

**UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES**

VERIFICATION OF THE ABSENCE OF *Wuchereria bancrofti*
TRANSMISSION AFTER TEN YEARS OF ALBENDAZOLE AND
IVERMECTIN ADMINISTRATION IN FOUR DISTRICTS, GHANA

BY

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THIS THESIS SUBMITTED TO UNIVERSITY OF GHANA, LEGON IN
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD
OF **PhD PUBLIC HEALTH DEGREE.**

DEPARTMENT OF EPIDEMIOLOGY AND DISEASE CONTROL

MARCH 2017

DECLARATION

I hereby declare that this submission is my own work towards the PhD and that, to the best of my knowledge includes nothing which is the outcome of work done in collaboration except where specifically indicated in text. This work has not been accepted for the award of any other degree.

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ABSTRACT

Lymphatic filariasis (LF) is one of the seven neglected tropical diseases (NTDs) identified by the World Health Organization (WHO) as controllable and potentially eliminable with effective interventions. It is a vector borne parasitic infection transmitted by mosquitoes which belong to the *Anopheles*, *Culex*, *Aedes*, *Mansonia*, *Coquillettidia* and *Ochlerotatus* genera. The nematode parasites responsible for causing the disease are *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. These parasites have no animal reservoir and are largely found in rural areas even though they can be found in urban areas. Mass drug administration (MDA) is the approved strategy for elimination of the disease. Between 4 and 6 rounds of yearly MDA with at least 65% treatment coverage has been found to be enough to interrupt transmission. Transmission Assessment Surveys (TAS) is the primary tool recommended for deciding when to stop MDA and for post-MDA surveillance in LF elimination programs. In Ghana, four districts, Awutu Senya, Effutu, Agona East and West have stopped MDA after ten rounds with microfilaria and antigen prevalence below 1% and 2% respectively. Surveillance is ongoing in these areas to ensure the detection of early recrudescence. These areas have passed the pre-TAS based on the WHO criteria for an area to pass pre-TAS or otherwise and therefore the need to conduct TAS to monitor for recrudescence in both humans and vectors of the disease. The aim of this study was to conduct post-MDA surveillance in four endemic areas that have stopped MDA to detect early recrudescence using periodic surveys. Three surveys were conducted; school-based, household and xenomonitoring surveys. Approximately 1,600 school children aged 6-10 years from selected schools and 1,000 community members aged 11-60 years were sampled annually for four years to

participate in the longitudinal school-based and household surveys respectively. Daytime finger-prick blood samples were collected from all consenting participants and tested using a rapid card test (ICT) and Enzyme Linked Immunosorbent Assays (OG4C3 and Wb123). Night time blood was collected for blood smear from participants who tested positive for ICT. Mosquitoes were captured from households using the Pyrethrum Spray Catch (PSC) method for the xenomonitoring survey. All mosquito species were pooled by community with an average pool size of fifteen mosquitoes. Loop Mediated Isothermal Amplification (LAMP) and Polymerase Chain Reaction (PCR) assays were conducted to detect *Wuchereria bancrofti* parasites in the mosquitoes. Results obtained show that microfilaria prevalence in humans remained below the 2% threshold required for post-MDA surveillance to be conducted and was fairly stable over the study period [2010=0.07%, 2012=0%, 2013=0.12%, 2014= 0.12%] among 6-10-year-old children sampled and tested as well as the older population [2010=1.1%, 2012=1.0%, 2013=0.17%, 2014=0.60%]. Mosquitoes collected in 2013 and 2014 numbered 7,268. This consisted of 4628 *Anopheles gambiae s.l.*, 146 *An. funestus*, 5 *An. pharoensis*, 2430 *Culex* spp. and 59 *Mansonia* spp. Six (0.08%) pools (five *An. gambiae* spp. and one *Culex* spp.) tested positive for *W. bancrofti*. Surveys in both humans and mosquitoes revealed very low antigen, antibody and microfilaria prevalence four years after last MDA. Mosquito biting rates were low (2013 = 0.057; 2014 = 0.058) but infection rates were high; 0.97 (CI: 0.17-3.17) in 2013 and 1.3 (CI: 0.36-3.65) in 2014 per 1,000 mosquitoes for *Anopheles gambiae* complex, while that for *Culex* species was 0.86 (CI: 0.05-4.15) in 2013 per 1,000 mosquitoes. These results depicted that mosquitoes harbored parasites making recrudescence possible. However, results from this study have

shown that four years post-MDA is not enough to detect recrudescence and therefore the earliest occurrence of recrudescence goes beyond four years post-MDA. Continued surveillance is therefore recommended.



DEDICATION

This work is dedicated to my mother, Miss Sophia Bonney and my brother Mr. Kwasi Offei-Owusu for believing in me and supporting me throughout the period of this study.



ACKNOWLEDGEMENT

I am very grateful to my supervisors Dr. Francis Anto, Professor John Gyapong and Professor Moses Bockarie for their guidance, advice and patience throughout this study.

I am also grateful to Professors Michael Wilson, Daniel Boakye and Maxwell Appawu for all the advice and guidance. I am also grateful to Drs. Dziejdom de Souza and Jewelna E.B Akorli for all the advice in data capture, analyses and taking time off their busy schedules to read and correct this thesis, and contribute substantially to the end-product. I thank Mr. Charles Quaye for all his help in the analyses of this data and Mrs. Yvonne Ashong and Dr. Paul Owusu Donkor for all the encouragement and support.

I would also like to acknowledge the immense contributions of the members of staff of the Parasitology Department of NMIMR, particularly Mr. Sampson Otoo for his immense help in field sample collections, Mr. Charles Narh and Miss Danielle Ladzekpo for teaching me how to conduct the LAMP assay, Miss Frances McCarthy for her immense support in running the ELISA assays, Mrs. Sarah Ayiku for all the data entry and all my colleagues who helped in diverse ways to make this work successful. My gratitude also goes to all the staff of the Neglected Tropical Disease Control Programme for all their support in sample collection, particularly the programme manager Dr. Nana Biritwum for the advice and provision of the national School Health data, Mr. Samuel Odoom in sample collection and provision of the entire national control programme data needed.

My appreciation goes to the heads and teachers of the various schools for their support and also to the parents who consented for their children to participate in this study. I am also grateful to the heads and people of the various communities and volunteers, whose cooperation made this work possible.

Many thanks go to Professor Kwadwo Ansah Koram, the immediate past director of NMIMR, for the opportunity offered me to work at the institute and encouraging me to pursue fulltime PhD studies. I am also grateful to the immediate past head of the Epidemiology department, NMIMR, Dr. Collins Ahorlu, for agreeing to allow me to pursue a full time PhD.

I would also like to express my gratitude to all loved ones, for their encouragement, support, patience and prayers.

Most of all, I thank GOD for His grace, mercy, favour, strength and good health to successfully complete this study.

This work was funded by with a grant from the Bill and Melinda Gates Foundation through the Task Force for Global Health and a grant (Grant Number: G1001337) from the UK Medical Research Council and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement, through the Liverpool School of Tropical Medicine and managed by the School of Public Health



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LIST OF ABBREVIATIONS

ABR	Annual Biting Rate
ADLA	Adenolymphangiodenitis
ALB	Albendazole
ATP	Annual Transmission Potential
BM14	<i>Brugia malayi</i> -14 antibody
BR	Biting Rate
CBSV	Community-based Surveillance Volunteer
CBV	Community- based Volunteer
CDC	Centre for Disease Control and Prevention
CHPS	Community-based Health Planning Services
CFA	Circulating Filarial Antigen
DALYs	Disability Adjusted Life Years
DDT	Dichlorodiphenyltrichloroethane
DEC	Diethylcarbamazine
DNA	Deoxyribonucleic Acid
DOLF	Death to Onchocerciasis and Lymphatic Filariasis
DRC	Democratic Republic of Congo
DOT	Directly Observed Treatment
EA	Enumeration Area
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
EMIS	Education Management Information System

EU	Evaluation Unit
GAELF	Global Alliance for the Elimination of Lymphatic Filariasis
GPELF	Global Programme for the Elimination of Lymphatic Filariasis
GSS	Ghana Statistical Service
HAT	Human African Trypanosomiasis
H ₂ O ₂	Hydrogen Peroxide
HRPO	Horse Radish Peroxidase
ICT	Immunochromatographic Test
IHC	In-house Control
IR	Infection Rate
IRS	Indoor Residual Spraying
IU	Implementation Unit
ITN	Insecticide Treated Net
IVM	Ivermectin
LAMP	Loop-mediated Isothermal Amplification
LF	Lymphatic Filariasis
LLIN	Long Lasting Insecticide Net
L ₃	Third Stage Larvae
MBR	Monthly Biting Rate
MDA	Mass Drug Administration
MF	Microfilaria
MIR	Minimum Infection Rate
MX	Molecular Xenomonitoring

NMIMR	Noguchi Memorial Institute for Medical Research
NPV	Negative Predictive Value
NTD	Neglected Tropical Disease
NTDCP	Neglected Tropical Diseases Control Programme
Og4C3	<i>Onchocerca gibsoni</i> Antigen
PA	Public Announcement
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
PSC	Pyrethrum Spray Catches
PTA	Parent Teacher Association
RAGFIL	Rapid Geographical Assessment of Bancroftian Filariasis
RAND	Random
RBM	Roll Back Malaria
RNA	Ribose Nucleic Acid
SHEP	School Health Education Programme
SPSS	Statistical Package for the Social Sciences
TAS	Transmission Assessment Survey
TBA	Traditional Birth Attendant
TDR	Special Programme for Research and Training in Tropical Diseases
TMB	3,3',5,5'-Tetramethylbenzidine
USA	United States of America
Wb123	<i>Wuchereria bancrofti</i> Specific Antigen

WHA World Health Assembly
WHO World Health Organization



CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Neglected tropical diseases (NTDs) are a medically diverse group of chronic parasitic, bacterial and viral diseases, and related conditions that represent the most common illnesses of the world's poorest people (WHO, 2010a). These afflict more than 2.7 billion of the world's population who live on less than US\$2 per day and threaten the lives of millions more. Africa carries about 500 million of the world's burden of these diseases (Hotez et al., 2007). Until the early 2000s, NTDs were largely ignored in the global health space with more than one billion people suffering from one or more NTD (WHO, 2006a).

