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COLLEGE OF HEALTH SCIENCES

ENTOMOLOGICAL ASSESSMENT OF LYMPHATIC FILARIASIS TRANSMISSION IN SELECTED COMMUNITIES IN GHANA

BY:

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THIS THESIS IS SUBMITTED TO THE DEPARTMENT OF MEDICAL MICROBIOLOGY, UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT FOR THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN MEDICAL MICROBIOLOGY.

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DECLARATION

I, Shittu Bunkunmi Dhikrullahi, declare that, with the exception of the references made to other people`s work, which have been duly acknowledged, this dissertation, either in whole or partially, have not been submitted for the award of any degree in any institution.

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DEDICATION

This work is dedicated to the Almighty Allah and my family for their unremitting support during the course of my study.

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LIST OF ABBREVIATIONS

µL	Micro Litre
µM	Micro Molar
ABR	Annual Biting Rate
ADL	Acute Adeno-Lymphangitis
AIBR	Annual Infectivity Biting Rate
ATP	Annual Transmission Potential
BR	Biting Rate
Ct	Cycle threshold
CCT	Counting Chamber Technique
CFA	Circulating Filarial Antigen
DEC	Diethylcarbamizine citrate
dH_2O	Distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EAS	Entomological Assessment survey
ELISA	Enzyme linked Immunosorbent Assay
GFEP	Ghana Filariasis Elimination Programme

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GPELF	Global Programme For the Elimination of Lymphatic Filariasis
gDNA	Genomic DNA
HLC	Human Landing Catches
ICT	Immunochromatographic Test
LDR	Long DNA Repeat
LF	Lymphatic Filariasis
MBR	Monthly Biting Rate
MDA	Mass Drug Administration
MF	Microfilariae
PC	Preventive Chemotherapy
PCR	Polymerase Chain Reaction
PSC	Pyrethrum Spray Collection
RT-PCR	Real Time PCR
SspI	Species Specific Primer
TAS	Transmission Assessment Survey
USAID	United State Agency for International Development
WHA	World Health Assembly
WHO	World Health Organization

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ABSTRACT

Resurgence of lymphatic filariasis (LF) infection after several rounds of Mass Drug Administration (MDA) has been the main reason behind constant monitoring of treatment. The MDA is designed to interrupt transmission by reducing microfilaraemia in a community to a threshold that local vectors may not be able to pick up microfilariae (MF) during a blood meal. The current study has been carried out to determine whether transmission had actually been interrupted following stoppage of MDA in the Anyakpor (Greater Accra region), Voggu Kpalsogo (Northern Region) and New Bakanta (Western region) communities, which had undergone several rounds of MDA treatment. Similar work was also done in Dwease in the Ashanti region, a site located within a non-endemic area to determine whether transmission is still absent there or not. This entomological assessment was done by collecting mosquitoes using the Pyrethrum spray collection (PSC), Human landing catches (HLC), and CDC light trap (LT) methods. In all, a total of 700 mosquitoes were collected with 251 (35.9%), 99 (14.1%), 97 (13.9%) and 253 (36.1%) obtained from Anyakpor, Dwease, Voggu Kpalsogo and New Bakanta respectively. The mosquitoes were dissected under a dissecting microscope and larval stages of *Wuchereria bancrofti* detected with the aid of a compound microscope. Following molecular identification of different species of mosquitoes, 10 mosquitoes of the same species were pooled together in a tube. A total of 74 pools were constituted, conventional polymerase chain reaction (PCR) and Real time PCR (RT-PCR) were performed on them for the detection of the parasite larvae. Microscopy revealed no larval stages of *W. bancrofti*, but 1 pool each from Anyakpor and New Bakanta communities revealed positives using the conventional PCR method. Using the RT-PCR method 17 (22.9%) of the pools were found to be positive. Of these 17 positive pools, 13 (76.5%), 3 (17.7%) and 1 (5.9%) belonged to the Anyakpor, Voggu Kpalsogo and New Bakanta communities respectively. The mosquito species in the positive pools consisted of *An. gambiae ss*, *An. coluzzii*

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and *An. melas,* all of which belong to the *An. gambiae* complex. In conclusion, this study has detected *An. gambiae ss, An. coluzzii* and *An. melas* and has revealed that active transmission of LF by them is ongoing in the Anyakpor, Voggu Kpalsogo and New Bakanta communities. The study also confirms the absence of transmission in Dwease notwithstanding the prevalence of *An. gambiae ss* and *An. coluzzii* there.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Lymphatic filariasis (LF) commonly referred to as elephantiasis is a neglected tropical disease. Infection which is usually acquired in childhood occurs when filarial parasites are transmitted to humans by female mosquitoes. The vectors are mosquitoes of the genera, *Aedes*, *Anopheles*, *Culex*, and *Mansonia* species (Koroma *et al.*, 2012; WHO, 2015). *Coquilletida* and *Ochlerotatus* species have also been reported as carriers of LF parasites (de Souza *et al*., 2010). Over 1 billion people in 55 countries are living in areas where preventive chemotherapy is required to stop the spread of the infection and the disease is known to infect over 120 million people worldwide (WHO, 2016). Elephantiasis, apart from being the leading cause of permanent and long term disability, appears to be the second most common cause of acute and chronic disability worldwide (Zeldenryk *et al.*, 2011). The adult worms live in the lymphatic system and obstruct the flow of lymph, causing local inflammation of the lymphatic vessels. The disease is characterized by abnormal swelling of the arms, breast (females), male and female genitalia, and legs (WHO, 2010). The disease is caused by infection with parasitic nematode (roundworm) of the Super family: *Filarioidea* and family *Filariidae*. Among the filarial worms involved in transmission, *Wuchereria bancrofti* is responsible for 90% of the LF cases in addition to *Brugia malayi* and *Brugia timori* that are responsible for the rest of the cases. The adult female mosquitoes become infected with microfilariae (MF) when they bite to ingest the blood of an infected human host. The MF mature into infective larvae within the mosquito. When infected mosquitoes bite people, mature parasite larvae are deposited on the skin from where they migrate into the body then to the lymphatic vessels for further development to adult worms.

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Several decades of research have identified LF as a disease that can be eliminated owing to three reasons. Firstly, it is a disease that exclusively affects humans. Secondly, the MF are unable to multiply in the vector and finally, the mechanism of transmission is relatively inefficient (Ottesen, 2000).

Recognizing the potentials of eliminating the disease, the World Health Assembly passed a resolution (WHA50.29) in 1997 which requested member states to initiate activities to eliminate LF. This led to the formation of the Global Programme to Eliminate Lymphatic Filariasis (GPELF), launched by The World Health Organization (WHO) in 2000 with the goal to eliminate the disease as a public health problem by 2020 (Ottesen, 2000; WHO, 2010). The programme has two principal objectives in achieving the goal. The first is to interrupt the transmission of LF through chemotherapy to all populations at risk and the secondly is to manage morbidity and prevent disability among individuals affected by the disease (WHO, 2010).

For GPELF to be successful, the ability of the various mosquito vectors to pick the MF (especially when the community MF load is low), and support the development of the ingested MF to the infective larva stage (L3), and to transmit those L3s to humans has to be understood.

1.2 Rationale of the study

The Ghana Filariasis Elimination Programme (GFEP) has completed at least six rounds of mass drug administration (MDA) which have seen the programme upscale from an initial 5 districts to 61 at the last round of distribution. Annual treatment coverage at the district level ranges from 61.8% to 88.8% and at the national level, 70% to 75% (GFEP, 2008). Despite this achievement, LF is prevalent in 61 out of 138 districts in 9 regions of Ghana. The antigen prevalence of LF is between 20% to 40% in the north and 10% to 20% in the south. The prevalence of hydrocoele

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varies from 0-35% while the prevalence of elephantiasis is from 0-4% with more females being affected than males (GFEP, 2008). There has been 5,000 recorded cases of elephantiasis and 10,000 cases of hydrocoele in the country. The incidence of the acute attacks is about 95.9 per 1000 patients with lymphoedema per annum (GFEP, 2008). About 90% of these attacks occur in people with existing lymphoedema with 3 days of total incapacitation and its attendant economic consequences. Occurrence of this condition is highest in the rainy season when most people are most productive on their farms (GFEP, 2008). Evidence exists that the widespread use of insecticide treated bednets may have an additional effect on the reduction of LF transmission through vector control (Bockarie *et al.*, 2009). Although a significant progress in initiating MDA programmes in endemic countries has been made, emerging challenges to this approach have raised questions regarding the effectiveness of using MDA alone, to eliminate LF, without the inclusion of supplementary vector control to reduce exposure to mosquitoes (Bockarie *et al.*, 2009), and morbidity management to alleviate suffering and prevent disability of those affected by the disease (WHO, 2006; 2016). According to Burkot (2006), with adequate sustained treatment coverage, MDA should meet the criteria for the elimination of LF. However, it may be difficult to sustain sufficiently high mass drug administration coverage to achieve lymphatic filariasis elimination in areas where *Aedes* species are the vectors. Despite the intervention of MDA, LF is still endemic in some parts of Ghana. Since vector control was effective in controlling and even eliminating LF transmission in some endemic areas, integration of vector control with MDA will ensure sustainability of transmission suppression, thereby better ensure the success of national filariasis elimination programmes (Burkot *et al.*, 2006). There is therefore the need to determine whether there is a break in LF transmission in areas where there is MDA treatment using entomological assessment survey (EAS) to complement transmission assessment survey (TAS). Data from EAS in addition to those of TAS will inform the WHO to take decisions as to when

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MDA should be discontinued and/or when an endemic area should be declared free of LF transmission.

