. M., Sinka, M. E., Minakawa, N., Mbogo, C. M., Hay, S. I., & Snow, R. W. (2010). Distribution of the main malaria vectors in Kenya. *Malaria journal, 9*, 69. doi:10.1186/1475-2875-9-69
- Okumu FO, Mbeyela E, Lingamba G, Moore J, Ntamatungiro AJ, Kavishe DR, *et al* (2013). Comparative field evaluation of combinations of long-lasting insecticide treated nets and indoor residual spraying, relative to either method alone, for malaria prevention in an area where the main vector is *Anopheles arabiensis*. *Parasites and vectors*, 6:46.
- Orograde A. (2012). Current issues in clinical and laboratory diagnosis of malaria. In: Okwa O, editor. *Malaria parasites*, vol 29. Rijeka: In Tech; pp. 161–172
- Orondo, P. W., Nyanjom, S. G., Atieli, H., Githure, J., Ondeto, B. M., Ochwedo, K. O., ... & Yan, G. (2021). Insecticide resistance status of *Anopheles arabiensis* in irrigated and non-irrigated areas in western Kenya. *Parasites & vectors*, 14(1), 335.
- Ottesen, E. A. (2008). The global programme to eliminate lymphatic filariasis. *Tropical Medicine & International Health, 5*(9), 591-594.
- Pousibet-Puerto, J., Salas-Coronas, J., Sánchez-Crespo, A., Molina-Arrebola, M. A., Soriano-Pérez, M. J., Giménez-López, M. J., Cabezas-Fernández, M. T. (2016). Impact of using artemisinin-based combination therapy (ACT) in the treatment of uncomplicated malaria from *Plasmodium falciparum* in a non-endemic zone. *Malaria journal, 15*(1), 339. doi:10.1186/s12936-016-1408-1
- President's Malaria Initiative. (2022). *Kenya malaria operational plan*. USAID.

- President's Malaria Initiative. Malaria Operational Plan FY 2016. 2016. <https://www.pmi.gov/docs/default-source/default-document-library/malaria-operational-plans/fy16/fy-2016-kenya-malaria-operational-plan.pdf?sfvrsn=5>. Accessed on 23 April 2020.
- Rajasekaram, S., Anuradha, R., Manokaran, G., & Bethunaickan, R. (2017). An overview of lymphatic filariasis lymphedema. *Lymphology*, 50(4), 164–182.
- Rogers, M. J., McManus, D. P., Muhi, S., & Gordon, C. A. (2021). Membrane technology for rapid point-of-care diagnostics for parasitic neglected tropical diseases. *Clinical microbiology reviews*, 34(4), e00329-20.
- Rougemont, M., Van Saanen, M., Sahli, R., Hinrikson, H. P., Bille, J., & Jaton, K. (2004). Detection of four *Plasmodium* species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays. *Journal of clinical microbiology*, 42(12), 5636–5643. <https://doi.org/10.1128/JCM.42.12.5636-5643.2004>
- Russell TL, Lwetoijera DW, Maliti D, Chipwaza B, Kihonda J, Charlwood JD, *et al* (2010). Impact of promoting longer-lasting insecticide treatment of bed nets upon malaria transmission in a rural Tanzanian setting with pre-existing high coverage of untreated nets. *Malaria Journal*, 9:187.
- Schoch, C. L., Ciufo, S., Domrachev, M., Hottot, C. L., Kannan, S., Khovanskaya, R., Leipe, D., Mcveigh, R., O'Neill, K., Robbertse, B., Sharma, S., Soussov, V., Sullivan, J. P., Sun, L., Turner, S., & Karsch-Mizrachi, I. (2020). NCBI Taxonomy: a comprehensive update on curation, resources and tools. *Database : the journal of biological databases and curation*, 2020, baaa062. <https://doi.org/10.1093/database/baaa062>
- Scott JA, Brogdon WG, Collins FH (1993). Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene*, 49:520–529.