Recent developments in science and research have profoundly changed the prospects of controlling these diseases. These new developments have also enabled people left behind on the socioeconomic ladder due to affliction by these NTDs to catch up (WHO, 2010a). Instead of waiting for these diseases to disappear as countries develop and living conditions improve, a deliberate effort is being made by all stakeholders to make them disappear. Efforts being made in this regard are alleviating poverty which can spur socioeconomic development (WHO, 2010a).

Currently, 17 NTDs of public health importance have been targeted for control, elimination or eradication. However, seven of these 17 NTDs attract the most attention because of their high prevalence and amenability to control worldwide. These are soil-

transmitted helminth infections, namely, hookworm, ascariasis, and trichuriasis, lymphatic filariasis, schistosomiasis, trachoma and onchocerciasis (Hotez et al., 2007).

Lymphatic filariasis (LF) the subject of this study is an ancient parasitic debilitating disease caused by the filarial parasites, *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. Ninety percent of all lymphatic filariasis infections are caused by *Wuchereria bancrofti*. Humans are the exclusive hosts of infection. *Brugia malayi* causes most of the remaining 10% of infections in humans. The disease adversely affects production and socioeconomic development in endemic countries (WHO, 2002a).

The two main clinical manifestations of the disease are elephantiasis which is characterised by swollen limbs (swollen legs and/or arms) and hydrocele characterised by an enlarged scrotum. Transmission of the parasite to humans is through the bite of an infected mosquito. Mosquito species which belong to the *Culex pipens* complex, *Anopheles*, *Aedes*, *Mansonia*, *Coquilletidia*, *Ochlerotatus* and *Downsiomyia* genera are implicated in the transmission of lymphatic filariasis (Ramalingam et al., 1968; Amuzu et al., 2010; Manguin et al., 2010; de Souza et al., 2012).

Mosquitoes belonging to the genus *Culex* particularly *quinquefasciatus* are responsible for transmission of LF in most urban and peri-urban areas of the world. *Anopheles* mosquitoes are the vectors in rural areas especially those of sub-Saharan Africa while *Aedes* are also the vectors found in southeast Asia and the islands of the Pacific. (Manguin & Boëte, 2011; Simonsen & Mwakitalu, 2013). Mosquitoes of the genus *Mansonia* are mainly responsible for transmission of *Brugia malayi* parasites but in some

areas anophelines have been found to also transmit the disease (Cholewiński et al., 2015). Brugian parasites are mainly found in northern and eastern Asia mainly, India, Indonesia, Malaysia and the Philippines (WHO, 2016a).

Approximately 1.34 billion people in 73 countries are at risk of infection of LF. Symptoms include acute inflammatory episodes, progressive chronic diseases of lymphoedema, chyluria and hydrocoele (Dissanayake, 2001). Lymphatic filariasis causes physiological and psychological pains as well as loss of productivity. The disease is mainly found in the tropics and affects the poor communities in developing countries. It contributes directly to household poverty since there is loss of productivity after clinical signs set in (Durrheim et al., 2004; Jones, 2014).

The Global Programme for the Elimination of Lymphatic Filariasis (GPELF) was launched in the year 2000 after the fifth World Health Assembly (WHA) meeting resolved to eliminate lymphatic filariasis as a global public health problem by the year 2020 (Mathieu et al., 2011; De-jian et al., 2013). The strategy for elimination is single dose of albendazole (ALB) and ivermectin (IVM) or diethylcarbamazine (DEC) to endemic populations and populations at risk of the disease for a period of between 4 and 6 years until adult worms have reached the end of their reproductive lifespan which is between 6 and 8 years (Turner, 1977; Aboagye-Antwi et al., 2015). Coverage of 65% or more is estimated to be good enough to achieve elimination after 4-6 rounds of MDA. In areas where coverage is low, the WHO recommends that MDA should be extended until transmission is interrupted (WHO, 2011a).

The postulation for the elimination strategy developed by the WHO is that the reservoir of microfilaria in the human host should be reduced to a threshold where *Wuchereria bancrofti* is no longer transmissible by vectors (Aboagye-Antwi et al., 2015) The threshold recommended is antigenemia prevalence of less than 1% using the immunochromatographic card test (ICT).



1.2 Epidemiology

Lymphatic filariasis (LF) is the second most common vector borne parasitic disease after malaria (Wynd et al., 2007). It is one of the 17 neglected tropical diseases (NTDs) with distinct characteristics that thrive mainly among poor populations. The disease was identified as eradicable by the Centres for Disease Control and Prevention (CDC), Atlanta in 1993 and since then it has since been targeted for elimination. Elimination efforts have been on-going using the World Health Organization (WHO) recommended strategy of preventive chemotherapy and transmission control (Ottesen, 2008; Koroma et al., 2013; Ramaiah & Vanamail, 2013).

The strategy of preventive chemotherapy is mainly by annual mass administration of albendazole and ivermectin/diethylcarbamazine for a period of four to six years. This is to cause a reduction in the reservoir of blood microfilaria (mf) below a threshold necessary for continuing transmission by local mosquito vectors (Ottesen, 2000; Molyneux & Zagaria, 2002). Mass drug administration (MDA) coverage has expanded from treatment of three million people in 12 countries since its inception in the year 2000 to more than 450 million in 53 countries in the year 2010 (WHO, 2010b, 2011a).

Lymphatic filariasis (LF) is prevalent in the tropical and subtropical regions of Africa, Asia, Western Pacific and parts of Central and South America (Figure 1). Currently, the disease is endemic in 73 countries with India, Indonesia, Nigeria and Bangladesh accounting for nearly 70% of cases (Ramaiah & Ottesen, 2014). Nine of these countries namely Burundi, Cape Verde, Mauritius, Rwanda, Seychelles, Costa Rica, Surinam, Trinidad and Tobago and Solomon Islands have however been recently reclassified due

to a natural decline in transmission intensity in areas where endemicity is low (Koroma et al., 2013; WHO, 2015).

Other regions of endemicity include Central Africa, the Nile Delta, Pakistan, Sri Lanka, Burma, Thailand, Malaysia, Southern China, the Pacific Islands, Haiti, the Dominican Republic, Guyana, French Guiana, and Brazil (John & William, 2006). In 1996, it was estimated that 119 million people were infected with LF with 43 million having clinical manifestations. Due to on-going efforts of the GPELF, the global prevalence of 3.55% has fallen to 1.47%; signifying a decrease of 59% in LF infections globally. Global microfilaria (mf) prevalence is currently 0.79%, hydrocele 0.42%, and lymphedema 0.36%. Africa carries about 32% of the global endemic population with an overall prevalence of 5.51% (Ramaiah & Ottesen, 2014).

In Africa, LF is endemic in 39 out of the 53 countries on the continent with Nigeria being the most endemic country. The mean prevalence of the disease in Nigeria is 23% and ranges from 4%-62% as per local government area (Richards et al., 2011). Spatial analysis to map the distribution of LF in four West African countries showed that in some communities, more than 70% of the adult population was infected with lymphatic filariasis (Gyapong et al., 2002). In Burkina Faso, prevalence ranged between 30% and 50% while most of Togo, southern Benin and Ghana were said to be free of infection when mapping for LF prevalence was done in West Africa (Gyapong et al., 2002).

In Ghana, mapping of LF showed that the disease was prevalent in the northern and southern sectors with prevalence of 30% and 15% respectively (Figure 2) (Dunyo et al.,

1996; Gyapong et al., 1996; Gyapong & Remme, 2001) . Some communities on the coast of Ghana had prevalence ranging between 10% - 30% but this prevalence did not extend into the coastal regions of Togo and Benin (Gyapong et al., 2002).



1.3 Clinical Presentation, Diagnosis and Management of Lymphatic Filariasis

1.3.1 Clinical presentation

Lymphatic filariasis (LF) infection is usually acquired early in childhood for people who live in endemic areas. The disease however, may take years to manifest. Most infections are asymptomatic and many people never have outward clinical manifestations. Asymptomatic infection is characterized by the presence of thousands of microfilariae circulating in the blood, the presence of adult worms in the lymphatic system and hidden damage to the renal and lymphatic system (Ottesen et al., 1990).

