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

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## Abstract

**INTRODUCTION**

**Lymphatic filariasis and malaria are mosquito-borne diseases and are co-endemic in coastal Kenya. We evaluated the occurrence of co-infections with *Wuchereria bancrofti* and *Plasmodium falciparum*, the causative agents of Lymphatic filariasis and malaria, respectively, in humans and known *Anopheles* vectors from Matayos constituency, Busia County, western Kenya.**

**MATERIALS AND METHODS**

**The samples were collected purposively based on clinical case reports. Members of the *An. gambiae* and *An. funestus* species complexes present and the presence of *W. bancrofti* and *P. falciparum* were detected using Polymerase Chain Reaction (PCR). Positive PCR amplicons were purified and Sanger sequenced.**

**RESULTS**

**In this study, 292 Anopheles mosquitoes were analyzed, and 288 of these (98.63%) were successfully identified by PCR. The majority of mosquitoes identified belonged to the *An. funestus* complex (85.76%) while *An. gambiae* complex mosquitoes comprised 14.24%. Two *W. bancrofti* infections were detected in the mosquitoes, one each in *An. leesoni* and *An. funestus* s.s. with an infection rate of 0.46% and 4.17% respectively. No *P. falciparum* was detected in the mosquito or human blood samples and only 2 blood samples (2.17%) were positive for *W. bancrofti*. Phylogenetic analysis based on the sequenced 18S rRNA gene showed that all *W. bancrofti* sequences from this study shared a close relationship with *W. bancrofti* sequences distributed in other regions. No case of co-infection of *P. falciparum* and *W. bancrofti* was found in the mosquito or blood samples analyzed.**

**CONCLUSION**

**This study confirms the presence of *W. bancrofti* infection in the human population and mosquito vectors in Matayos. The study also indicated that the majority of *Anopheles* mosquitoes from the study area belong to *An. funestus* complex. This study recommends additional screening before Mass Drug Administration, and that mosquito control programs be strengthened in the study area.**

**Keywords**: *Co-infections, Wuchereria Bancrofti, Plasmodium Falciparum, Anopheles Vectors, Polymerase Chain Reaction (PCR)*

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## Introduction

Malaria and Lymphatic Filariasis (LF) are important mosquito-borne parasitic infections which can simultaneously infect mosquitoes and humans [1, 2, 3]. In 2021, WHO reported an estimated 247 million cases of malaria worldwide and 619000 deaths [4]. On the other hand, 863 million people in 47 countries are threatened by LF and require preventive chemotherapy to prevent the spread of the infection [4].

Malaria is caused by parasites from the genus *Plasmodium* which are *P. falciparum, P. vivax, P. ovale* and *P. malariae* with humans being their intermediate hosts. *Plasmodium falciparum* is the most common causal agent of malaria in Africa, Asia and South America, while *P. vivax* is common in America, Asia and North Africa [5].In Kenya, malaria is endemic to the coastal, Nyanza and Western regions where Busia was reported to have the highest prevalence in 2019 [6]. Transmission is high in these regions as compared to other low-transmission regions such as Machakos, Kitui and Ngurumani [7,8].

Lymphatic filariasis, on the other hand, is one of the neglected tropical vector-borne diseases that cause lymphedema, hydrocele and elephantiasis [9, 10]. Damage to the lymphatic system causes the fluids to accumulate in the tissues resulting in disabling swellings. The thread-like nematodes responsible for the damage to the lymphatic system in filariasis are *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* which are transmitted by mosquitoes of the genus *Culex* and *Anopheles* in the African rural areas.

In Kenya, Lymphatic Filariasis (LF) is endemic to the coastal region. Between 1998 and 1999, a thorough mapping of the disease was conducted throughout Kenya, and only the coastal regions were found to have a prevalence that, following WHO guidelines, could be classified as endemic [11,12,13]. The coastal areas of Kilifi, Mombasa, and Malindi [8] continue to be endemic for the disease to date.