1.3 Aim of the study

The aim of the study is to assess the transmission of LF in selected communities in Ghana.

1.4 Specific objectives

The specific objectives are to:

1. identify and characterize the mosquitoes caught in the study areas using microscopy and molecular methods.
2. recover and characterize *W. bancrofti* parasites from female mosquitoes caught.
3. monitor LF transmission in areas where MDA has been discontinued owing to the assertion that transmission has been interrupted.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Distribution of Lymphatic filariasis

Lymphatic filariasis, ranked as the second most common vector-borne parasitic disease after malaria, is found in over 80 tropical and subtropical countries (Wynd *et al.*, 2009). Approximately 80% of people infected with LF are living in the following 10 countries: Bangladesh, Democratic Republic of Congo, Ethiopia, India, Indonesia, Myanmar, Nigeria, Nepal, Philippines and the United Republic of Tanzania (WHO, 2015). One third of these infected persons live in India and another one-third in Africa (with prevalence rates exceeding 10% in 17 of 34 endemic countries). In Africa, the prevalence of lymphatic filariasis is especially striking, affecting over 40 million people in the sub-Saharan region alone. Most of the remainder occurs in Asia, the Pacific and the Americas. *Wuchereria bancrofti* causes ninety percent of these infections and most of the remainder by *Brugia malayi* (WHO, 2016). In Ghana, lymphatic filariasis is endemic mostly in the rural areas. In most urban and semi-urban areas, the major vectors for *W. bancrofti* are *Culex* mosquitoes. *Anopheles* species is the major vector in rural areas of Africa and elsewhere, while *Aedes* mosquitoes serves as the vectors in many of the endemic Pacific Islands. (Gbakima *et al*., 2005; Simonsen and Mwakitalu, 2013). The disease is prevalent in 61 out of 128 districts, in 9 out of 10 regions in Ghana.

2.2 Disease manifestation and symptoms

The filarial parasite transmitted by the female mosquitoes to a susceptible host migrate into the lymphatic system and live in the lymph nodes. In the lymph nodes the adult filarial parasite can cause **As the filarial parasites live in the lymph nodes, they can** cause damage and blockage of

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the lymph channels thereby preventing proper circulation of lymph in the body. The accumulation of lymph leads to the chronic manifestation of LF in the extremities of the body, a condition referred to as elephantiasis of the upper and lower limbs, which affects about 4.6 million people in Africa (Michael *et al.,* 1996). Some manifestation of LF begins with acute adenolymphagitis (ADL) which are often characterized by constitutional signs and symptoms such as fever, general malaise, pain, tenderness and swelling (WHO, 2000).

Other chronic disease manifestations of LF includes elephantiasis, lymphoedema, hydrocoele, genito-urinary lesions, chyluria, chylous ascites, adenopathy, microhaematuria and macrohaematuria (Dreyer *et al*., 1999). The WHO and its partners have established strategies and activities both for managing lymphoedema through community home-based care, and for increasing access to surgery for hydrocoele (Lee *et al*., 2011).

Additionally, researchers, Melrose and Goldsmid (2005) also explained that, in other instances, there is the passing of chyluria (a milky-coloured urine), which is caused by leakage of lymph into the urinary tract. These analysts further explained that, this observation is seen from time to time after the consumption of a high-fat diet and after vigorous physical exercises.

2.3 Diagnosis of lymphatic filariasis

Definitive diagnosis of lymphatic filariasis requires direct demonstration of the MF, since the adults worms are difficult to detect. Several diagnostic tools exist for doing this. The commonest method employed depends on the detection of the MF in stained blood films. Blood samples are usually collected between 10:00 p.m. and 4:00 a.m. when the MF are detectable (because of their exhibition of nocturnal periodicity). Other diagnostic tools are available and are used for the detection of circulating filarial antigens, anti-filarial antibodies and parasite genomic DNA in

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blood samples and in the mosquito. These tools are also being employed in various endemic regions for LF control programmes.

2.3.1 Giemsa-stained thick smear

The method is used to identify and quantify the number of MF in blood samples. This method involves the preparation of a thick blood film from either capillary or venous blood following dehaemoglobinization of red blood cells with clean tap water. The film is dried and fixed in methanol and stained in 10% Giemsa solution for about 10 minutes (The stain can be diluted and the time for staining increased). The film is washed in tap water and allowed to dry thoroughly. The film is examined under the ordinary light microscope for the detection of the MF. McMahon *et al.* (1979) identified two limitations of this method; loss of MF during the dehaemoglobinization step and low sensitivity due to small volume of blood sample used.

2.3.2 Counting Chamber Technique (CCT)

This method involves the use of a counting chamber which is 1 ml deep over an area of 50 X 20 mm and holds 100 cubic ml of liquid. This diagnostic technique also detects MF directly in the peripheral blood. One hundred (100) microliters of the venous blood or capillary blood is collected and transferred into a tube containing 900 µl of 3% acetic acid (Simonsen *et al.*, 2014). The base of the counting chamber is divided into 1 mm squares. A cover slip traps liquid to the correct depth of the chamber is placed on it. A known volume of capillary blood washed into 3% acetic acid is poured onto the chamber. By observing the liquid through a low magnification of the ordinary light microscope, objects contained in each cubic mm can be identified and counted.

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2.3.3 Knott's concentration method

This method is very sensitive in detecting MF (Knott, 1939). In this method, 1 ml of venous blood collected in a tube containing anticoagulant is mixed with 10 ml of 2% formalin. The mixture is left for, at least, 15 minutes before centrifugation. The supernatant is discarded for the remaining sediment to be examined for the detection of MF under the ordinary light microscope.

2.3.4 Immunodiagnostic tests

Filarial nematodes are known to release antigenic products and elicit strong humoral antibody responses in human hosts (Ottesen, 1982). The immunochromatographic test (ICT) and the Og4C3 enzyme linked immunosorbent assay (ELISA) are the two commercially available techniques that detect circulating filarial antigen (CFA). These diagnostic tests have some advantages in their employment; they are highly sensitive and specific and they can be employed during the day time (Weil *et al.*, 1997). However, according to Lammie *et al.* (1988), these antibody detection assays also have their drawbacks in that antibody responses appear to be persistent even after clinically defined cure has been achieved and thus are unable to discriminate between past and present infections.

2.3.5 DNA detection assays

DNA-based technology is now used for the diagnosis of filarial infections both in humans and in the mosquito vectors. The identification of a *W. bancrofti* repeated DNA sequence (Zhong *et al.*, 1996) has facilitated the design of a PCR based assay capable of detecting *W. bancrofti* genomic DNA in human blood (Zhong *et al*., 1996) and in the mosquito vectors (Chanteau, 1994). The PCR is an enzyme-catalysed biochemical reaction in which small quantities of specific DNA segments are amplified into large quantities, using oligonucleotide primers, and DNA polymerase (Mullis, 1990). Species-specific (SspI) DNA and the long DNA repeat (LDR) probes developed so far

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detect DNA sequences that are highly repeated in the filarial genome. According to WHO (1992), these probes are theoretically sensitive enough to detect DNA from a single filarial larva.

2.3.6 Ultrasonography

Adult worms can be visualized in the lymphatic system using ultrasonography. Amaral *et al* (1994) used this method to describe adult worms performing "filaria dance" in the lymphatics.