- Shenoy, R. K. (2019). Clinical management of lymphatic filariasis complications. *Filaria Journal*, 18(1), 1–10.
- Shretta, R., Liu, J., Cotter, C., Cohen, J., Dolenz, C., Makomva, K., Newby, G., Ménard, D., Phillips, A., Tatarsky, A., Gosling, R., & Feachem, R. (2017). Malaria Elimination and Eradication. In K. K. Holmes (Eds.) et. al., *Major Infectious Diseases*. (3rd ed.). The International Bank for Reconstruction and Development / The World Bank.
- Sinclair, D., Donegan, S., Isba, R., & Lalloo, D. G. (2012). Artesunate versus quinine for treating severe malaria. *Cochrane Database of Systematic Reviews*, (6).
- Sinka, M. E. (2013). Global Distribution of the Dominant Vector Species of *Anopheles mosquitoes: new insights into malaria vectors*, 109.
- Sinha, A., Kumar, S., Dayal, D., Yadav, V., Pramanik, A., Chaubey, K. K., & Kumar, S. (2023). Elimination of lymphatic filariasis: Where do we stand so far? *Asian Pacific Journal of Tropical Medicine*, 16(9), 385-399.
- Slater, H., & Michael, E. (2012). Predicting the current and future potential distributions of lymphatic filariasis in Africa using maximum entropy ecological niche modelling. *PloS one*, 7(2), e32202. doi: 10.1371/journal.pone.0032202
- Slater, H. C., Gambhir, M., Parham, P. E., & Michael, E. (2013). *Modelling co-infection with malaria and lymphatic filariasis*. *PLoS Computational Biology*, 9(6), e1003096. <https://doi.org/10.1371/journal.pcbi.1003096>
- Small, S. T., Tisch, D. J., & Zimmerman, P. A. (2014). Molecular epidemiology, phylogeny, and evolution of the filarial nematode *Wuchereria bancrofti*. *Infection, genetics, and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases*, 28,

- Sougoufara, S., Ottih, E. C., & Tripet, F. (2016). The need for new vector control approaches targeting outdoor biting malaria vector communities. *Parasites & Vectors*, 9, 295. <https://doi.org/10.1186/s13071-016-1575-1>
- Speers, D. J., Ryan, S., Harnett, G., & Chidlow, G. (2003). Diagnosis of malaria aided by polymerase chain reaction in two cases with low-level parasitaemia. *Internal medicine journal*, 33(12), 613–615. <https://doi.org/10.1111/j.1445-5994.2003.00500.x>
- Standring, S. (2020). *Gray's anatomy: The anatomical basis of clinical practice* (42nd ed.). Elsevier
- Stolk, W. A., Stone, C., & de Vlas, S. J. (2015). Modelling lymphatic filariasis transmission and control: modelling frameworks, lessons learned and future directions. *Advances in parasitology*, 87, 249-291.
- Stone, W., Grabias, B., Lanke, K., Zheng, H., Locke, E., Diallo, D., ... Kumar, S. (2015). A comparison of *Plasmodium falciparum* circumsporozoite protein-based slot blot and ELISA immuno-assays for oocyst detection in mosquito homogenates. *Malaria journal*, 14, 451. doi:10.1186/s12936-015-0954-2
- Supali, T., Rahmah, N., Djuardi, Y., Sartono, E., Rückert, P., & Fischer, P. (2004). Detection of filaria-specific IgG4 antibodies using Brugia Rapid test in individuals from an area highly endemic for Brugia timori. *Acta tropica*, 90(3), 255–261. <https://doi.org/10.1016/j.actatropica>.
- Sutcliffe, A. C., Irish, S. R., Rogier, E., Finney, M., Zohdy, S., & Dotson, E. M. (2021). Adaptation of ELISA detection of *Plasmodium falciparum* and *Plasmodium vivax* circumsporozoite proteins in mosquitoes to a multiplex bead-based immunoassay. *Malaria journal*, 20(1), 377. <https://doi.org/10.1186/s12936-021-03910-z>
- Tangpukdee, N., Duangdee, C., Wilairatana, P., & Krudsood, S. (2009). Malaria diagnosis: a brief review. *The Korean journal of parasitology*, 47(2), 93–102. doi:10.3347/kjp.2009.47.2.93