The LF elimination program was first launched by the Ministry of Health in Kilifi district where the entire population was treated with a single dose of diethylcarbamazine citrate in addition to albendazole. The program had by 2011 extended to the districts of Tana River and Lamu counties [18]. However, infection is still a concern for about 2.5 million Kenyans [18, 14]. Because it is only in the areas where the disease has been discovered to be endemic that all control efforts have been focused and intensified [14]. However, there are compelling anecdotal reports that lymphatic filariasis circulates in other areas, such as the Lake Victoria region, notably in the counties of Western Kenya, Kisumu, and Kisii. Both malaria and LF are transmitted by *Anopheles* mosquito vectors [16, 17]. In tropical Africa, these parasitic diseases are transmitted by various vectors such as *Anopheles arabiensis*, *An. funestus*, *An. merus*, *An. gambiae*, and *An. melas*, among others [2, 3]. In Kenya, the major anopheles vectors *An. gambiae* and *An. funestus* are co-infected with *Plasmodium falciparum* and *Wuchereria bancrofti*, especially in the coastal region [3]. The presence of these two vectors has also been reported in western and Nyanza provinces which are closest to Lake Victoria as well as the coastal region [20].

Previous studies on co-infection of *Anopheles* mosquitoes with malaria and LF observed that *W. bancrofti*-infected *An. gambiae* had a significantly higher number of *Plasmodium* sporozoites [3]. This was also observed in Papua New Guinea, where *Plasmodium*-infected *An. punctulatus* had more *W. bancrofti* larvae compared to the LF-negative controls [16].

Previous studies have also shown that co-infection of mosquitoes with filarial and malarial parasites reduces the vector's survival rates as well as transmission of malaria and filariasis [2, 3,]. This observation led to the hypothesis that the control of one of the diseases for example, malaria, in endemic regions could lead to increased rates of transmission of Lymphatic filariasis [21].

Given the reports of filarial transmission in Busia County as well as the increase in malaria infections in Kenya and Uganda [4], studies on co-infection of mosquito vectors and humans with *W. bancrofti* and *Plasmodium* species are key in providing information to guide policymakers in the control of filariasis and malaria.

## Materials and Methods

### Study site

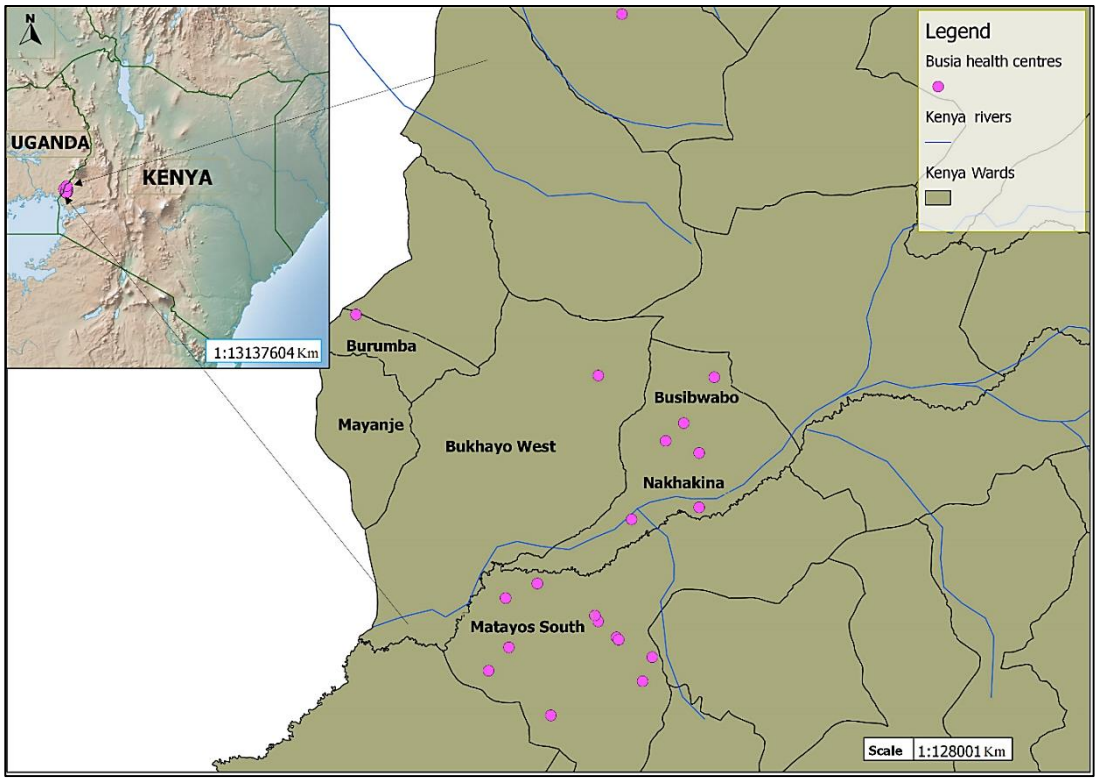
Due to prior reports of filarial-like lymphedemas, Matayos South and Busibwabo