Clinical manifestations can be classified into 4 phases: asymptomatic microfilaraemic, symptomatic microfilaraemic, acute, and chronic phase. In patients who manifest clinical symptoms, the illness starts as “filarial fevers”. These occur as episodes of fever with chills and also inflammation of the lymph glands particularly in the groin. They occur at least 6 to 10 times a year and last for about three to five days after which it subsides on its own (Dreyer et al., 2000). Repeated episodes of these filarial fevers cause damage to the lymph glands, swollen extremities and visible changes in the appearance of the skin.

Hardening of the skin causes the skin to resemble that of an elephant, thus the name elephantiasis (Burri et al., 1996). Hydrocele, an enlargement of the scrotum, is found only with *Wuchereria bancrofti* infections. It is the most common clinical manifestation of lymphatic filariasis. It is not common in childhood but is seen more frequently post-puberty and with a progressive increase in prevalence with age. In many endemic communities, between 40% and 60% of all adult males have hydrocele (Wijers, 1977; Estambale et al., 1994). Lymphedema, which is a condition of localized fluid retention

and tissue swelling caused by a compromised lymphatic system, which normally returns interstitial fluid to the thoracic duct, then the bloodstream is also another clinical presentation of LF. Chronic lymphoedema is often accompanied by acute episodes of local inflammation involving skin, lymph nodes and lymphatic vessels. Some of these episodes are caused by the body's immune response to the filarial parasite while most are a result of secondary bacterial skin infection where normal defences have been partially lost due to underlying lymphatic damage. These acute attacks are debilitating, may last for weeks (<http://www.who.int/mediacentre/factsheets/fs102/en/>).

1.3.2 Diagnosis

Diagnosis of LF has undergone changes over the years. Diagnosis is important in deciding where to undertake MDA, where to stop MDA and when to stop MDA. It is used in defining targets and endpoints for MDA and monitoring endemic populations for resurgence of transmission after MDA has been stopped (Weil & Ramzy, 2006). The success of MDA is dependent on the interruption of transmission of the parasite by reducing the prevalence of microfilariae circulating in the blood of persons living in endemic areas (Ottesen et al., 1997). This can only be determined with diagnostic tools that can detect low levels of microfilariae, to be able to decide whether MDA can be stopped or not.

Not too long ago, diagnosis of filariasis was limited to clinical diagnosis, detection of microfilariae and detection of antibodies to crude native antigen preparations (Weil, 2005). These tools were good but were inadequate in answering the basic questions that are crucial to LF elimination programmes. Significant advances in diagnosis have

however been made over the years and more sensitive and specific tools have been developed which help in detection of LF infection at very low levels of prevalence. These new tools are especially important in areas where MDA activities have stopped and post-MDA surveillance activities are being undertaken.

Successful MDA campaigns have brought several countries near the point of LF elimination. Current WHO Guidelines for determining national LF elimination programme endpoints and for establishing post-MDA surveillance utilize diagnostic tools for verification. Making a choice of diagnostic tools for monitoring and evaluating national elimination programmes is dependent on the sensitivity and specificity of the tools in assessing potential on-going transmission of parasites that cause LF; as well as their feasibility in terms of being fairly rapid, relatively inexpensive to perform on a large number of samples either at a point of care or in centralized laboratory and requiring minimal technical skills in its use (Gass et al., 2012; Steel et al., 2013).

The tools now available and frequently used to assess the impact of MDA according to the WHO guidelines are:

1. Blood film microscopy to detect the presence of microfilaraemia;
2. Immunochromatographic Card Test (ICT) to detect circulating antigen to *Wuchereria bancrofti*
3. Laboratory-based antigen Enzyme Linked Immunosorbent Assay (ELISA)
Og4C3: *Onchocerca gibsoni* filarial antigen based detection test
Bm14 and Wb123: filarial antibody based detection tests to detect antibodies of *Brugia malayi* and *Wuchereria bancrofti*.

4. Polymerase chain reaction (PCR) techniques to detect parasite DNA in humans and mosquitoes (WHO, 2011a).

As programmes reduce the prevalence of diseases through mass drug distribution, more sensitive techniques are required to define endpoints. For lymphatic filariasis in particular where the programmatic goal is elimination, sensitive surveillance tools are required particularly in those areas where such risks of recrudescence are high.

1.3.3 Management

The secondary goal of the Global Programme for the Elimination of Lymphatic Filariasis (GPELF) is alleviation of the suffering caused by lymphatic filariasis. This is because in areas where transmission has been interrupted, the adult worms may still continue to induce lymphatic pathology and consequently morbidity, thus causing suffering in infected persons (Ottesen et al., 1997; Ottesen, 2000). Three filariasis related conditions are addressed by this objective; acute inflammatory episodes, lymphedema and hydrocele (Addiss & Brady, 2007).

Management of lymphoedema is a rigorous regime which includes skin hygiene and self-help measures such as limb elevation, exercise, application of topical antibiotics and antifungals. These methods are employed because there is enough evidence to show that bacteria and fungus are the cause of acute inflammatory episodes known as acute adenolymphangiodenitis (ADLA) (Suma et al., 2002; Addiss & Brady, 2007). These simple management procedures have been proven to result in dramatic reduction in

ADLA attacks and reduced inflammation of the lymphs, thus improving quality of life of infected individuals (Chandrasena et al., 2004; Adhikari et al., 2015).

MDA to some extent has been documented to impact filarial morbidity especially in the initial stages of development of the symptoms. Reductions in acute inflammatory episodes, lymphoedema and hydrocoele have been reported (Bockarie et al., 2009). Anti-wolbachial treatment therapy has been shown to reduce standard hygiene treatment of lymphoedema, hydrocele and lymphatic vessel pathology (Addiss & Mackenzie, 2004; Bockarie et al., 2009).



1.4 Problem Statement

In 1997, the World Health Assembly (WHA) passed Resolution 50.29 calling on member states to take advantage of current developments in the understanding of lymphatic filariasis and the new opportunities for its elimination by developing national plans. The resolution also called for monitoring and evaluation of programme activities, in order to implement simple, affordable, acceptable and sustainable activities based on community-wide treatment strategies (WHO, 1999; Ichimori et al., 2014).

The principal strategy to achieve elimination was to deliver single-dose, two-drug treatment once yearly to all individuals in populations at risk of LF infection through MDA for an estimated 4-6 years so that transmission of infection will be reduced to levels below which there is no risk of recurrence even after the yearly MDAs have been stopped. This is because continued annual mass drug administration (MDA) reduces the density of microfilaria circulating in the blood of infected persons.

This leads to a reduction in the prevalence of infection to such low levels that transmission is no longer sustained and therefore new infections do not occur. According to the WHO "Stop MDA" criteria, a reduction of infection below the 1% target threshold level implies that MDA is no longer required. What then is required is surveillance to ensure the detection and treatment of any cases of early recrudescence (WHO, 2011a).

For effective detection of recrudescence, highly sensitive and specific tools are needed to detect infection in humans and the presence of microfilaria in vectors. The selection of the best diagnostic tool for the detection of early recrudescence is important and requires

consideration of each tool's accuracy, technical requirements, programmatic feasibility and reliability, as well as confidence in their performance (Gass et al., 2012).

Transmission Assessment Survey (TAS) is the approved methodology for post-MDA surveillance. The purpose of TAS is to provide a standard methodology to assess whether continued annual MDA has reduced the prevalence of infection to levels equal to or below the critical cut-off threshold, to decide when MDA should be stopped and when to conduct post-MDA surveillance (WHO, 2011b). For every lymphatic filariasis elimination programme, TAS which has three main objectives should be a standard component of monitoring and evaluation.

The first objective of TAS is the provision of a simple but yet robust survey design for documenting that the prevalence of LF among children born during and after the start of MDA is below a predetermined threshold; the second is the provision of evidence for programme managers on when MDA can be stopped and the third is the provision of assurance to governments that national programmes have achieved the set elimination goals (WHO, 2012b).

Humans are the most appropriate means of detecting recrudescence in a treated population but the decline of infection in humans poses a challenge. Molecular xenomonitoring provides an indirect assessment of infection. This serves as an appropriate means of post-MDA surveillance in areas where humans are not willing participants. Conduct of post-MDA surveillance in both humans and vectors helps

estimate how early recrudescence is likely to occur in a treated population after stopping MDA.

Mass Drug Administration (MDA), using albendazole and ivermectin in Ghana began in the year 2001 in five endemic districts including the Awutu-Effutu-Senya district in the Central Region. MDA has now expanded to all endemic regions with 100% geographic coverage. The Agona East and West districts joined the programme a year later in 2002. After 9-10 rounds of mass drug administration (MDA) in these districts, prevalence has been reduced to below one percent; from 23.8% to 0.6% in Awutu Senya and Effutu and 23.5% to 0.1% in the Agona East and West districts (NTDCP, unpublished).