- Taylor, W. R., Thriemer, K., von Seidlein, L., Yuentrakul, P., Assawariyathipat, T., Assefa, A. ... & Price, R. N. (2019). Short-course primaquine for the radical cure of *Plasmodium vivax* malaria: a multicentre, randomised, placebo-controlled non-inferiority trial. *The Lancet*, 394(10202), 929-938.
- Wilairatana, P., Kotepui, K. U., Mala, W., Wangdi, K., & Kotepui, M. (2022). *Prevalence, probability, and characteristics of malaria and filariasis co-infections: A systematic review and meta-analysis*. PLoS Neglected Tropical Diseases, 16(10), e0010857. <https://doi.org/10.1371/journal.pntd.0010857>
- Wang, J. (2021). Genomics of the parasitic nematode *Ascaris* and its relatives. *Genes*, 12(4), 493.
- Wanjala, C. L., & Kweka, E. J. (2018). Malaria vectors insecticides resistance in different agroecosystems in Western Kenya. *Frontiers in public health*, 6, 55.
- White, N. J., Pukrittayakamee, S., Hien, T. T., Faiz, M. A., Mokuolu, O. A., & Dondorp, A. M. (2014). Malaria. *The Lancet*, 383(9918), 723–735. [https://doi.org/10.1016/S0140-6736\(13\)60024-0](https://doi.org/10.1016/S0140-6736(13)60024-0)
- White NJ & Chotivanich K. (2024). Artemisinin-resistant malaria. *Clin Microbiol Rev*. 37(4):e0010924. doi: 10.1128/cmr.00109-24. Epub 2024 Oct 15. PMID: 39404268; PMCID: PMC11629630.
- Wijesinghe, R. S., Wickremasinghe, A. R., Ekanayake, S., & Perera, M. S. (2007). Physical disability and psychosocial impact due to chronic filarial lymphoedema in Sri Lanka. *Filaria Journal*, 6, 4. doi:10.1186/1475-2883-6-4
- Wilairatana, P., Kotepui, K. U., Mala, W., Wangdi, K., & Kotepui, M. (2022). Prevalence, probability, and characteristics of malaria and filariasis co-infections: A systematic review and meta-analysis. *PLoS neglected tropical diseases*, 16(10), e0010857.

- Williams, S. A., Laney, S. J., Bierwert, L. A., Saunders, L. J., Boakye, D. A., Fischer, P., Goodman, D., Helmy, H., Hoti, S. L., Vasuki, V., Lammie, P. J., Plichart, C., Ramzy, R. M., & Ottesen, E. A. (2002). Development and standardization of a rapid, PCR-based method for the detection of *Wuchereria bancrofti* in mosquitoes, for xenomonitoring the human prevalence of bancroftian filariasis. *Annals of tropical medicine and parasitology*, 96 Suppl 2, S41–S46. <https://doi.org/10.1179/000349802125002356>
- Wilson, A. L., Courtenay, O., Kelly-Hope, L. A., Scott, T. W., Takken, W., Torr, S. J., & Lindsay, S. W. (2020). The importance of vector control for the control and elimination of vector-borne diseases. *PLoS neglected tropical diseases*, 14(1), e0007831. <https://doi.org/10.1371/journal.pntd.0007831>
- Wirtz, R. A., Burkot, T. R., Graves, P. M., & Andre, R. G. (1987). Field evaluation of enzyme-linked immunosorbent assays for *Plasmodium falciparum* and *Plasmodium vivax* sporozoites in mosquitoes (Diptera: Culicidae) from Papua New Guinea. *Journal of medical entomology*, 24(4), 433–437. <https://doi.org/10.1093/jmedent/24.4.433>
- World Health Organization (2002). Preparing and implementing a national plan to eliminate Lymphatic filariasis in countries where onchocerciasis is not co-endemic. doi:(WHO/CDC/CPE/2000.15) http://apps.who.int/iris/bitstream/10665/66899/1/WHO_CDS_CPE_CEE_2000.16.pdf
- World Health Organization (2015). Global Programme to Eliminate Lymphatic Filariasis: progress report, *Weekly epidemiological record*. 2016; 39: 441–460.
- World Health Organization (2015). Guidelines for the Treatment of Malaria. 3rd edition Geneva: Annex 6, Treatment of *Plasmodium vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* infections. Retrieved from: <https://www.ncbi.nlm.nih.gov/books/NBK294430/#>
- World Health Organization (2016). Global Programme to Eliminate Lymphatic Filariasis: progress report, *Weekly epidemiological record*, 39: 441–460.