wards in Matayos Constituency in Busia County (**Figure 1**) were chosen for the study (N. Kinyatta; Personal observations). Based on the WHO-AFRO guidelines for mapping lymphatic filariasis [15] such as the presence of chronic cases of the disease suggesting a high likelihood of lymphatic filariasis transmission in the area, the two wards were purposefully chosen. Busia county (00 27 11N, 34 07 30E) is located in the Western part of Kenya, approximately 431 km west of Nairobi and East of Busia district in Uganda (**Figure 1**). According to the 2019 population census, the population of Busia was estimated to be 893,681. The main economic activity is agriculture, tourism, and fishing [22].

## Sampling design and sample collection

This study used a purposive sampling study design where only *Anopheles* mosquitoes were selected from all mosquitoes sampled from an ongoing study because malaria parasites are only transmitted by these mosquitoes. *Anopheles* mosquitoes were identified using standard morphological character keys to distinguish them from other species [23].

The samples used in this study were collected in June 2019 and March 2020. Adult mosquitoes were collected inside the identified houses of participants who tested positive for Filarial Test Strips (FTS) as well as outdoors. CDC light traps were used for the indoor and outdoor collections of mosquitoes. Ten traps were deployed per night (5 indoors and 5 outdoors) for 10 nights and were mounted within one metre of the bed indoors while the outdoor traps were hung near windows and doors. The light traps were set up at 6 pm and retrieved the following day at 6 am. Mosquitoes collected were preserved dry in silica gel for transportation to the Centre for Biotechnology Research and Development (CBRD) at the Kenya Medical Research Institute (KEMRI) for storage before laboratory analyses.




**F*igure 1:***
A Map of Kenya showing the sampling sites in Matayos Constituency, Busia County generated using the QGIS Software.

The study included participants who had chronic clinical signs of lymphatic filariasis and living close to affected individuals, particularly members of the same homestead. For blood sampling, first, approximately 75 µL of blood was obtained through finger pricks to test for the presence of circulating filarial antigens using the Filarial Test Strip (FTS) following user instructions. The blood obtained was gradually added to AlereTM Filariasis Test Strip (FTS) (Alere©, Waltham, United States). The test cards were then set out on a table, and the results were read after ten minutes. If a case tested positive, an additional 4ml of blood was drawn into vacutainers and taken to CBRD at KEMRI for parasite characterization.

## DNA extraction

**DNA extraction from blood samples.** **T**he DNA was extracted from the blood sample, using the procedure of Lahiri and Nurnberger [25]. The pellet obtained was washed with 70% alcohol, dried, and suspended in 50µL elution buffer and stored at -20°C.

**DNA extraction from mosquitoes.** The DNA was extracted from mosquitoes' heads and thoraces following the alcohol precipitation method of Collins *et al.* [26]. The DNA pellet obtained was dried and re-suspended using 100µL of PCR water and stored at -20°C.

## Molecular identification of mosquitoes

The members of the *Anopheles gambiae* s.l. and *An. funestus* s.l. species complexes were identified as per protocols by Scott *et al*., [17] and Koekemoer *et al*. [28], respectively. The primers used for the *Anopheles gambiae* complex were UN-GTGTGCCCCTTCCTCGATGT, *GAM-CTGGTTTGGTCGGCACGTTT*, *MER-TGACCAACCCACTCCCTTGA*, ARA-AAGTGTCCTTCTCCATCCTA, QUA-CAGACCAAGATGGTTAGTAT [29]. For the *Anopheles funestus* complex, the primers used were UV- TGT GAA CTG CAG GAC ACA T, FUN- GCA TCG ATG GGT TAA TCA TG, VAN- TGT CGA CTT GGT AGC CGA AC, RIV- CAA GCC GTT CGA CCC TGA TT, PAR- TGC GGT CCC AAG CTA GGT TC, LEE- TAC ACG GGC GCC ATG TAG TT [30]. The reaction was performed in 30µL volumes including 6µL HOT FIREPol® Blend Master Mix (Solis BioDyne, Tartu, Estonia), 1.5µL of 10µM primers and 25ng of template DNA and topped up to the reaction volume using nuclease-free water.