2.3.7 Millipore (Nuclepore) Technique

This technique involves the use of a Millipore filter which consists of a base (holder), a cap and a gasket. A Millipore membrane of pore size of 3-5 µm is put on the holder, the gasket placed on it and the base covered with the cap. About a half milliliter anticoagulated blood sample is dehaemoglobinised with 1% detergent such as teepol. The dehaemoglobonised blood sample is expressed through the filter to filter out MF. After filtration, the filter is uncapped and the membrane is removed with a pair of forceps. The membrane is placed face up on a filter paper to dry. It is then examined under the ordinary light microscope to detect and enumerate MF. The membrane can also be stained in Giemsa solution before it is examined under the microscope (Wamae and Njenga, 2008).

2.4 Transmission of Lymphatic filariasis

In 1877, Patrick Manson in India incriminated mosquitoes as vectors of *W. bancrofti*. This was the first time that an insect was associated with the active transmission of an agent of any animal disease (Service, 1978). In Ghana, various species of the *Anopheles* mosquitoes have been identified as important vectors of LF. For example, *An. melas* and *An. funestus* are important in the Coastal Western Region (Dunyo *et al.,* 1996). *An. gambiae* s.s. has been reported to be the most important vector followed by *An. funestus* and *An. pharoensis* in the central region

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(Dzodzomenyo *et al.*, 1999; Aboagye-Antwi *et al.*, 2015), whiles in the Upper East region *An. arabiensis* was found to be the major vector (Appawu *et al.*, 2001). Even though certain strains of *B. malayi* can also infect feline and monkey species, humans are the exclusive host for *W. bancrofti*. The life cycle in humans and in these animals remain epidemiologically distinct that show very little overlap (WHO, 2000). Vector control for LF is particularly attractive because transmission of the parasite is inefficient. There is no multiplication of the parasite in the mosquito vector and only continuous exposure to bites of many infected mosquitoes maintains the infection in humans (Bockarie *et al.*, 2009).

The biological features of the vector-parasite relationship should be understood in order to define the entomological variables critical to LF transmission and the threshold for interrupting transmission. Unlike the transmission of malaria and arboviruses, a large number of bites from infectious mosquitoes is required to initiate a new infection with microfilaremia (de Meillon *et al.*, 1968).

An endemic community is said to have a low MF density when the density of circulating MF is less than 200 MF per ml of blood, an amount which cannot be detected in a significant number of instances when commonly used blood sampling techniques are employed (Southgate, 1992). By this definition, a high MF density was observed in the work done by Aboagye-Antwi *et al.*, (2015) in the Hwida, Dago, and Mampong communities where monitoring was carried out. This was due to occupational activities farming and fishing that exposed the inhabitants to the bites of the anopheline mosquitoes. Studies on the relationship between MF density in blood meals and the percentage of *Anopheles mosquitoes* that ingest MF have not provided consistent results (Boakye *et al.*, 2004; Bryan *et al.*, 1990; Coulibaly *et al.*, 2013; McGreevy *et al.*, 1982). Although many of the MF ingested by *Anopheles* mosquitoes are reportedly damaged by the mosquito`s foregut armature, the proportion of the MF destroyed is dependent of the number of MF ingested and

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varies between members of the *An. gambiae* complex and *An. funestus* (Amuzu *et al.,* 2010; Southgate and Bryan 1992).

In order to interrupt transmission, the intensity of MF or the vector density must be driven below a threshold to ensure that no new infection occurs. Local environmental conditions also affect transmission: rainfall, temperature, humidity and soil type can all affect the production of breeding sites and the survival of adult mosquitoes (GPELF, 2012).

2.5 Vector biology and profile

The features/ habits of the mosquito vectors described below are based on the keys prepared by Gilles and de Mellion (1968).

2.5.1 *Anopheles* mosquitoes

They are identified morphologically by the presence of dark and pale band patterns on the coastal margin on their wings. The femora, tibiae and the 1st tarsal segment is speckled to a variable degree. Speckling may rarely fuse to form short lines.

Anopheles occurs in a wide range of habitats, including puddles, drainage channels, ponds and rice fields with small amounts of surface vegetation. It also occurs in polluted breeding habitats in urban environments. It is nocturnal (i.e. bites at night) in its habit. Some of its species are endophagic (feed indoors) whiles others are exophagic (feed outdoors). Again, some of the species are endophilic (seek shelter indoors) whiles others are also exophilic (rest outdoors) e.g. in rodent burrows, vegetation, cracks and crevices in trees, the ground, under bridges and sometimes caves.

Some species of *Anopheles* are both anthropophilic (feed on humans), and zoophilic (feed on animals) while others are exclusively anthropophilic or zoophilic. They have a flight range of 1000 m to 3000 m.

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2.5.2 *Culex* mosquitoes

Culex are identified by the absence of the dark and pale band pattern on their wings. These mosquitoes are mostly nocturnal, zoophilic and/or anthropophilic and are also endophilic. They have a flight range of less than 200 m. They are known to breed in still water bodies ranging from drainage basins, large water bodies to artificial containers.

Culex quinquefasciatus which is the main vector for LF transmission in Asia is known to breed in polluted waters with organic materials as found in septic tanks, pit latrines, canals, abandoned wells and blocked drains. In urban areas *Culex* is found to breed in places where drainage and sanitation are inadequate.

2.5.3 *Mansonia* mosquitoes

These mosquitoes also lack dark and pale patterns on their wings but they have a pale bands on their legs that differentiate them from *Culex* species. They are commonly found in marshy areas and usually oviposit in masses. The eggs are glued to the lower side of plants and are found floating or hanging near water bodies.

The larvae and pupae depend on their attachment to the underwater parts of floating aquatic weeds and grasses for breathing and therefore they are found in water bodies such as ponds, grassy ditches, irrigation canals and swamps. They often attached to the underwater parts of floating aquatic weeds and grasses.

Mansonia species usually bite at night. They are mostly exophagic and generally exhibit exophilic behavior. They have a flight range of less than 2000 m.

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2.6 Life cycle of *Wuchereria bancrofti*

Patrick Mason in 1878, in China became the first person to work out the life cycle of *W. bancrofti* in *Culex quinquefasciatus*. The life cycle involves both the human and the insect vector as definitive and intermediate hosts respectively. The female worm is viviparous liberating embryos into the lymphatic system in large numbers. The embryos or MF are enclosed in a membrane (sheath) and are therefore said to be sheathed. They ultimately escape from the lymphatics and appear in the peripheral blood. The mosquito acquires the infection by ingesting the MF with the blood meal. The MF lose their sheath on arrival in the stomach of the mosquito. The larvae migrate into the thoracic muscles within 2 days where they develop into thicker, shorter, "sausage" forms. They then undergo two moults before developing into elongated infective larvae measuring about 1.5 X 0.002 mm over a period of 6-14 days at high temperatures and humidity. Moulting is delayed for 6 weeks at low temperatures. The larvae then migrate to the mouthparts of the mosquito and enter the skin of the definitive host through the puncture wound when a blood meal is taken. The infective larvae enter the lymphatics where they grow into mature male and female worms. After mating, the gravid females release millions of small immature MF which circulate in the blood after 3-6 months following initiation of infection. The worm is estimated to have an active reproductive life span of 4-6 years. The tail of the MF tapers to a delicate point and exhibits no terminal nuclei thus, it can easily be distinguished from the MF of *Brugia malayi* and *Loa loa*. The sheathed MF of *W. bancrofti* which measures 230-275µ in length stains pink with Giemsa.