- World Health Organization (2019). Global programme to eliminate lymphatic filariasis: progress report, 2018. *Weekly epidemiological record*, 94(41), 457-470.
- World Health Organization. (2017). Guideline: alternative mass drug administration regimens to eliminate lymphatic filariasis. WHO/HTM/NTD/PCT/2017.07. 50p.
- World Health Organization. Global programme to eliminate lymphatic filariasis: progress report, 2020. *Wkly Epidemiol Rec.* 2021; 96:497–508
- World Health Organization. (2022). *World malaria report 2022*. World Health Organization.
- World Health Organization. (2023). *Global programme to eliminate lymphatic filariasis: Progress report*. WHO.
- World Health Organization. (2023). *Lymphatic filariasis: Epidemiological update and global programme report*. WHO.
- World Health Organization. (2024). *Global programme to eliminate lymphatic filariasis: Progress report*. WHO.
- World Health Organization. (2015). *World malaria report 2015*. WHO.
- World Health Organization. (2021). *Global technical strategy for malaria 2016–2030: Update*. WHO.
- World Health Organization. (2024). *World malaria report 2024*. WHO.
- World Health Organization. (2025). *World malaria report 2025*. World Health Organization.
- World Health Organization. (2023). *Malaria*. <https://www.who.int/news-room/fact-sheets/detail/malaria>

- World Health Organization. (2023). *Malaria microscopy and rapid diagnostic tests: Implementation guidelines*. WHO Press.
- World Health Organization. (2022). *Lymphatic filariasis: Progress report and elimination strategies*. WHO Press.
- World Health Organization. (2023). *Guidelines for malaria treatment*. WHO Press.
- World Health Organization. (2022). *Lymphatic filariasis fact sheet and global programme update*. WHO Press.
- World Health Organization (WHO). (2023). *World malaria report 2023*. Geneva: WHO.
- Worrall, E., Were, V., Matope, A. *et al* (2020). Coverage outcomes (effects), costs, cost-effectiveness, and equity of two combinations of long-lasting insecticidal net (LLIN) distribution channels in Kenya: a two-arm study under operational conditions. *BMC Public Health* **20**, 1870. <https://doi.org/10.1186/s12889-020-09846-4>
- Zhao, H., Xu, T., & Shen, H. (2025). Global, regional, and national disability-adjusted life years and prevalence of lymphatic filariasis from 1990 to 2021: A trend and health inequality analysis based on the global burden of disease study 2021. *PLOS Neglected Tropical Diseases*, *19*(4), e0013017.
- Zhang, R., Zhu, Z., Zhu, H., Nguyen, T., Yao, F., Xia, K. ... Liu, C. (2005). SNP Cutter: a comprehensive tool for SNP PCR-RFLP assay design. *Nucleic acids research*, *33*(Web Server issue), W489–W492. doi:10.1093/nar/gki358

APPENDICES

Appendix I: Ethical Approval Letter from SERU


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

KEMRI/RES/7/3/1 **September 26, 2020**

TO: CAROLINE WACERA MAINA,
PRINCIPAL INVESTIGATOR.

THROUGH: THE DEPUTY DIRECTOR, CBRD,
NAIROBI.

Dear Madam,

RE: KEMRI/SERU/CBRD/208/4088 (RESUBMITTED INITIAL SUBMISSION):
CO-INFECTION OF ANOPHELES MOSQUITOES WITH WUCHERERIA
BANCROFTI AND PLASMODIUM SPECIES AND DETECTION OF SNPS ON THE
β-TUBULIN GENE ASSOCIATED WITH ALBENDAZOLE RESISTANCE IN BUSIA
COUNTY, WESTERN KENYA

Reference is made to your letter dated September 18, 2020. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on September 19, 2020.

This is to inform you that the Committee notes that the issues raised during the 302nd Committee B meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **August 19, 2020** have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **September 26, 2020** for a period of **one (1) year**. Please note that authorization to conduct this study will automatically expire on **September 25, 2021**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **August 14, 2021**.

Please note that only approved documents including (informed consents, study instruments, Material Transfer Agreement) will be used. You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

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 faithfully,


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

In Search of Better Health