The reactions were run in a 96-well Gene Amp® PCR system with the conditions, initial denaturation at 94°C for 12 minutes followed by 35 cycles 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.

All PCR products were resolved in ethidium bromide-stained 2% agarose gel electrophoresis and visualized under ultraviolet light transillumination using a gel documentation system (EZ Imager, Bio Red, CA).

## Molecular detection of *Wuchereria bancrofti*

NV1-CGTGATGGCATCAAAGTAGCG and NV2-CCCTCACTTACCATAAGACAAC primers [31] were used to detect *W. bancrofti* by PCR. The reaction mix contained 12µl of 10x buffer Bioline (with $MgCl_2$ and dNTPs), 5pmol/µl of the primers each, 5µl DNA template and PCR water to top up the reaction volume to 25µl. The reaction was run with the conditions, initial denaturation at 95°C for 5 minutes, 40 cycles 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. Representative positive PCR amplicons were purified using ISOLATE II PCR and gel kit (Bioline, UK) according to the manufacturer's protocol and outsourced for Sanger sequencing (Macrogen Inc., The Netherlands).

## Molecular detection of Plasmodium falciparum

To detect the presence of *Plasmodium falciparum* by PCR, the primers rFAL1-

TTAAACTGGTTTGGGAAAACCAAATAT ATT and rFAL2-ACACAATGAACTCAATCATGACTACCC GTC [32] were used to amplify the 18S rRNA sequence target of *Plasmodium falciparum*, approximately100 base pair long. The PCR reaction mix contained PCR buffer, dNTPs, Taq polymerase, rFAL1 and rFAL2 primers, each, a DNA template, and water to top up the reaction volume to 30µl. The reaction was run with the conditions, 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 final extension step of 72°C for 10 minutes.

## Phylogenetic analysis

The sequences obtained from this study were edited and aligned alongside reference sequences from the GenBank nr database using the MAFFT plugin in Geneious Prime software. PhyML v. 3.0 plugin in Geneious software was used to compute nucleotide similarities and phylogenetic relationships estimated using the maximum likelihood (ML) method with 1000 replications in the bootstrap test. Phylogenetic trees were visualized using FigTree v1.4.4.

## Ethics statement

Before implementation, this study was reviewed and approved by the Scientific and Ethics Review Unit (SERU) at KEMRI, Approval no. SERU/3561. The study participants were provided with adequate information about the study to allow independent informed written consent to adults over 18 years to participate in the study. For the children below 18 years of age, informed consent was obtained from the parents or guardians, with children above 7 years of age giving assent.

## Results

A total of 92 human blood samples and 292 *Anopheles* mosquitoes collected indoors, were analyzed in this study. Of the 292 mosquitoes analyzed, a total of 288 mosquitoes representing 98.63% were successfully identified by PCR while those not identified by PCR were not processed further. Of the 288 mosquitoes, none were found to be infected with *P. falciparum*, while only two were found to be infected with *W. bancrofti* and represented an infective rate of 0.68%.