Based on the WHO criteria for stopping MDA (WHO, 2012), Awutu Senya, Effutu, Agona East and Agona West were eligible to stop MDA in 2010. The purpose of the current study therefore was to conduct post MDA surveillance using the transmission assessment survey (TAS) and xenomonitoring to determine if transmission has ceased in endemic districts in Ghana which have achieved prevalence below 1% and estimate how early recrudescence is likely to occur after stopping MDA. The study also sought to determine the most appropriate tool(s) for post-MDA monitoring and surveillance activities. The ability to detect early recrudescence prevents re-infection, reduces morbidity caused by the disease and helps in achieving the elimination goal.

1.5 Justification

Monitoring and evaluation is necessary because it assesses the impact of MDA and whether the indicators of the impact, namely; microfilaria prevalence in the population, vector infection rate and antigenemia prevalence in children born during the MDA period, have fallen below the recommended threshold. Post-MDA monitoring is also important because it monitors changes in LF infection and transmission to be sure that the fall in infection is sustained, interruption in transmission is complete and if there is the incidence of new infections, it is contained easily to remain below permissible levels in both humans and vectors (Ramaiah & Vanamail, 2013).

Zanzibar was the first country in Africa to complete five rounds of MDA and achieve 100% geographic coverage with effective treatment coverage of over 65% in each round in 2006. However, due to the lack of continuous surveillance, in 2012 when TAS was conducted it was shown that transmission had not been interrupted and microfilaria prevalence levels gone above the 1% required to stop MDA. MDA therefore had to be resumed in 2013 to break the cycle of transmission (Rebollo et al., 2015).

Secondly, a comprehensive study of the tools available and a comparison of their effectiveness in demonstrating the absence of LF transmission will help facilitate the determination of the most appropriate approach for defining the LF elimination programme's endpoint. Elimination of LF will provide a better quality of life for affected populations. This is because the prevalence and intensity of infection of the disease are linked to disability which leads to poverty (Durrheim et al., 2004). Elimination of LF will contribute to achieving the United Nations Sustainable Development Goal 1 which states

that “end poverty in all its forms everywhere”. Specifically target 1.1; “by 2030 eradicate extreme poverty for all people everywhere” and SDG Goal 3 which states that “ensure healthy lives and promote well-being for all at all ages” specifically target 3.8 “achieve universal health coverage including financial risk protection, access to quality essential health care services, and access to safe effective, quality and affordable essential medicines and vaccines for all”.



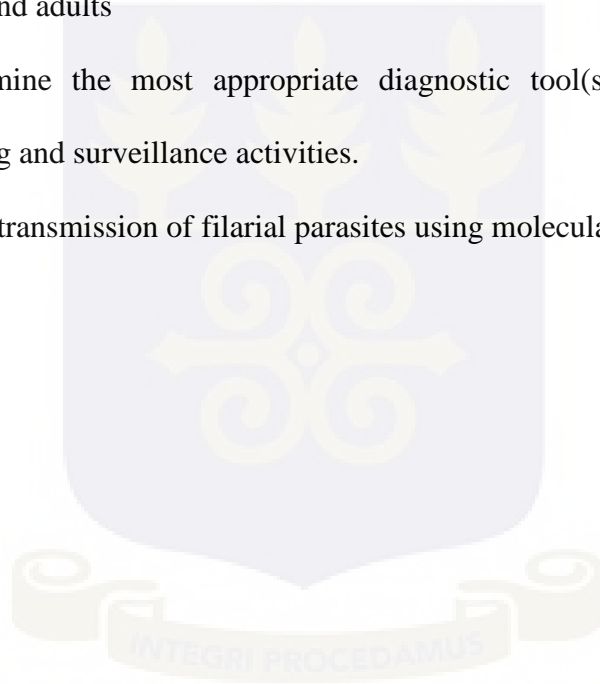
1.6 Objectives

1.6.1 General objective

The general objective was to conduct post-MDA surveillance in four endemic areas where MDA has been stopped to detect early recrudescence in both humans and vectors.

1.6.2 Specific objectives

1. To determine if transmission is on-going after stopping MDA in school-aged children and adults
2. To determine the most appropriate diagnostic tool(s) to use in post-MDA monitoring and surveillance activities.
3. To detect transmission of filarial parasites using molecular xenomonitoring



CHAPTER TWO

LITERATURE REVIEW

2.1 Lymphatic Filariasis – A Neglected Tropical Disease

2.1.1 Background

Neglected Tropical Diseases (NTDs) have afflicted humanity for centuries and have acquired notoriety as chronically disabling and deforming. These diseases are diverse, have distinct characteristics and are mostly found in the low and middle-income countries of Africa, Asia and Latin America (WHO, 2013). So far however, there has been no consensus definition for this group of diseases (Choffnes & Relman, 2011).

These diseases have frustrated the achievement of the then Millennium Development Goals and impede global development outcomes. Currently, there are 17 diseases that are classified as Neglected Tropical Diseases (NTDs) by the WHO in 149 endemic countries. These can be grouped according to the causative organism (Table 1). These 17 diseases affect more than 1.4 billion people living in 149 endemic countries. At least 100 of these endemic countries are endemic for two or more of these diseases and 30 countries are endemic for six or more.

The affected population themselves may be classified as ‘neglected’. This is because they are usually in the lowest socioeconomic status, have little or no access to clean water, do not have proper ways of disposing of waste and live in unsanitary environments (Hotez et al., 2007; WHO, 2013). Seven of these 17 listed diseases have been identified as being controllable and potentially eliminable with effective interventions (WHO, 2010a);

lymphatic filariasis (LF) is one of the seven. Its burden is high in Sub-Saharan Africa, constitutes about 40% of the global disease burden.

Five public health strategies have been recommended for the control and prevention of these NTDs (Table 2). One approach may predominate for the control of a specific disease or group of diseases but available evidence suggests that control is more effective and better results are obtained when all five approaches are combined. For example, in 2008, approximately 670 million people in 75 countries benefitted from preventive chemotherapy even though not everyone was given the full package of medicines (Allen & Parker, 2016).

Table 1: Neglected tropical diseases (NTDs) classified by causative organism

Helminth Infections	Taeniasis/Cysticercosis, Echinococcosis, Dracunculiasis, Food-borne trematodiasis, Lymphatic filariasis, Onchocerciasis, Schistosomiasis, Soil transmitted helminthiasis
Viral Diseases	Dengue fever, Rabies
Protozoan Infections	Chagas disease, Human African Trypanosomiasis, Leishmaniasis
Bacterial Infections	Buruli Ulcer, Leprosy, Trachoma, Yaws

Source: WHO, 2009

Table 2: Strategies recommended for the control and prevention of neglected tropical diseases (NTDs)

Intervention Strategy	Disease
Preventive chemotherapy	Lymphatic filariasis, Onchocerciasis, Schistosomiasis, Soil transmitted helminthiasis, Trachoma, Leprosy, Leishmaniasis
Intensified case management	Buruli Ulcer, Chagas disease, Human African Trypanosomiasis, Visceral Leishmaniasis, Dengue fever, Leprosy, Lymphatic filariasis, Trachoma
Vector Control	Chagas disease, Human African Trypanosomiasis, Lymphatic filariasis, Leishmaniasis, Onchocerciasis, Dengue fever
Provision of safe water, sanitation and hygiene	Trachoma
Veterinary public health	Human African Trypanosomiasis (HAT), Brucellosis, Cysticercosis, Echinococcosis, Foodborne Trematodiasis, Leishmaniasis, Rabies

Source: (Gyapong & Boatin, 2016)

Lymphatic filariasis (LF), also known as elephantiasis is an ancient parasitic disease that was identified as one of the seven potentially eradicable diseases by the Centres for Disease Control and Prevention (CDC) in Atlanta, USA in 1993. The World Health Organization (WHO) in 1997 at its 50th World Health Assembly (WHA) meeting initiated a plan for its elimination as a public health problem by the year 2020. It called on member states to:

1. Take advantage of the recent advances in the understanding of the disease and the new opportunities for its elimination.
2. Develop national plans leading to the elimination of LF, as well as monitoring and evaluation of programme activities.
3. Strengthen local programmes and their integration with the control of other diseases particularly at the community level. This will aid in the implementation of simple, affordable, acceptable and sustainable activities based on community-wide treatment strategies. These activities may be strengthened by vector control activities and improved sanitation.
4. Strengthen capabilities for training, research, laboratory diagnostic, disease management and data management in order to improve clinical, epidemiological and operational activities geared toward the elimination of LF.
5. Mobilise the support of all relevant sectors, affected communities and non-governmental organizations for the elimination of the disease (WHA 50.29)

This led to the formation of the Global Programme for the Elimination of Lymphatic Filariasis (GPELF) (Melrose, 2004). Control activities began globally in the year 2000; however, only 8 out of the 38 endemic countries in Africa had active LF elimination programmes by 2005. Of these, Ghana, Burkina Faso, Togo and Zanzibar were the only countries treating their entire national populations at risk of the disease (Gyapong & Boatin, 2016).