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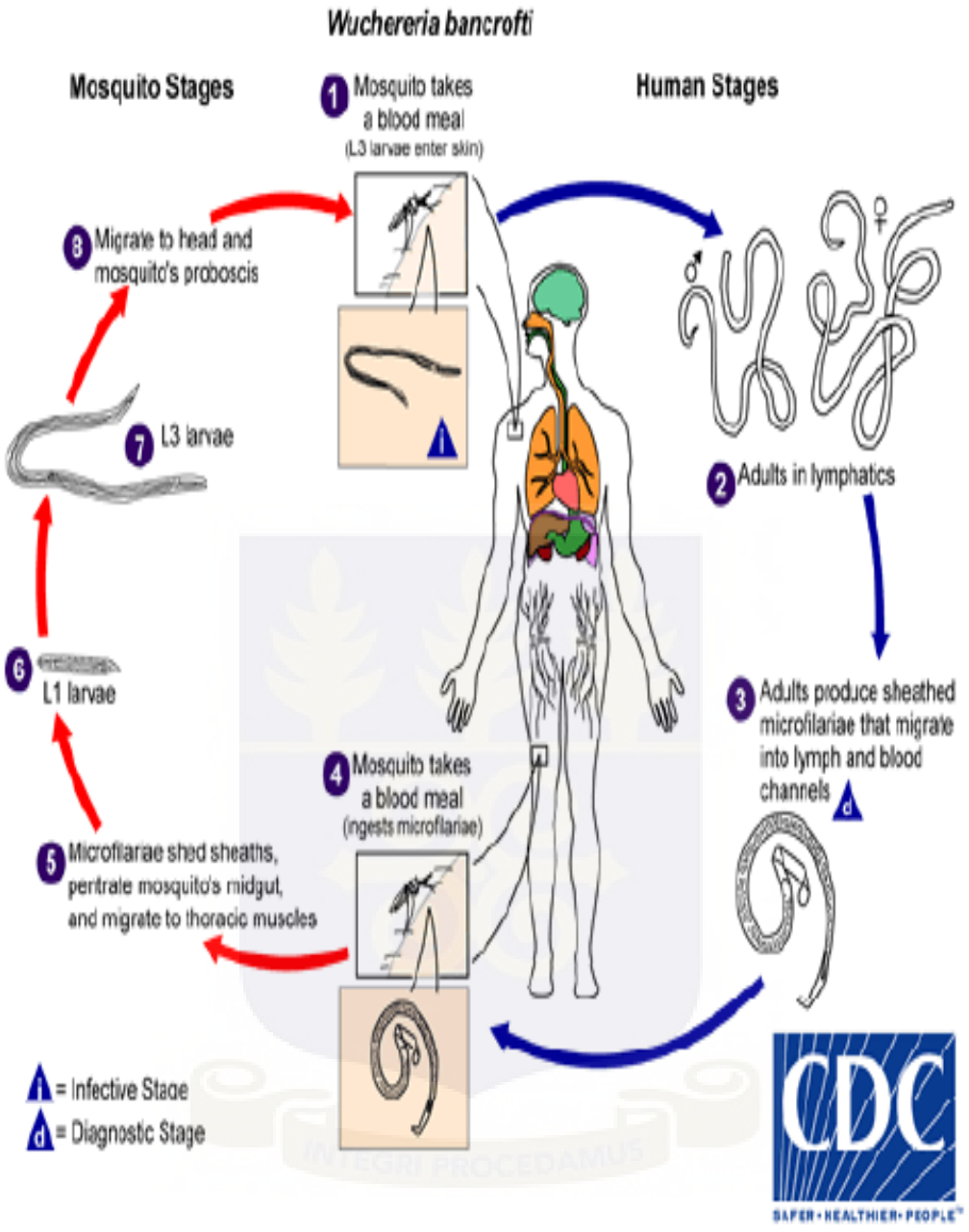

Figure 1: Image showing the Life cycle of *Wuchereria bancrofti* (USAID, 2015; CDC, 2013).

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2.7 Control of lymphatic filariasis

The GPELF was launched in 2000 with the principal objective of breaking the cycles of the transmission of *W. bancrofti* and *Brugia malayi* through the application of annual MDA to entire at-risk populations. The WHO`s strategy is based on 2 key components: stopping transmission through large-scale annual treatment of all infected people in an area or region where infection is present, and alleviating the suffering caused by lymphatic filariasis through increased morbidity management and disability prevention activities (Ottesen *et al*., 1997).

There are strategies that are used in the control of LF. These include vector control, morbidity control and chemotherapy (Ottesen *et al.,* 1997). However, depending on the location or endemic area, controlling LF could be difficult irrespective of which strategy is being adopted (Ottesen *et al.,* 1997). Of all these control strategies, it is documented that vector control, which involves the use of strategies that reduce human vector contact, has traditionally played an important role, since the infection is transmitted through the bite of the mosquito. Prevention of lymphatic filariasis is possible by stopping the spread of the infection. Large-scale treatment involves a single dose of 2 medicines given annually to an entire at-risk population by administering albendazole (400 mg) together with ivermectin at 150-200 mg/kg body weight or with diethylcarbamazine citrate (DEC) at 6 mg/kg body weight. These preventive chemotherapy (PC) medicines have a limited effect on the adult stage of the parasite but they can effectively clear the microfilaria from the blood stream and prevent the spread of the parasite by mosquitoes (Slatko *et al*., 2010). By 2012, 56 countries had started implementing large-scale treatment through MDA. Of these 56 countries, 13 have moved to the post-MDA surveillance phase (WHO, 2012). During the last half century, several countries including Japan, China (De-jian *et al*., 2013), South Korea (Cheng *et al.,* 2009), the Solomon Islands, Egypt (Ramzy *et al*., 2005), and Togo (Sodahlon *et al*., 2013) have successfully

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eliminated LF. Although significant progress in initiating MDA programmes in endemic countries has been made, emerging challenges to this approach have raised questions regarding the effectiveness of using MDA alone, to eliminate LF, without the inclusion of supplementary vector control to reduce exposure to mosquitoes (Bockarie *et al*., 2009), and morbidity management to alleviate suffering and prevent disability of those affected by the disease (WHO, 2012).

2.8 Impact of vector control on Lymphatic filariasis transmission

The treatment of enclosed water bodies with floating layers of expanded polystyrene beads can prevent mosquito breeding for extended periods (Curtis *et al.*, 2002; Maxwell *et al.*, 1990; Minjas, 1984; Reiter, 1978). Layers of polystyrene beads in pit latrines persist if the pit does not flood. In a survey of *Cx. quinquefasciatus* breeding sites conducted in a section of Dar es Salaam, Tanzania, sanitation structures were the most prolific breeding places, totaling 2,324 (Chavasse *et al.*, 1995). When all the enclosed breeding sites were treated with polystyrene beads and checked seven months later, only one site (from which polystyrene had been removed during emptying) contained immature stages of *Cx. quinquefasciatus*. Expanded polystyrene beads are capable of preventing breeding in sanitation structures for at least five years (Curtis *et al.*, 2002), although the periodic emptying of these structures is likely to reduce the effective life of a single treatment. Maxwell and others (1990) applied polystyrene beads to all the wet, *Culex*-infested pit latrines in Makunduuchi, a community of 12,000 people in Zanzibar, Tanzania. This treatment reduced the number of bites per person per year from approximately 25,000 to 440 (a reduction of 98%). Mass treatment of the community with DEC rapidly reduced the MF rate from 49% to 10%, which had the effect of reducing the proportion of L3 infective mosquitoes from 2.4% to 0.4%; in so doing, the number of infective bites per person per year went down by 99.7%. After this single campaign

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of DEC treatment plus sustained vector control, follow-up surveys showed continued decline to an MF rate of 3% after five years (Maxwell *et al.*, 1999).

Evidence that vector control had contributed to this long-term decline was obtained by comparison with another town where DEC was used without vector control. Treatment with DEC without vector control resulted in resurgence of MF three to six years after the drug campaign. Whereas long-term prevention of resurgence of infection could probably have been achieved by annual rounds of drug treatment, the 98% reduction in the biting nuisance achieved by the vector control greatly increased public appreciation of this integrated programme.

2.9 Role of vector control in lymphatic filariasis elimination

The challenges to MDA programmes have led to growing concerns regarding the effectiveness of using MDA alone to eliminate LF without the inclusion of vector control (Burkot *et al*., 2002; 2006). This is especially pertinent given that vector control was once advocated as the primary tool to control filariasis (Sasa, 1976). According to Sunish and others (2007), annual MDA alone have the ability to decrease the filarial infection load in the community if there were no lapses. However, residual microfilaraemia of 0.4% and antigenaemia positivity of 4.6% were observed even after 36 years of filariasis control in French Polynesia (Esterre *et al.*, 2001). Mass drug administration with DEC drug combination was found to be more effective than DEC alone in decreasing filarial infection variables (Rajendran *et al.*, 2004). Vector control was found to be important during any lapse in the MDA programme (Maxwell *et al.*, 1999; Sunish *et al.*, 2002). The importance of vector control methods has been emphasized, as they play a key role in the prevention of disease transmission (Townson *et al.*, 2005). In China, the campaign against LF turned successful when vector control was integrated with other intervention measures, such as DEC (selective and mass treatment, and as fortified salt), resulting in the interruption of filarial

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transmission without any resurgence (Sun *et al.,* 1992). The impact of MDA in combination with vector control has been extensively studied in Brazil (Regis *et al.,* 1996), Zanzibar (Maxwell *et al.,* 1990 and 1999) and India (Reuben *et al.,* 2001). Furthermore, it has been reported that even a lower drug coverage can achieve the set control criteria with the inclusion of a vector control component to MDA, by decreasing the number of years required to attain the target of infection elimination (Michael *et al.,* 1996). The maintenance of low transmission levels for a sufficiently long period to interrupt transmission is a more affordable and sustainable way to eliminate filariasis, especially when communities can be empowered to carry out simple vector control operations along with MDA (Sunish *et al.,* 2007).