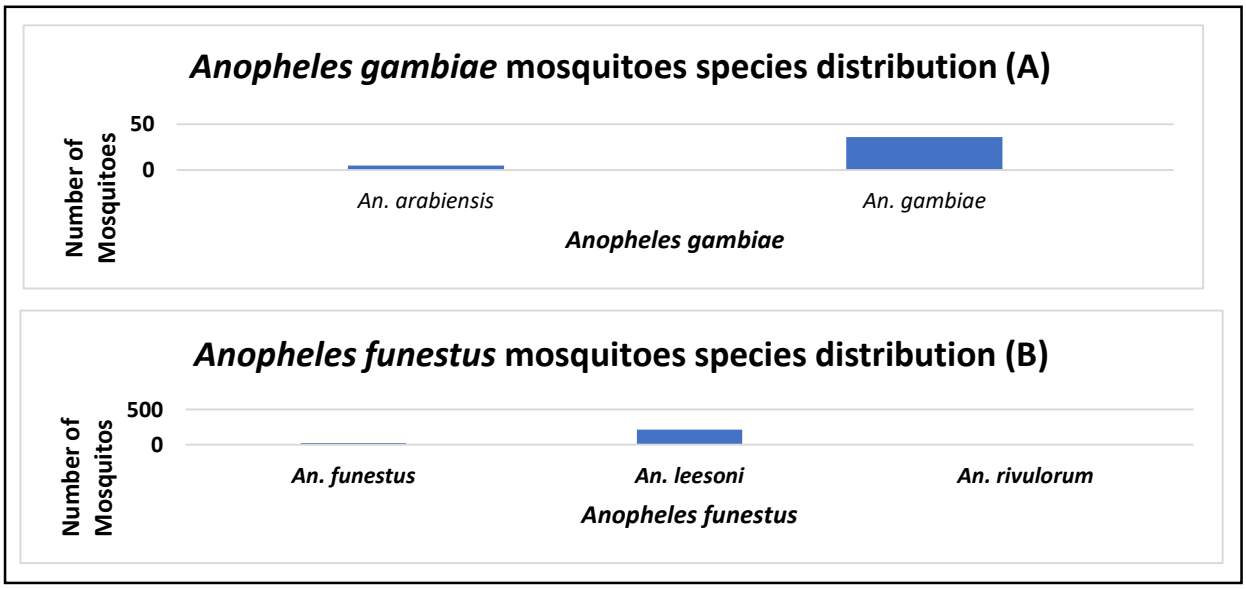



***Figure 2:***
The above graph shows the mosquito species distribution. A shows the distribution of the sibling species in the *Anopheles gambiae* complex, and B shows the distribution of *Anopheles funestus* sibling species in the complex.

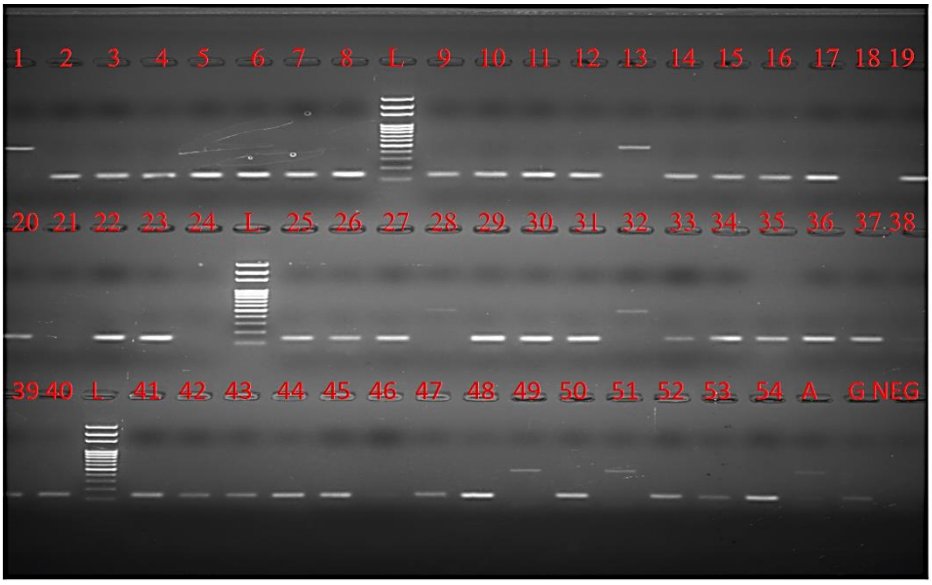



***Figure 3***:
Gel Electrophoresis of PCR products of *An. funestus* species complex mosquito samples. Wells indicated as L indicate 100 base pair (bp) DNA ladder/marker. Wells 1, 13,28,32,49, 51 and A: *An. rivulorum* showing 400bp band. Well 18, 21, 24, 28, 38, 46 and NEG did not amplify for *An. funestus*. The rest of the wells from 2 to 54 and G: *An. leesoni* showing a 150 bp band.