Lymphatic filariasis (LF) is a mosquito transmitted parasitic infection which is caused by nematode species *Wuchereria bancrofti*, *Brugia malayi* or *Brugia timori* (Zagaria & Savioli, 2002). These parasites have no animal reservoir and are largely rural even though currently they can be found in urban areas (Richards et al., 2011). Mosquito species belonging to the genera *Anopheles*, *Culex*, *Aedes*, *Mansonia*, *Coquillettidia* and *Ochlerotatus* are the vectors of the disease. *Anopheles* mosquitoes are the main vectors for LF in West Africa including Ghana (Dunyo et al., 1996; Dzodzomenyo et al., 1999; Appawu et al., 2001) while *Culex* mosquitoes have been found to transmit LF in East Africa (Anosike et al., 2005; Agi & Ebenezer, 2009).

The parasite which causes the disease is transmitted through the bite of an infective mosquito which contains the third stage infective larvae (L₃ larvae). More than a thousand infective bites are needed to facilitate active infection in humans (Stolk, 2005) and infection, even though not fatal is long lasting and insidious. Signs and symptoms of the disease include swelling of the limbs and genitalia. In endemic areas, three groups of persons are normally found:

- a) persons with active infections who can be identified by the presence of circulating filarial antigen and microfilaria but are free of chronic manifestations of the disease
- b) persons who show one or more chronic disease manifestation but do not have demonstrable filarial infection when tested
- c) persons free of both infection and disease (Ravindran et al., 2003).

Lymphatic filariasis (LF) causes major disability worldwide. It affects socioeconomic development and also causes major psychological problems in infected persons (Cox, 2000). The disease is responsible for more Disability Adjusted Life Years (DALYs) than any other infectious disease with the exception of malaria (Zagaria & Savioli, 2002) which is responsible for the largest proportion of DALYs globally. In spite of all its characterizations, LF is considered one of the “tool ready” neglected tropical diseases (NTDs) which can easily be eliminated (WHO, 2008; Zhang et al., 2010). This is because its transmission can be interrupted by administering safe medications orally which have the ability to markedly reduce microfilaraemia levels in the blood. This results in fewer mosquitoes picking up parasites when a blood meal is taken (Molyneux & Zagaria, 2002; Ottesen, 2006; WHO, 2010b, 2011a).

The WHO strategy for the elimination of LF prior to 2012 was primarily chemotherapy. However, after the London Declaration in January 2012, vector control activities have been incorporated into the strategy for elimination (WHO, 2013). Vector control has been shown to be an important component of the filariasis elimination strategy that should not be overlooked (Webber, 1975; Webber, 1977; Webber, 1979). It has been proven to successfully eliminate LF when implemented alone or with MDA.

In the Pacific, vector control was the primary means of controlling LF before antifilarial drugs were made available. Even after the drugs were made available, the Ministries of Health preferred vector control because they considered MDA campaigns labor intensive as compared to vector control activities (Burkott et al., 2002). In Australia, *W. bancrofti* was eliminated through campaigns that controlled the vector, *Culex quinquefasciatus* (Boreham & Marks, 1986).

In Japan as well, vector control played a crucial role in LF elimination (Sasa, 1976). In Zanzibar and India, polystyrene beads have been found to have an impact on *Culex quinquefasciatus* populations in areas where pit latrines or soakage pits are the main breeding sites. This has been found to augment MDA to suppress transmission of *W. bancrofti* in these areas (Maxwell et al., 1990; Maxwell et al., 1999; Curtis et al., 2002).

There are challenges associated with LF elimination efforts and vector control has been shown to address some of these challenges. Some of the challenges associated with LF elimination are the uncertainty about the exact level and duration of microfilarial suppression required for elimination, the mobility of infected persons, consistent non-participation of some infected persons in MDA activities and the possibility of the development of resistance to the antifilarial drugs. The treatment strategies employed in areas that are co-endemic with loasis is also a possible challenge (Burkot et al., 2006). However, treatment for onchocerciasis has been beneficial to LF elimination efforts. Distribution of ivermectin in areas co-endemic for onchocerciasis has helped in reducing microfilaria levels in the affected population (Hoerauf et al., 2011).

To address these challenges, integration of vector control with MDA is necessary. Some of the potential benefits of vector control are firstly, the ability to suppress transmission of filariae without necessarily identifying all “foci of infection”. Secondly, vector control will help minimize the risk of re- infection from imported persons who are microfilaria positive. Thirdly, vector control will help decrease the risk of dengue and/or malaria transmission where *Aedes* or *Anopheles* are lymphatic filariasis vectors (Burkot et al.,2006).

The success of vector integration into the elimination programme depends on the ability to understand the different roles of the different vectors in transmission of the disease (Amuzu et al., 2010; D. K. de Souza et al., 2012). The ability to understand the way filarial parasites are transmitted by mosquitoes is essential when planning control, monitoring and evaluation and surveillance measures.

In Africa, where malaria and LF are co-endemic, indoor residual spraying (IRS) and distribution of long-lasting insecticide treated nets (LLINs) is the main means of controlling malaria. This has had a significant impact on LF transmission because the same vector is responsible for transmission of both diseases (Kelly-Hope et al., 2013). In areas where dengue is co-endemic with LF, *Aedes* mosquitoes are the vectors of transmission and integrated control efforts have been put in place to curb both diseases (WHO, 2013). Integrated vector management has been found to be cost effective and efficient. In areas where LF and malaria co-exist and are transmitted by the same vector,

the massive roll-out of LLINs for malaria control have shown to have an impact on the transmission and control of LF (Stone et al., 2014).

2.1.2 Epidemiology of lymphatic filariasis

Lymphatic filariasis (LF) is endemic in 73 countries. India, Indonesia, Nigeria and Bangladesh account for nearly 70% of cases (John & William, 2006; Koroma et al., 2013). *Wuchereria bancrofti* is responsible for 90% of the cases while *Brugia malayi* and *Brugia timori* cause the remaining 10% of cases. Even though LF is one of the most debilitating conditions recognized globally, it has been largely ignored in policy-making decisions in many affected countries (Perera et al., 2007). One reason for neglecting LF is because generally it is not fatal and is restricted to countries in the tropics and subtropics where the poor population are mainly the ones affected.

The extent of disability caused by the disease is hidden and not recorded in the International Classification of Impairments, Disabilities, and Handicaps. For neglected diseases like LF, morbidity totals about 56 million cumulative disability-adjusted life years (DALYs) which is more than what is estimated for malaria and tuberculosis (Perera et al., 2007). Cases of obvious manifestations of the disease are usually enlargement of the leg or arm, the genitals, vulva and breasts. The psychological and social stigma associated with these physical manifestations is immense (Zeldenryk et al., 2011).

Visible symptoms like lymphoedema and elephantiasis are found in about 41 million people (Durrheim et al., 2004) while about 76 million people have occult infections of LF mostly with microfilariae in their blood. The number with occult infections makes up

about two-thirds of infected persons. They do not manifest any evidence of the disease but when tested, show some degree of parasite-associated immunosuppression. Some may also demonstrate evidence of some level of renal dysfunction (Wynd et al., 2007).

In women, chronic manifestations which affect the genitals are rare and even when they occur, their effects are often hidden. There may also be some manifestations in the breast but that is also rare (Gyapong et al., 2000). In men however, the most profound manifestation is hydrocoele. This is the accumulation of fluid in the tunica vaginalis in the scrotum. The peak incidence of this manifestation occurs in early adulthood when men are physically active and formulating social and sexual identities (Gyapong et al., 2000).

There may also be some form of hidden damage to the internal organs, the lymphatic and renal systems. About 44 million infected patients have recurrent infections and abnormalities of renal function (WHO 2004; Bockarie & Molyneux, 2009). Approximately 10% to 50% of men and up to 10% of women are likely to be affected with the disease in endemic countries and show visible signs of it (WHO, 2002b) but the numbers with the hidden disease is not documented.

Lymphatic filariasis is chronically disabling and the only known reservoir host for the disease is man. Prolonged exposure to multiple infective mosquito bites (about 1,000 infective bites) is required to establish infection in a new human host (Ottesen et al., 2008). Globally, more than 1.39 billion people are at risk of the disease. Approximately 65% of those infected live in the WHO's South-East Asia Region, 30% in the African

Region, and the remaining 5% in other tropical areas. More than 25 million men are afflicted with genital disease and approximately 15 million people with lymphoedema (WHO, 2012).

Tropical and subtropical countries tend to be endemic for LF due to the availability of an optimal habitat for the vectors of the disease. Survival of the infective larva stage of the microfilaria is dependent on the presence of ambient humidity (Smith, 2006). Populations at high risk of contracting LF infection are primarily poor with their habitats normally providing the ambience that the vectors need to survive and thrive.

The disease is often associated with areas with poor sanitation and housing quality (WHO, 2006b; Brady & GAELF, 2014). Majority of the cases of LF are concentrated in rural areas. These communities are also typically built around optimal environments for vectors, these include marshes or rivers. These communities most of the time lack the resources or capabilities to control for vectors and as a result of this high transmission occurs in these areas (Alirol et al., 2011). On the other hand, the disease could serve as an indicator of poverty and its gradual elimination partially reflects the progress in achieving global poverty alleviation (Durrheim et al., 2004).