2.10 Challenges to lymphatic filariasis control

Apart from technical, logistic and financial challenges, challenges associated with vectors, coverage rates, compliance and initial prevalence levels cannot be ignored. The global elimination of LF as a public health problem has been operationally interpreted as the reduction in the prevalence of infection with *W. bancrofti, B. malayi, or B. timori* in all endemic countries, to target thresholds below which transmission of the infection cannot be sustained. These thresholds were earlier empirically observed to be less than 1.7% MF prevalence for bancroftian filariasis and less than 1.5% MF prevalence for malayan filariasis though current targets for GPELF are considerably more conservative (Ichimori *et al.,* 2014). In bancroftian filariasis where *Aedes* is the primary vector, the target threshold is less than 1%.

Achieving the elimination of LF through MDA alone would be difficult if culicines are involved in the transmission cycle, in that culicines can transmit LF even at microfilaria rate of less than 1.5%. Recent studies in Ghana have shown that *An. melas* and species of *Mansonia* are capable of

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transmitting at low MF levels thus, keeping residual transmission of the disease even more after more than seven rounds of LF MDA (Amuzu *et al.*, 2010; Ughasi *et al.*, 2012).

The national programme in Ghana is based on MDA treatment with ivermectin and albendazole combination administered annually to all endemic villages. Though MDA for LF elimination is comparatively inexpensive in relation to most other public health programmes (Goldman *et al*., 2007), the total cost of drug treatment is relatively high and may affect coverage for treatment (Ramzy *et al.,* 2005). Compliance being far behind the required coverage, non-compliance was mostly due to the fear of side effects, lack of awareness of the benefits of MDAs, and non-attendance to health staff in villages (Hussain *et al.*, 2014). The long intervals between MDAs, and a lower than optimal treatment coverage, including the significance of treatment intervals and compliance must be researched into, in order to optimize efforts to control LF in sub-Saharan Africa (Simonsen *et al.*, 2010). Challenges associated with the quality of data reported such as uncompleted data, poor writing, and lack of confidentiality of data lead to inaccurate data (de Souza *et al.,* 2016). Inaccurate data does not tell the real state of disease and this may retard elimination (de Souza *et al.,* 2016).

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CHAPTER THREE

3.0 METHODOLOGY

3.1 Study sites

Mosquitoes for the study were collected between January and May, 2017 from four (4) study sites. Ethical approval was sought from the Ethics and Protocol Review Committee (EPRC) of the School of Biomedical and Allied Health Sciences (SBAHS) and that of the College of Health Sciences of the University of Ghana. The mosquitoes were collected from Anyakpor in the Ada East District of the Greater Accra Region, Dwease in the Asante Akim District of the Ashanti Region, Voggu Kpalsogo in the Kumbungu District of the Northern Region and New Bakanta in the Ellembele District of the Western Region. The Greater Accra, Northern and Western regions are classified as endemic regions for LF while the Ashanti region is classified as a non-endemic region.

The Ada East district which forms part of the sixteen (16) territorial entities (metropolis/municipalities /districts) in the Greater Accra Region, is located in the Eastern part of the region. It falls between Latitudes 05° 45´ south and 06° 00´ north and between Longitudes 00° 20´ east to 00° 35´ west. In the south lies the Gulf of Guinea, which stretches over 45 kilometres (27.9 miles). Ada Foah**,** the district capital, is located at the south-eastern part, about 20 km off the Accra-Aflao road, along the coast and about 2 km from the Volta River Estuary. Other major settlements are Big Ada, Kasseh, Got, Anyamam, Lolonya, Akplabanya, Wokumagbe and Koluedor. The district shares boundaries with Central Tongu to the North, Gulf of Guinea to the South Tongu to the East, Dangbe to the West. The population of the district according to the 2010 population and housing census stands at 71,671 comprising 34,012 males and 37,659 females.

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The Asante Akim Central Municipality (formerly Asante Akim North Municipality) is one of the 30 Districts in the Ashanti Region. It was carved out of the erstwhile Asante Akim District Council in 1988 as part of the Ghana's Decentralization Process.
It has Konongo as its Capital Town. The Municipality is located in the eastern part of the Ashanti Region and lies between Latitudes 60° 30´ North and 70° 30´ North and Longitudes 00° 15´ West and 10° 20´ West. It covers a land area of 1,160 sq. km.
It shares boundaries with the Sekyere East district on the north, Asante Akim South on the south, Kwahu South on the east, and Ejisu-Juaben on the west. The population of the district according to the 2010 Population and Housing Census stands at 71,508 with 33,942 males 37,566 females.

The Kumbungu district with its capital Kumbungu was carved from the former Tolon-Kumbungu district. It now forms part of the new districts and municipalities created in the year 2012. It was inaugurated at the various locations simultaneous.l.y on 28th June, 2012. The district shares boundaries with the Mamprugo/ Moaduri to the North, Tolon District to the South, Savelugu-Nanton Municipality to the East and North Gonja District to the West. The population of the district according to the 2010 population and housing census stands at 39,341 comprising 19,686 males and 19,655 females.

The Ellembele district being one of the twenty-two (22) districts in the Western Region of Ghana, was established by a Legislative Instrument (L.I) 1918. It is located on the southern part of the region between Longitudes 02º 05´ W and 02º 35´ W and Latitudes 04º 40´ and 05º 20´ N.
The district has Nkroful as its capital and shares boundaries with the Jomoro District to the West, Wassa Amenfi West District to the North, the Nzema East Municipality to the South, the Tarkwa-Nsuaem Municipality to the East and a 70 km stretch of sandy beaches to the south. It covers a total area of about 1,468 Square kilometers, which constitutes about 9.8% of the total land mass

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of the Western Region. The population of the district according to the 2010 population and housing census, stands at 86,501 comprising, 42,317 males and 45,184 females.

3.2 Adult Mosquito Collections

Sixty houses were randomly selected in the four selected communities in Ghana; 14 houses were selected from Anyakpor, 18 from Dwease, 16 from Voggu Kpalsogo and 12 from New Bakanta communities. Three main mosquito sampling methods; Pyrethrum spray collection (PSC), Human landing catches (HLC), and CDC light traps were used to collect the mosquitoes for the work.

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Figure 2. Map of Ghana showing mosquito collection sites (arrows).

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3.2.1 Pyrethrum Spray Collection (PSC)

Indoor resting mosquitoes were collected daily for 16 days from 4 randomly selected houses from the 60 houses employed in this study. This procedure was carried out in the morning between 6:00 am and 7:00 am. Consent of the occupant of the room to be sprayed in a particular house was sought in the evening preceding the collection day.

For each house, information pertaining to each room which includes the number of s.l.eepers in the room was recorded. Prior to the spraying procedure, windows and doors were closed and other exit sites for the mosquitoes were blocked. Also, a cream spreadsheet cloth was laid on the floor. The room was then sprayed with pyrethrum and the door of the room is opened 10-15 min later to collect the mosquitoes which had been knocked down by the insecticide, using an aspirator starting from the entrance door to other parts of the room.

3.2.2 Human Landing Catches (HLC)

This method involved providing a human bait for the mosquitoes and collecting them when they land to bite. The method was carried out between 6:00 pm and 6:00 am.

Prior to the collection of the mosquitoes, the collectors were trained and the risk of the process was explained to them. For each collection, 4 people were made to work in a house. Two of them sat indoors and two outdoors. In all, a total of sixteen collectors were recruited for four collection sites. They exposed their arms and legs and each of them held a torchlight and a test tube.

The torchlight was switched on anytime a bite was felt by the collector and mosquitoes were collected from the body using a test tube whose mouth was applied to the skin. After successfully getting the mosquitoes into the tube, the tube was covered with the thumb. The mosquitoes were then transferred into the paper cup and the time of collection labelled on the cup. With this process, mosquitoes were collected in each hour through a 12 hour cycle with a 10 min break in the hour.

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3.2.3 CDC Light Traps

Mosquito collection using the CDC light traps was carried out in four randomly selected houses in each community. In each house, a miniature CDC light trap with a standard 6 V 100mA incandescent bulb powered by dry cell batteries was hanged from 6:00 pm the previous day to 6:00 am the following day. Mosquito collections were made with 8 traps (4 indoor and 4 outdoor) each night.

All the mosquitoes caught in this and the other methods mentioned above were kept in paper cups in moist chamber and transferred to the laboratory of the Noguchi Memorial Institute for Medical Research for laboratory work to commence

3.3 Morphological identification of specimens

3.3.1 Mosquitoes

In the laboratory, the mosquitoes were killed when a piece of cotton wool was dipped into chloroform and placed on a mesh on the paper cup. The cup was covered with another cup that was empty to increase the effect of the chloroform on the mosquitoes. The cup was left for 30 – 60 sec for the mosquitoes to be effectively killed.