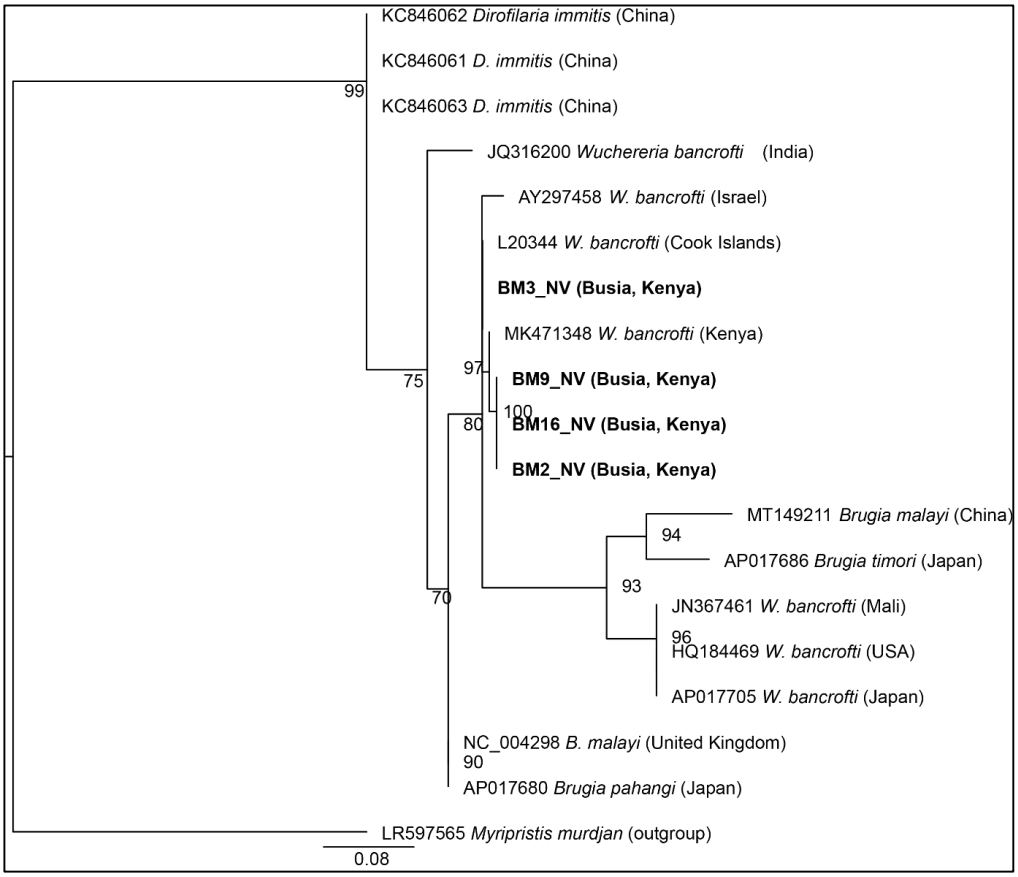



***Figure 4***:
Maximum likelihood phylogenetic tree of *Wuchereria bancrofti* 18S rRNA gene sequences. The information provided above includes GenBank accession numbers, species identifications, and country of origin. Sequences obtained from this study are in bold labelled as BM3, BM2, BM16 and BM9_NV

Of the 92 blood samples tested, again none was positive for *P. falciparum* while two specimens were positive for *W. bancrofti,* representing an infection rate of 2.17%.

The majority of mosquitoes identified belonged to the *An. funestus* complex and comprised 85.76% (247 out of 288), while *An. gambiae* complex mosquitoes comprised 14.24% (41 out of 288). Among the *An. funestus* complex mosquitoes identified, the majority were *An. leesoni* comprising 87.04% (215 out of 247) while 9.72% (24 out of 247) were *An. funestus* s.s. and 3.24% (8 out of 247) were *An. rivulorum* (Figure 2). No *An. parensis* and *An. vaneedeni* were identified. For the *An. gambiae complex,* the majority (36 out of 41, representing 87.80%) were identified as *An. gambiae* s.s. while 12.20% (5 out of 41) were identified as *An. arabiensis*.

The two *W. bancrofti* infections that were detected in the mosquitoes represented an overall infection rate of 0.68% (2 out of 288), with one each in *An. leesoni* 0.46% (1 out of 215, within the species) and *An. funestus* 4.17% (1 out of 24).

Sequence analysis of the 18S rRNA gene of *W. bancrofti* obtained from this study was 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). Phylogenetic analysis based on the sequenced 18S rRNA gene (188 bp) showed that all *W. bancrofti* sequences from this study shared a close relationship with *W. bancrofti* sequences distributed in Kenya, Cook Island, and Israel (Figure 4).