The presence of the disease has tremendous economic burden on endemic countries. People carrying heavy infections of either *W. bancrofti*, *B. malayi* or *B. timori* have a high risk of developing chronic symptoms leading to a decrease in productivity and facing life-threatening infections if not properly cared for. They may also have mobility and functionality problems (Smith, 2006). It has been estimated that the health burden of

LF is responsible for more than 1% of all disability adjusted life years (DALYs) lost due to infections and parasitic diseases; 44% of which occurs in India (Ramaiah et al., 2000). Indeed, economic development and improved sanitation alone are credited with interrupting transmission of the parasite in some countries such as the United States of America (Chernin, 1987; Ramaiah et al., 2000; Addiss, 2005).

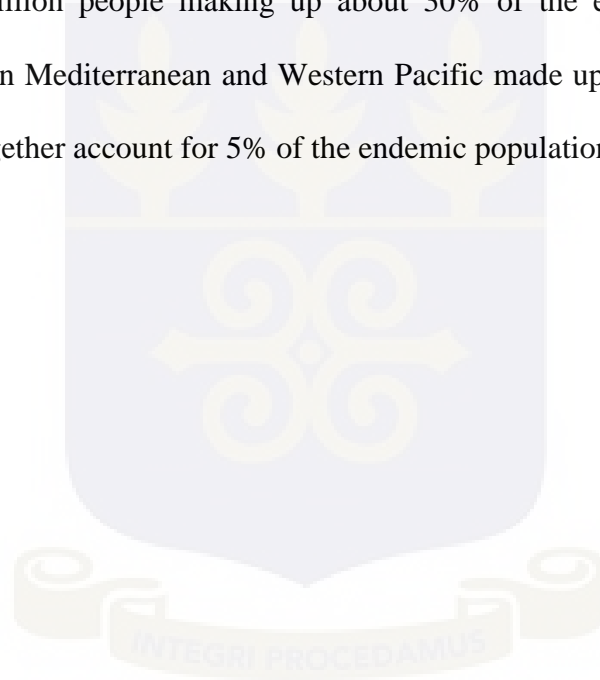
2.1.3 Global distribution and burden of lymphatic filariasis

After mental illness, which thought to be the leading cause of disability in adults worldwide, lymphatic filariasis is the second leading cause of permanent and long-term disability (Gyapong et al., 2005; Fox, 2011). Globally, 73 countries are considered to be endemic for the disease (WHO, 2015) with 856.4 million people in 58 countries throughout the tropical and subtropical regions of Africa, Asia, the Americas and the Pacific at risk. Eighty percent of these people live in 10 countries.

India accounts for over half a billion of the population at risk followed by Bangladesh, the Democratic Republic of Congo (DRC), Ethiopia, Indonesia, Myanmar, Nepal, Nigeria, the Philippines and Tanzania (Country Leadership and Collaboration on Neglected Tropical Diseases, 2015). Global estimates show that at the inception of the GPELF, 120 million people were infected while more than 40 million people were disabled by the disease. Some 80 million people suffered from covert lymphatic changes or kidney disease (WHO, 2006; Zeldenryk, et al., 2011). About 15 million people were known to suffer from lymphoedema while 25 million men had urogenital swelling, principally scrotal hydrocoele globally (WHO, 2012; Adhikari et al., 2015).

An approximate 1,341 million people live in the 73 countries known to be endemic for LF (Figure 1). Before the start of the launch of the GPELF, 115 million people were estimated to be infected with *W. bancrofti* and 13 million with *Brugia* spp. Presently, 95% of the population living in endemic areas and 98% of the infected population are found in the WHO's African and South-East Asia regions (Ramaiah & Ottesen, 2014).

Nine countries are endemic in the WHO's South-East Asia Region, with a population of 873 million. These make up 65% of the population in the region. In the African region there are 406 million people making up about 30% of the endemic population. The American, Eastern Mediterranean and Western Pacific made up of 7, 3 and 23 countries respectively altogether account for 5% of the endemic population (WHO, 2008).



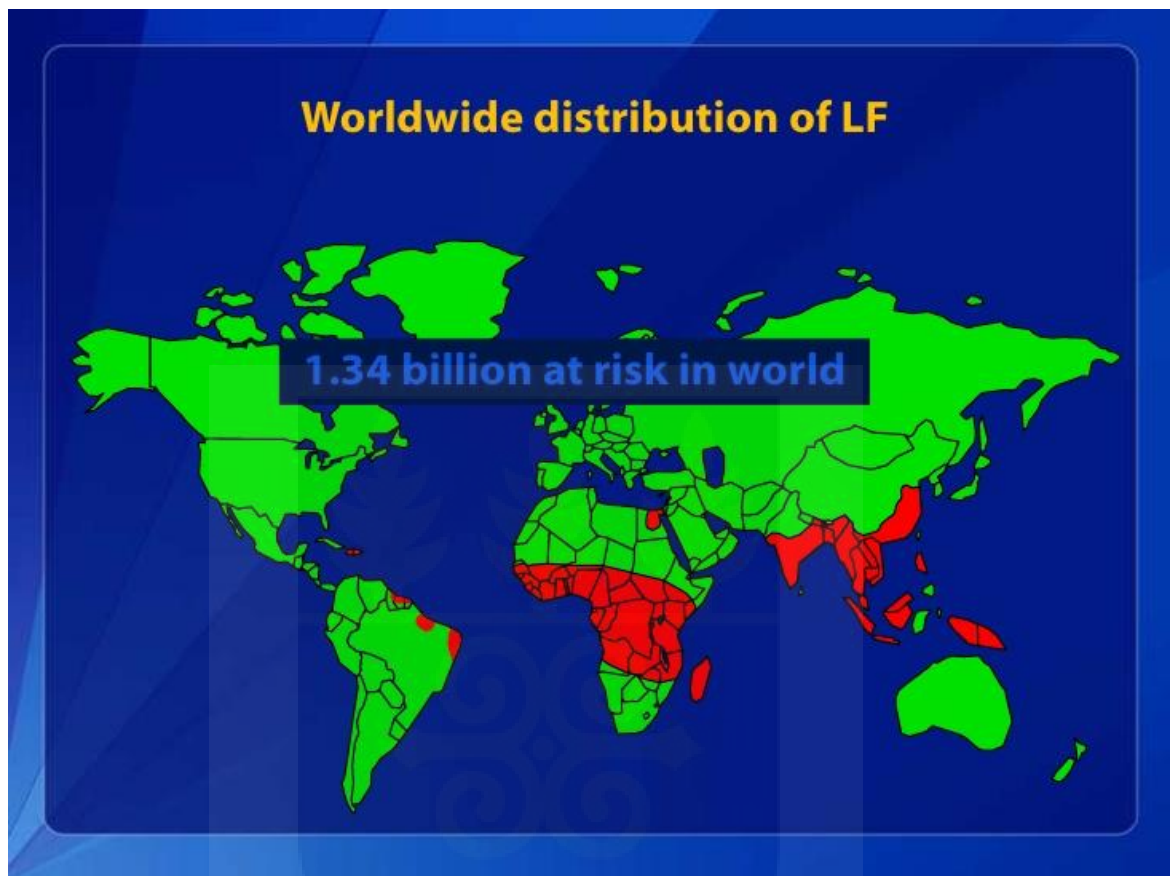


Figure 1: Global distribution of lymphatic filariasis

Source: Centres for Disease Control and Prevention, Atlanta, 2013

- Lymphatic filariasis endemic areas
- Lymphatic filariasis non-endemic areas

2.1.4 Distribution of lymphatic filariasis in Africa

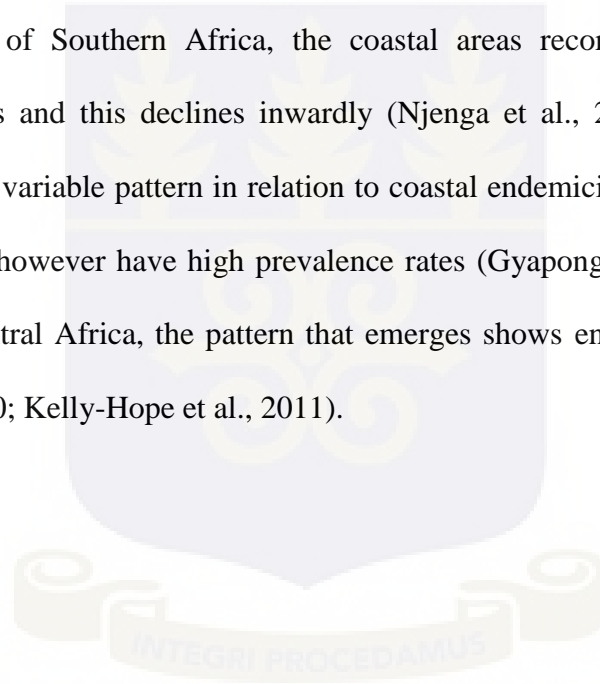
Currently, 34 out of the 54 countries in the African Region including South Sudan are classified as endemic for lymphatic filariasis. Approximately 477 million people are estimated to be at risk of the disease; this makes up about 30% of the global disease burden (WHO, 2011b; WHO 2015). Initially 39 countries were classified as endemic but there was very little evidence of active transmission in many of these countries while others did not require MDA (WHO, 2011b). This led to the review and reassessment of the status of five countries, namely, Burundi, Cape Verde, Mauritius, Rwanda and Seychelles. These were reclassified as non-endemic in 2011 (WHO, 2012b).