The mosquitoes were transferred into petri dishes and identified morphologically with the aid of a dissecting (Stereo) microscope (Olympus SZ60, Japan) and the keys prepared by Gilles and de Mellion (1968) to separate the mosquitoes according to their various species.

On a well labelled glass slide, the head, thorax and abdomen of each mosquito were separated from each other. Using a Pasteur pippete, a drop of distilled water was delivered onto each segment and the segments were teased out using dissecting pins with the aid of a dissecting microscope.

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The legs were detached from the thorax and placed in 1.5 ml Eppendorf tubes for species identification using molecular methods.

3.3.2 *Wuchereria bancrofti*

The larval stages of the *W. bancrofti* parasite were searched for with the aid of the low power (X10) of the compound microscope after mosquitoes were dissected.

3.4 Deoxyribonucleic acid extraction

Genomic deoxyribonucleic acid (DNA) was extracted from the body and legs of the mosquitoes and used for the detection of *W. bancrofti* and the identification of mosquito species respectively, using the conventional and real time PCR techniques. For the extraction, the direct PCR method, which requires the use of mosquito legs and the extraction of DNA using 10% chelex were employed. DNA samples were extracted from pools of 10 mosquitoes or less where the numbers for a given species were not enough. In a particular tube, only *Anopheles* mosquitoes were pooled together and the same was done for the culicines. In the identified *Anopheles gambiae* complex, *An. coluzzii* were sorted and pooled together. *An. gambiae* s.s. were as well sorted and pooled together for the process.

3.4.1 The direct PCR method

The direct PCR method was used for the molecular identification of *An. gambiae* to confirm the morphological identification. One to two legs detached from each mosquito was placed in a well labelled 1.5 ml Eppendorf tube using a pair of forceps. The detached legs was used as a DNA template for PCR.

3.4.2 DNA Extraction using 10% Chelex

The carcasses of dissected mosquitoes were scrapped off the glass slides and put into pools of 10 or less into the 1.5 ml Eppendorf tube and macerated with sterile pestles after which 200 µl of

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chelex reagent was added into the tube. The pools were then incubated at 55ºC overnight in an oven. The samples were removed and vortexed thoroughly for 30 sec at X2500 followed by centrifugation at 8000 rpm for 1 min. The samples were re-incubated at 95ºC for 30 min after which they were removed and vortexed thoroughly for 30 sec and centrifuged again at 8000 rpm for 2 min. The supernatant of the samples were transferred into new tubes by pipetting. The DNA obtained was used as a template for the PCR reaction.

3.5 Molecular analysis

3.5.1 Identification of *Anopheles gambiae* complex

The direct legs of mosquito was used for species identification. Three sets of oligonucleotide primers were used in PCR for the identification of members of the *Anopheles gambiae* species complex. The primer sequence detail are shown in Table 1.

The PCR mix contained 7.5 µM SYBR Mix (a mixture of AccuStart Taq DNA polymerase, stabilizers, fluorescin, a SYBR Green dye, Buffer $MgCl_2$, and dNTPs), 0.2 µM of each primers, 6.9 µM of dH_20 and direct legs was used as template for the reaction. The amplification was carried out in a thermal cycler. The cycling conditions for the reactions were; initial pre-heating step for 5 min at 94ºC followed by 45 cycles, each consisting of 30s denaturation step at 94ºC, 30s annealing at 50ºC and 40s extension at 72ºC. The final cycle products were extended for 5 min at 72ºC.

After amplification, 10 µl of each of the amplicons were mixed thoroughly with 2 µl gel loading dye and loaded onto wells of 2% agarose gel for electrophoresis. The gel was observed and photographed in a BIO-Doc -lt Imaging System. The primer sequence used shown in Table 1.

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3.5.2 Identification of *Anopheles gambiae* sensu stricto

The PCR products obtained from above was used as template for digestion. The reaction mix contained 1.0 µl Restriction enzyme buffer, 3.5 µl of dH_20, 0.5 µl of Restriction enzyme (HhaI) and 5.0 µl of DNA samples. Incubation was done overnight at 37ºC, deactivation of the enzyme was carried out in a thermal cycler at 65ºC for 20 mins. The digested products were mixed with 2 µl of gel loading dye and run through a 2% agarose gel. The products were observed and photographed in a Bio-Doc-lt Imaging System.

3.6 Molecular identification of *Wuchereria bancrofti*

3.6.1 Conventional PCR

DNA extracts from the mosquito carcasses were used as template for the PCR reaction. In the identification of the *W. bancrofti* parasite, conventional PCR was used to amplify the *Ssp I* repeats for the detection of the parasite given a band size of 90 bp. The PCR-mix contained 0.2 µM each of primers WbLDR1 (5`→ ATT TTG ATC ATC TGG GAA CGT TAA TA-3`) and WbLDR2 (5`→ CGA CTG TCT AATCCA TTC AGA GTG A-3`), 5 µM SYBR Mix (a mixture of AccuStart Taq DNA polymerase, stabilizers, fluorescin, a SYBR Green dye, Buffer $MgCl_2$, and dNTPs), 2.6 µM of ddH_2O, and 2 µl DNA template giving a final volume of 10 µl. The cycling conditions used were 95ºC for 5 min followed by 45 cycles of denaturation of 94ºC for 30 sec, annealing at 55ºC for 30 sec and extension at 72ºC for 42 sec, then a final extension at 72ºC for 5 min.

After DNA amplification, the PCR products were electrophoresed on 2% agarose gel stained in ethidium bromide and run for 1 hr 20 min after loading 10 µl of the PCR product, thoroughly mixed with 2 µl of gel loading dye. The agarose gel was observed and photographed in a Bio-Doc-lt Imaging System.

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3.6.2 Real Time PCR

The "long DNA repeat" of *W. bancrofti* (LDR: GenBank accession number AF093510) was used as a detection target with extracted mosquito genomic DNA (gDNA) templates (Rao *et al.* 2006). The RT-PCR-mix contained 1.0 µM each of primers WbLDR1 and WbLDR2, 25 µM SYBR Mix (a mixture of AccuStart Taq DNA polymerase, stabilizers, fluorescin, a SYBR Green dye, Buffer $MgCl_2$, and dNTPs), 18 µM of ddH_2O, and 5 µl DNA template giving a final volume of 50 µl. the cycling conditions used were 95ºC for 5 min followed by 45 cycles of denaturation of 94ºC for 30 sec, annealing at 55ºC for 30 sec and extension at 72ºC for 42 sec, then a final extension at 72ºC for 5 min.

3.8 Data management and analysis

Data entry and validation on parasitological surveys were done in the Microsoft excel 2013 version. Graphs were drawn using the Microsoft software.

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Table 1. DNA sequence of the oligonucleotide primers used for *An. gambiae* identification

Primers used	Primer sequence 5`→3`	Product size
Universal (UN)	GTGTGCCCCTTCCTCGATGT	468
An. gambiae (GA)	CTGGTTTGGTCGGGCACGTTT	390
An. melas (ME)	TGACCAACCCACTCCCTTGA	464

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CHAPTER FOUR

4.0 RESULTS

4.1 Distribution of mosquitoes

Table 2 shows the number of the mosquitoes species caught from the various study sites. A total number of 700 mosquitoes was caught in the study sites, 35.8% was from Anyakpor, 14.1% from Dwease, 13.9% from Voggu Kpalsogo and 36.2% from New Bakanta. Out of the total number of mosquitoes collected, 87.8% were *Anopheles* complex, 8.6% were *Culex* and 3.6% were *Mansonia.*

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Table 2. Number of mosquitoes species caught from the study sites.

Community	*An. gambiae* s.l.	*Culex* spp	*Mansonia* spp	Total
Anyakpor	236	15	0	251
Dwease	84	15	0	99
Voggu Kpalsogo	82	15	0	97
New Bakanta	213	15	25	253
Total	615	60	25	700

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4.2 Mosquito Dissection

No MF or larval stage of *W. bancrofti* was recovered from the dissected mosquitoes.