## Discussion

Cases of lymphatic filariasis infections in Busia County have been reported recently but information on the occurrence of coinfections of lymphatic filariasis and malaria in the *Anopheles* vectors as well as in humans is scanty. There are efforts to implement integrated control strategies targeting these two diseases, especially in countries where they are both endemic [34]. To achieve this goal, there is a need to obtain information on the occurrence of co-infections and their prevalence. The present study aimed to generate information to guide the design and implementation of an integrated control of filariasis and malaria in the study area. We found *W. bancrofti* lymphatic filariasis parasite infection rates of 0.68% and 2.17% in mosquitoes and blood samples analysed, respectively, but neither the mosquitoes nor blood samples were positive for *P. falciparum* malaria parasite infection.

According to the findings of the current study *An. funestus* mosquitoes plays an important role in the transmission of *W. bancrofti* in Matayos constituency, Busia County. Previous studies in Tanzania collaborated well with these observations where infection with *W. bancrofti* was detected in all four siblings of *An. funestus* complex [47].

Widespread use of effective vector control interventions, such as insecticide-treated bed nets or indoor residual spraying, may have reduced mosquito populations or interrupted the transmission of malaria parasites in the study area (35). A study by Worrall and his colleagues found that the use of several control methods such as mass campaigns, had resulted in high LLIN ownership and use in Busia County [19] thus increasing the recommended universal coverage. This could have contributed to the lower parasite prevalence and explain the absence of positive findings in our study.

The presence of a higher number of vectors belonging to the *An. funestus* complex recorded in our study area compared to those from the *An. gambiae* complex could have resulted in the low infection rates in the mosquitoes and humans considering that several studies have documented *An. gambiae* as the main vector of malaria and Lymphatic filariasis with *An. funestus* playing a minor role [41, 3]. Other studies have also reported that a reduction in the number of vectors taking their blood meals from humans suppresses the risk of *W. bancrofti* transmission [41, 42].

The absence of co-infection in the vector samples in the current study resonates with the findings in several studies that demonstrated that transmission of such infections of malaria and filarial parasites was not only rare but also seemed to occur more frequently in *An. gambiae;* our study recorded a low number of *An. gambiae* [3]. Other studies that have reported similar findings include a study with samples from the coastal region in Kenya in which only 0.4% of the *An. gambiae* were found to be concomitantly infected with both parasites [43]. The other study from Tanzania reported that among the 15 *An. gambiae* mosquitoes found to be infected with either filaria or malaria, only one of them was found harbouring both parasites [44].

The rare occurrence of concomitant parasite infections in *Anopheles* mosquitoes has been associated with a reduced life span of mosquitoes that pick up both parasites; this results in the mosquitoes dying long before the parasites reach their infective stages, thus reducing the simultaneous transmission of the parasites [2,3, 16]. A study under laboratory conditions in which a large proportion of *B. pahangi* did not develop to the second stage in *Ae. aegypti* mosquitoes infected with *P. gallinaceum* parasites further support this theory [45]. In addition, it has been reported that an enhanced susceptibility of mosquitoes to co-infection does not offer an added advantage to the transmission of either of the parasites [46].

The tree topology from the phylogenetic analysis also corroborates earlier studies, with sequences of *W. bancrofti* from this study branching with other sequences reported from Kenya (MK471348) and Israel among others [48, 49]. The results of 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.

## Study Limitation

Some cases may have gone unnoticed during mass screening due to the disease's asymptomatic nature and slow progression to chronic stages; before the parasite grows to detectable levels, infected people may initially test negative. Furthermore, some patients with chronic illnesses may not have circulating filarial antigens, the worms having died a long time ago and no longer reproducing and the patients may consequently test negative for antigens despite having long-term deformities.

## Conclusion

This study confirms the presence of *W. bancrofti* infection in the human population and the mosquito vectors with no co-infection or malaria infection identified. The study also confirms that the majority of mosquitoes identified belonged to the *An. funestus* complex with *An. leesoni* being the majority.

## Recommendations

Based on the results of this study, widespread screening and treatment for lymphatic filariasis are recommended in Busia County. Additionally, more extensive studies, potentially utilizing a longitudinal sampling design, are suggested to provide further insights into the prevalence and dynamics of the infection. There is also the necessity of prioritizing control and prevention initiatives as well as Morbidity Management and Disability Prevention (MMDP), for the study area.

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**Data Availability.** All data sets are available from the corresponding author on request.

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