An estimated 50 million people are infected in the African Region (Addiss & GAELF, 2010; WHO, 2012). The prevalence of the infection exceeds 10% in 17 of these countries with Guinea Bissau topping with a prevalence of 37%. Comoros follows with a prevalence of 27% followed by Seychelles with a prevalence of 24% and finally Nigeria, with a 22% prevalence (Michael & Bundy, 1997; Ramzy, 2002; Richards et al., 2011).

Mosquitoes responsible for transmission of the disease vary depending on the region in which they are found. *Anopheles gambiae* and *funestus* are the main vectors found south of the Sahara, *Culex quinquefasciatus* are found in coastal East Africa and *Culex pipiens* is known to be the sole vector in Egypt (Ramzy, 2002). Nigeria bears the highest burden of LF in sub-Saharan Africa with an estimated 110 million cases. In developing countries in Africa, the rapid rate at which urbanization occurs does not take into account the provision of proper sanitary conditions thereby promoting the efficient transmission of many vector borne diseases including LF (Gratz & Jany, 1994).

Mapping of LF in Sub-Saharan Africa has been accelerated by the development of the Rapid Assessment of Geographical Distribution of Filariasis (RAGFIL) methodology by the Special Programme for Research and Training in Tropical Diseases (TDR) (Gyapong & Remme, 2001) and the development of the filarial antigen based diagnostic test which can be used at any time of the day (Weil et al., 1997). Data generated from mapping done so far suggested that LF has a focal distribution and there is variability in regional infection (Cano et al., 2014).

Across the east of Southern Africa, the coastal areas record the highest levels of endemicity levels and this declines inwardly (Njenga et al., 2010) while across West Africa, there is a variable pattern in relation to coastal endemicity levels along the coast. Inland countries however have high prevalence rates (Gyapong et al. 1996; Gyapong et al. 2002). In Central Africa, the pattern that emerges shows endemicity to be relatively low (Carne, 2010; Kelly-Hope et al., 2011).



2.1.5 Distribution of lymphatic filariasis in Ghana

In Ghana, LF is mainly a rural disease with some cases found in urban settings. Studies done by Dunyo et al., 1996 and Gyapong et al., 1996 showed that the prevalence of the disease varies in the different parts of the country. Generally, mapping of LF in Ghana showed it was most prevalent in the northern guinea savannah and the southern coastal savannah areas (Fig 2). The middle forest belt was found to be relatively free of the disease.

Within the different regions however, there was some variation in the distribution of the disease (Gyapong et al., 1996) but it still remains a major public health issue in the northern and coastal savannah areas of Ghana. Mapping done in 1999 showed that 49 of the then 110 districts were endemic for the disease. This corresponds presently to 98 of the present 216 districts in the country (www.ghanabusinessnews.com). Work done by Dunyo et al. 1996, found that prevalence in the northern sector was 30% while in the southern sector it was 15%.

Along the coast of Ghana, reported prevalence of microfilaria ranged between 9% and 25%, while the prevalence of hydrocoele and elephantiasis were found to be between 8.5% - 25% and 5.6% - 6.6% respectively (Dunyo et al., 1996). Presence of microfilaria in the rural areas was found to be between 26% and 32% (Dzodzomenyo et al., 1999; Gyapong et al., 1994). The prevalence of LF in urban areas in the northern part of Ghana was comparable to that reported for rural northern Ghana; between 1% and 4% (Gbakima et al., 2005). This is in contrast to the prevalence in southern Ghana, where prevalence in urban areas was lower as compared to rural areas and between 2% and 6%

of the population were seen to have developed elephantiasis (Gyapong et al., 1994 ; Gyapong et al., 1998).

Disease manifestation patterns reveal that in the northern part of the country, the prevalence of elephantiasis was 1.7%, microfilaraemia - 11.3%, hydrocoele - 20.3% and breast lymphoedema - 6.6% while in the south the prevalence was 0.3%, 0.6%, 5.2% and 6.1% respectively (Gyapong et al., 1996; de Souza et al., 2014). The vector responsible for transmission still remains mosquitoes of the *Anopheles gambiae* species complex (Sasa, 1976; Appawu et al., 1994; Appawu et al., 2001; Zagaria & Savioli, 2002; Boakye et al., 2004).

Mansonia species were found to carry the infective stages of *W. bancrofti* in two communities in the Western Region in studies conducted by Ughasi et al., 2012 to determine if *Mansonia africana* and *Mansonia uniformis* are vectors in the transmission of LF in Ghana. The Western Region is highly endemic for LF with a microfilaraemia prevalence of 2.1% (Gyapong et al., 1996). *Wuchereria bancrofti* is the parasite known to cause the disease in Ghana.

A study conducted by de Souza et al., 2014 showed that there is some genetic variability in the *W. bancrofti* parasite in Ghana and therefore there is the need for further studies to understand this genetic heterogeneity. Due to migration and rapid urbanization the pattern of LF prevalence seems to be changing in recent years as some cases are being seen the middle forest belt. These previously non-endemic areas need to be re-looked at to establish their non-endemicity or otherwise.



Figure 2: Map of Ghana showing the lymphatic filariasis endemic districts

Source: Ghana National Lymphatic Filariasis Control Program, Ministry of Health, 2002.

2.2 Biology, Life Cycle and Transmission of *Wuchereria bancrofti*

This section focuses on *Wuchereria bancrofti* which is the parasite of interest in Sub-Saharan Africa.

2.2.1 Biology of *Wuchereria bancrofti*

Wuchereria bancrofti is specifically transmitted by *Ochlerotatus*, *Anopheles*, *Aedes* and *Culex* mosquitoes. The parasite is responsible for more than 90% of the global burden of LF globally (Zagaria & Savioli, 2002). Its distribution is patchy. *Wuchereria bancrofti* is mainly found in the rural areas but can be found in some towns and coastal cities as well. Generally, the parasite is nocturnally periodic and occurs in the hottest and humid Sub-Saharan areas of the African continent but over the years they have diversified into a large number of strains which is evident from the different periodicities of the microfilaraemic reactions they cause (WHO, 1992).

Three genetically determined physiological strains exist in *W. bancrofti* depending on the microfilarial periodicity. They are the nocturnally periodic, nocturnally subperiodic, and diurnally sub periodic forms. The adult *W. bancrofti* parasites are thread-like and are found mainly in the lymphatic system, especially the lymphatic vessels and the nodes. The adult male worm is 23.8-30.6mm long and 90-120 μ m wide while the female is 42.2-46.3mm long and 160-188 μ m wide. Microfilariae are 309-346.8 μ m long and 5.3 μ m wide (Paily et al., 2009).

2.2.2 Transmission and life cycle of *Wuchereria bancrofti*

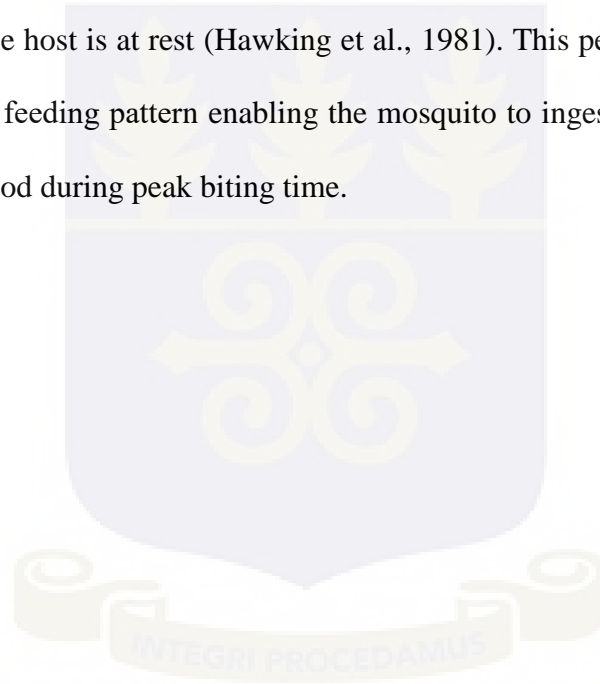
Complete development of filarial parasites takes place in two both man and the mosquito vector; man is the definitive host. The microfilariae (mf) produced by the adult worms are found circulating in the blood. They can be found in peripheral blood at night but during the day are lodged in the lymph. Parasite development in man takes between 5-8 months while in mosquitoes it takes between 10-14 days. The life cycle of the parasite begins when microfilaria (mf) are ingested by a mosquito vector during blood feeding on an infected human host (Figure 3).