4.3 Molecular Identification of *Anopheles gambiae* complex

Table 3 shows the number and percentage of *Anopheles* species identified from the various study sites. A total of 320 *Anopheles* species (80 from each site) were identified. Two hundred and thirty-eight (238) of the mosquitoes were of *An. coluzzii* and *An. gambiae* s.s. both of which were formerly considered as M and S forms respectively and 82 were *An. melas*. Among the *Anopheles* collected from Anyakpor 8.8% were *An. gambiae* s.s., 86.2% were *An. coluzzii*, and 5.0% were *An. melas*. From Dwease 11.2% *An. gambiae* s.s. and 88.8% *An. coluzzii* were collected. At Voggu Kpalsogo, 8.8% *An. gambiae* s.s. and 91.2% *An. coluzzii* were collected, whilst at New Bakanta 2.5% *An. gambiae* s.s. and 97.5% *An. melas* were also collected. Out of the 320 *Anopheles* identified, 7.8% were *An. gambiae* s.s., 66.6% were *An. coluzzii* and 25.6% were *An. melas*. Figure 4 shows the amplified DNA for the restriction of both *An. gambiae* s.s. and *An. coluzzii*. The restriction enzyme HhaI recognizes a restriction site on the 390 bp of *An. gambiae* s.s. at position 367 bp which resulted in two separate bands 367 bp and 23 bp. On the *An. coluzzii* DNA. The HhaI, recognizes two restriction sites at position 257 bp and 110 bp which resulted in three separate bands 257 bp, 110 bp and 23 bp. The 23 bp is insignificant on the gel images because the ladder used is 50 bp. The restriction results for *An. gambiae* s.s. revealed one band at position 367 bp whiles that of *An. coluzzii* showed two bands at position 257 bp and 110 bp.

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Figure 3. Gel image following identification of *An. gambiae* species. Lanes 1, 3, 6-13 represent *An. melas* bands and lanes 2 and 5 represent *An. gambiae* bands.

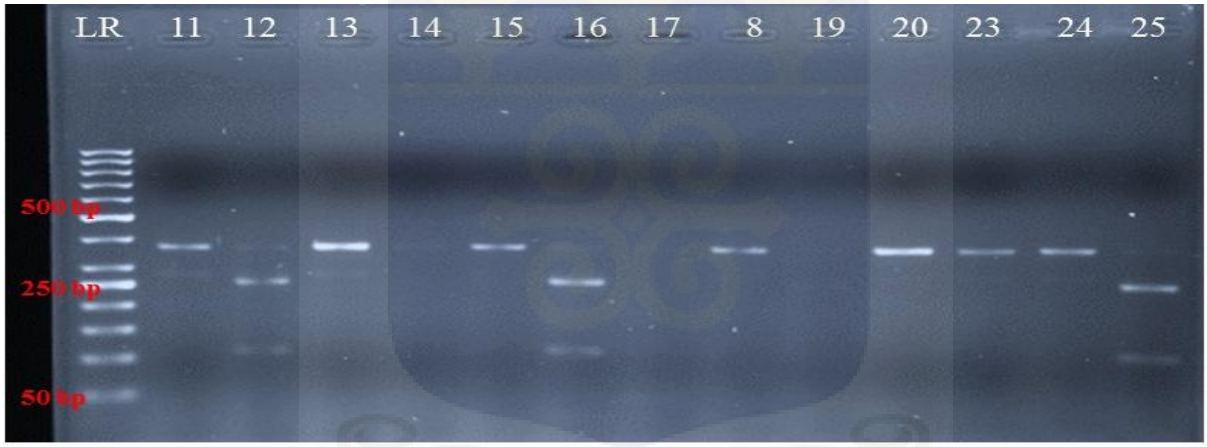

Figure 4. Gel image following restriction of *An. gambiae* s.s. and *An. coluzzii.* Lanes 11, 13-15, 17, 18, 20-23 represent *An. gambiae* s.s. bands and lanes 12, 16, 19, 25 also represent *An. coluzzii* bands.

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Table 3. Number (percentage) of *Anopheles* species identified from the study sites

Community	*An. gambiae* s.s.	(%)	*An. melas*	(%)	*An. coluzzii*	(%)	Total
Anyakpor	7	28	4	4.9	69	32.4	80
Dwease	9	36	0	0	71	33.3	80
Voggu Kpalsogo	7	28	0	0	73	34.3	80
New Bakanta	2	8	78	95.1	0	0	80
Total	25	100	82	100	213	100	320

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4.4 Molecular detection of *Wuchereria bancrofti* using conventional PCR

Table 4 shows the different mosquito species present in a pool according to the various study sites. In all, 74 pools were constituted and two amplifications were detected using conventional PCR. A weak positive was detected in the pools made from the samples obtained from New Bakanta and a distinct positive was detected in the pools from the Anyakpor community. The positive pool belonging to New Bakanta was that of *An. melas* whiles the one belonging to Anyakpor was that of *An. gambiae* s.l. Figures 5 and 6 show the amplified 90 base pair (bp) product which was loaded onto wells of 2% agarose gel for electrophoresis. The gel was observed and photographed in a BIO-Doc -lt Imaging System.

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Table 4 Number of pools (and positive pools) of mosquito from PCR analysis.

Community	No. of positive pools for conventional PCR					No. of positive pools for RT-PCR				
	An. gambiae s.l.	*An. gambiae* s.s.	*An. coluzzii*	*An. melas*	*Mansonia* species	*An. gambiae* s.l.	*An. gambiae* s.s.	*An. coluzzii*	*An. melas*	*Mansonia* species
Anyakpor	15 (1)	1	7	1	0	15 (7)	1	7 (5)	1(1)	0
Dwease	0	1	8	0	0	0	1	8	0	0
Voggu Kpalsogo	0	1	8	0	0	0	1(1)	8(2)	0	0
New Bakanta	12	1	0	8 (1)	1	12	1	0	8(1)	1
Total	27	4	23	9	1	27	4	23	9	1

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Figure 5. Gel image for the detection of *W. bancrofti* DNA. Band showing a weak positive pool for *W. bancrofti* obtained from New Bakanta at lane 10 (arrow). Lanes 1-9, 11 and 12 show no bands.

Figure 6. Gel image for the detection of *W. bancrofti* DNA. Band showing a distinct positive pool for *W. bancrofti* obtained from Anyakpor at lane 11 (arrow). Lanes 1-10 show no band.

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4.5 Molecular detection of *Wuchereria bancrofti* using Real-Time PCR

Table 5 shows the number of pools of mosquitoes used for RT-PCR**.** The RT-PCR revealed 22.9% (17/74) positive pools; of the number positive, 76.5%, 17.7% and 5.9% were from the Anyakpor, Voggu Kpalsogo and New Bakanta communities respectively. Figure 7 represents the amplicons above the threshold line, the curves indicate samples that were positive for *Wuchereria bancrofti* DNA. The lines below the threshold line, were the negative samples.

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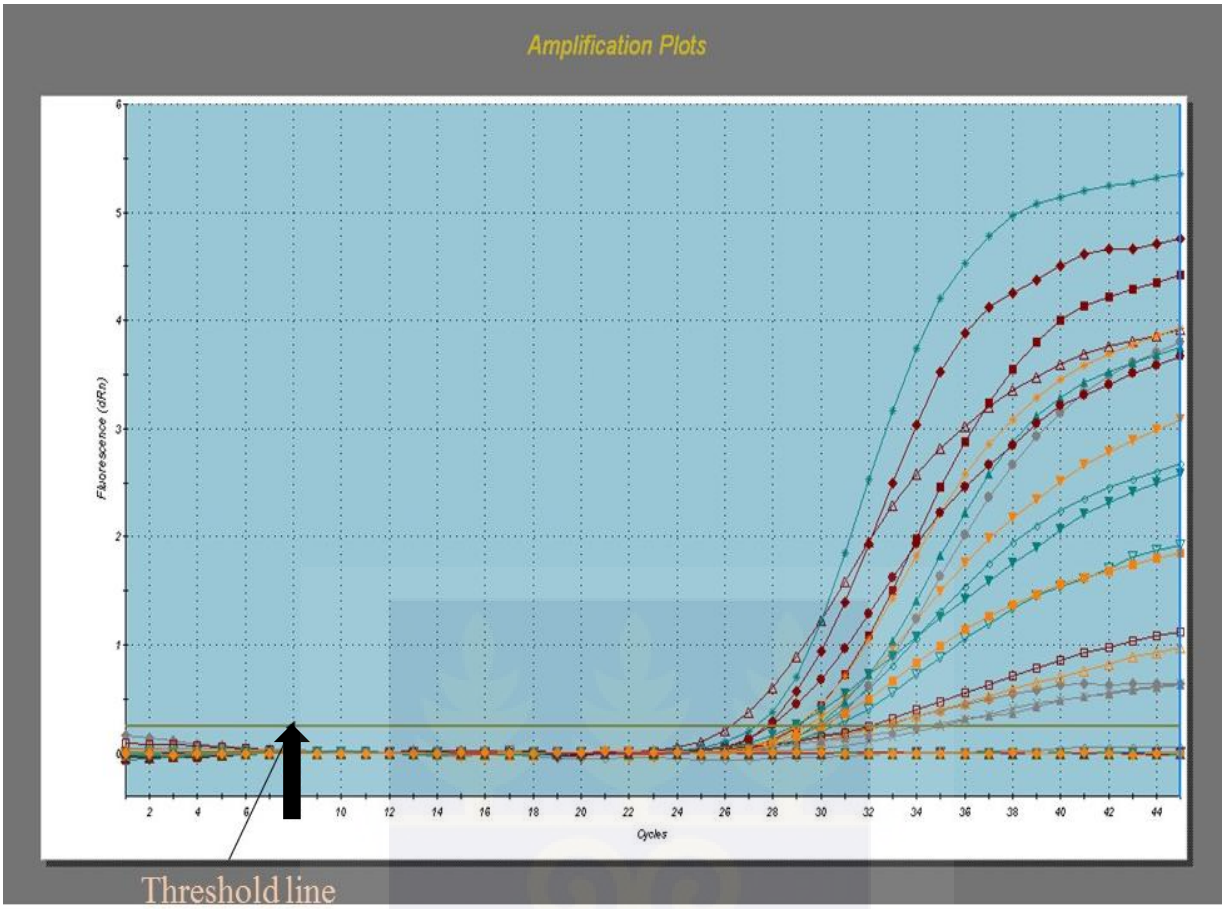

Figure 7. RT-PCR plot for *W. bancrofti* detection. The arrow points to the threshold line. Curves below the threshold line represent samples that were not amplified and those above the line represent samples that amplified.

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CHAPTER FIVE

5.0 DISCUSSION

The understanding of LF transmission dynamics by different species of mosquitoes is essential for the rational planning of control measures and assessment. For filariasis transmission to be interrupted, vector density or MF intensity needs to be lowered below a threshold that ensures that no new infection can occur (WHO, 2012). In Ghana, the last three decades has been filled with a lot of education, morbidity management, mass drug administration (MDA) and transmission studies for lymphatic filariasis (Dunyo *et al.,* 1996; Dzodzomenyo *et al.,* 1999; Gyapong *et al.,* 2000 and 2004; Appawu *et al.*, 2001; Boakye *et al.,* 2004 and 2007; Gbakima *et al.*, 2005; Amuzu *et al.,* 2010; de Souza *et al.,* 2010 and 2012, Ughasi *et al.,* 2010 and 2012). The WHO recommends that before lymphatic filariasis elimination in an area can be fully confirmed, an additional survey should be performed, at least, 5 years after stoppage of MDA (WHO, 2012). A successful transmission assessment survey in a particular evaluation unit leads to the stoppage of MDA there, but this does not necessarily mean that elimination of LF has been achieved in the evaluation unit. Even after MDA has stopped, the LF elimination programme should continue for a further period of at least four years. Post MDA activities are supposed to take place in each evaluation unit as individual units stop MDA and then at a country level, once the entire country has stopped MDA. Despite the absence of specific entomological data from many countries, available bibliography suggests that *Anopheles gambiae* s.l. and *An. funestus* are the major vectors of *W. bancrofti* throughout Sub-Saharan-Africa and the Indian Ocean Islands (Brengues, 1975; Boakye *et al.,* 2004; Simonsen *et al.,* 2008). The same vectors are involved in the transmission of malaria which offers a good opportunity for integrated vector control (Boakye *et al.,* 2009; Ashton *et al.,* 2011). The distribution, ecological and biological characteristics of the genera of a vector differ widely. Within a genus, differences in biting and feeding behavior, resting and breeding preferences,

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seasonal abundance and affinity to human habitations determine their transmission potential. Even though, vectors are most abundant in the rainy season, they are mostly less infective. However, in the dry season when their numbers are low, transmission is higher owing to the fact that at this time, they are more infective (Dogara *et al.,* 2015; James, 2015).

In general, thatched houses seem to harbor more mosquitoes than houses built with concrete with aluminium roofs and occupational activities such as farming and fishing expose inhabitants to the bites of Anopheline vectors (Dunyo *et al.,* 1996; Appawu *et al.,* 2001). Both Anyakpor and New Bakanta are fishing communities, which are sited close to water bodies. These water bodies support the development of mosquito larvae. In these communities many of the houses are covered with thatch. At the time of the mosquito collection the weather at Voggu Kpalsogo was very dry and all the water detainment sites during rainfall were all dried out hence, the low numbers of mosquitoes collected from this particular site. Dwease was observed to have a good drainage system which allows the flow of water into a small stream. Houses were built of concrete with aluminium roofs and most of the inhabitants slept under mosquito bed nets. The environment was tidy and locating mosquito breeding sites was very difficult, which probably explains the low numbers of mosquitoes collected from this site also.

Anopheles gambiae, *Mansonia* and *Culex* species which were sampled for this study have been incriminated as LF vectors in various part of Africa (Dzodzomenyo *et al.,* 1999; Onapa *et al.,* 2001; Anosike *et al.,* 2005; Dogara *et al.,* 2015). The mosquitoes that made up the positive pools from both New Bakanta and Anyakpor were *An. melas* and *An. gambiae* and this is in agreement with findings from previous studies that suggested that *An. gambiae* was more involved in the transmission of LF in Ghana (Dzodzomenyo *et al.,* 1999; Ughasi *et al.,* 2012). *Anopheles gambiae* is known to be efficient in transmission only when parasitaemia levels are high. Since this species

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of mosquito has been found to harbour MF in the Anyakpor community, it gives an indication that the parasitaemia level is high in the inhabitants of that community.

Effective monitoring of the LF programme requires tools that are sensitive and specific to facilitate decision on stopping MDA. For the purpose of LF elimination, the sensitivity of a test should increase at the expense of specificity. A case missed due to inadequate test could lead to the continuation of the transmission cycle (Owusu *et al.*, 2015). The RT-PCR employed in this study revealed more positive results compared to the conventional PCR and this confirms the findings from previous studies that suggested that RT-PCR is more sensitive when monitoring LF transmission after MDA (Nyarko, 2013; Owusu *et al.*, 2015; GPELF, 2015; Rao *et al.*, 2006). Pools of *An. melas* which are known to transmit LF at low parasitaemia (Duerr *et al.*, 2005), in addition to *An. coluzzii* and *An. gambiae* were all found to be positive with the RT-PCR. This suggests active transmission ongoing in the Anyakpor and New Bakanta communities. The *Mansonia* mosquitoes which were obtained in this study were obtained from New Bakanta. Since samples from this area were found to be positive for *An. melas*, the area should be monitored closely as *Mansonia*, according to Ughasi *et al.* (2010), could also transmit LF at low microfilaraemia levels.

CONCLUSION AND RECOMMENDATION

Conclusion: This study has detected the presence of *An. gambiae* s.s. *An. coluzzii* and *An. melas* and has revealed that active transmission of LF by these mosquitoes is ongoing in the Anyakpor, Voggu Kpalsogo and New Bakanta communities. The study also confirms the absence of transmission in Dwease notwithstanding the prevalence of *An. gambiae* s.s. and *An. coluzzii* there.

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Recommendation: Following the detection of *An. melas* in the New Bakanta community, monitoring of LF transmission should be considered seriously since *Mansonia* spp and *An. melas* are known to transmit at low parasitemia. Highly sensitive test should be used in monitoring LF transmission and vector control should be adopted to complement the MDA treatment that has been carried out or still being carried out in some endemic areas. This study should be extended to other endemic areas within the country. Monitoring in these areas would allow programme managers to know the species composition of the vector in the various study sites and the phenomenon of transmission that is being adopted by these vectors.

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APPENDIX

Transmission Indices

The transmission indices include biting rates, infection rates, infectivity rate, worm load, infective biting rate, annual infective biting rate, transmission potential and annual transmission potential. These indices help in determining the transmission status of an endemic area.

Biting Rate

The biting rate refers to the estimated number of vector mosquitoes coming to bite one person exposed during the sampling night. Biting rate (BR) may be expressed as the number of mosquitoes caught or collected divided by the product of the number of persons s.l.eeping in the room during the sampling and the catch nights. To estimate the monthly biting rate (MBR) or annual biting rate (ABR), the man biting rate is either multiplied by 30 or 365 respectively.

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Table 5: Biting rate estimation

Community	No. of mosquitoes	No. of sleepers	No. of Catch Nights	BR	MBR	ABR
Anyakpor	235	240	4	0.25	7.65	91.25
Dwease	123	115	4	0.27	8.37	98.55
Voggu Kpalsogo	94	63	4	0.37	11.47	135.05
New Bakanta	248	96	4	0.65	20.15	237.25