The ingested microfilaria sheds its sheath and migrates to the thoracic muscle of the mosquito within 24 hours of ingestion. Here the mf undergoes two moulting stages. During the first stage also known as the L₁ stage, the parasite is short, thick and has a sausage like form. At about the 5th - 7th day, the L₁ moults to the L₂ stage where it increases in length and has slow movements which is more active than the L₁ stage. By the 10th - 11th day the parasite loses its cuticle, is very active and shows oscillatory pattern of movement. This is the L₃ stage of the parasite which is also the infective stage larva. The L₃ larvae then migrate to the head and proboscis of the mosquito.

The infective stage or L₃ larvae have the ability to remain alive and active for about 46 - 50 days or for as long as the mosquito survives. When the infective mosquito takes a blood meal the larvae penetrate the wound. The L₃ larvae which get into the wound travel to the lymphatic vessels of the human host. About 9-10 days after entry the L₃ moult to become the fourth stage larvae. The developmental stages of the L₄ takes place over several days or even months and then they undergo the final moult to become the adult

worm. The life span of the adult female *W. bancrofti* is 10.2 years while its rate of production has been found to be stable for a period of at least 5 years (Vanamail et al.,1990).

Microfilariae in humans show periodicity in the course of the circadian cycle. The worms mainly live in the pulmonary capillaries from where they move to peripheral blood and can be detected during their hours of periodicity. The periodic fluctuation of microfilariae has been attributed to greater oxygen tension in the lungs during the day as compared to the night when the host is at rest (Hawking et al., 1981). This periodicity is synchronized with the vector's feeding pattern enabling the mosquito to ingest mf in large numbers in the peripheral blood during peak biting time.



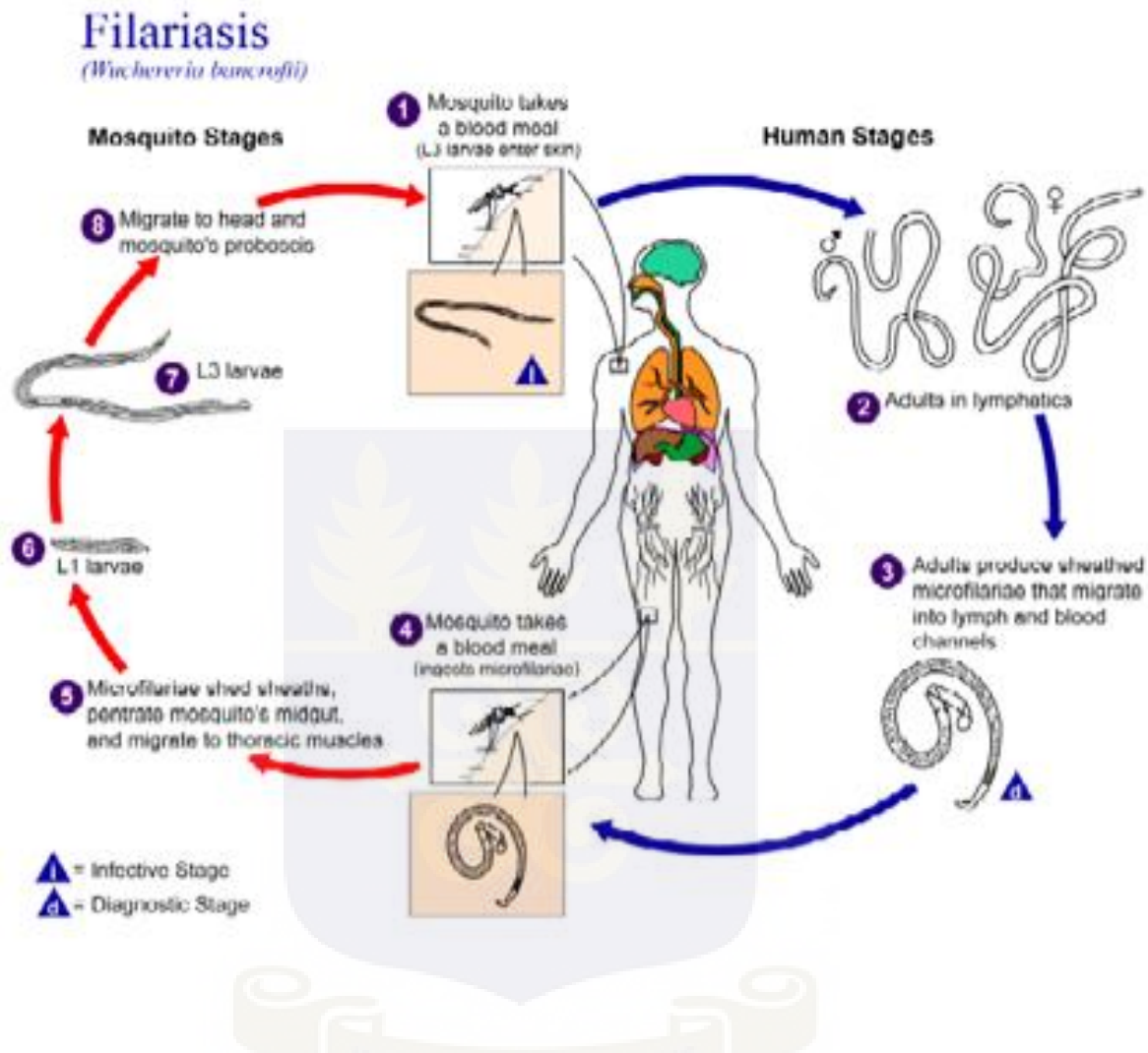


Figure 3: The life cycle of *Wuchereria bancrofti* showing the various developmental stages of the parasite in the mosquito and in the human host.

Source: <https://phil.cdc.gov/phil/home.asp> , 2016

2.3 Vectors of Lymphatic Filariasis

This section focuses the distribution, capacity, competence, composition and abundance of the vectors of lymphatic filariasis.

2.3.1 Distribution of the vectors of lymphatic filariasis

The spread of lymphatic filariasis is determined by how widespread the vectors of the parasite are. The mosquitoes responsible for carrying the *W. bancrofti* parasite include *Aedes*, *Anopheles*, *Culex*, *Coquilletidia*, *Mansonia* and the *Ochlerotatus* genera. Geographical location and type of habitat determine which mosquito species serve as vectors in particular endemic areas (Erickson et al., 2009).

In East Africa, *Anopheles* and *Culex* mosquitoes are the known vectors of lymphatic filariasis. West Africa is home to the *An. gambiae* complex, *An. funestus*, *An. pharoensis* and *Mansonia* species while in the Americas, Pacific and Asia *Aedes*, *Culex* as well as *Mansonia* mosquitoes are responsible for transmission (Dunyo et al., 1996; Dzodzomenyo et al., 1999; Appawu et al., 2001; Weil et al., 2008; Zhou et al., 2009).

In Ghana, mosquitoes of the *An. gambiae* complex and *An. funestus* have been found to be the principal vectors responsible for transmission of LF (Dzodzomenyo et al., 1999; Appawu et al., 2001; Boakye et al., 2004). *Anopheles pharoensis* has also been reported as a vector harboring the *W. bancrofti* parasite. However, information on its vectorial ability is not adequate (Dzodzomenyo et al., 1999; Amuzu et al., 2010). *Mansonia africana* and *Mansonia uniformis* have also been reported to harbor the infective stage (L₃ stage) of *W. bancrofti* (Ughasi et al., 2012). This buttresses results from an earlier

study done in Boké, Guinea which reported that *Mansonia uniformis* was a carrier of the L₃ stage of *W. bancrofti* (Toumanoff, 1958). *Mansonia* species are known vectors of filariasis in some endemic countries in Asia but until recently they had not been considered as vectors in Africa.

2.3.1.1 *Anopheles gambiae* complex mosquitoes

Anopheline mosquitoes are the known vectors of LF in Sub-Saharan Africa, specifically, *Anopheles gambiae s.l.* and *Anopheles funestus* (McMahon et al., 1981) They are heterogeneous and exhibit differences across species. Their heterogeneity is expressed in their choice of vertebrate hosts, biting and resting behavior and selection of oviposition sites (Amuzu et al., 2010).

Most anophelines feed at night and prefer to take their blood meals from humans but they may feed during the dusky hours of the morning and evening. Some of the mosquitoes of this complex are zoophilic - they prefer feeding on animals to humans as a source of food; while others are anthropophilic - they are attracted to humans as a source of food. The night biting and anthropophilic behaviour of the mosquitoes of the complex puts humans at greatest risk of infection at night during sleep. Some mosquitoes of this complex are also endophagic - they prefer to take their blood meal indoors while others are exophagic - they prefer to take their blood meal outdoors (Bøgh et al., 1998; Manguin et al., 2010).

After taking a blood meal, an important distinction is seen between mosquitoes. Some prefer to rest inside after a blood meal- endophilic, while others are exophilic - they

prefer to rest outside. The resting habits of the mosquito are an important factor to consider in control efforts (Williams & Pinto, 2012; <http://www.infectionlandscapes.org/2012/05/lymphatic-filariasis.html> accessed on 24/10/2016; <http://www.idph.state.il.us/envhealth/pcmosquitoes.html> , accessed on 24/10/2016).

2.3.1.2 *Culex* mosquitoes

Culex quinquefasciatus is the most efficient vector for lymphatic filariasis. This is because of its ability to pick up filarial parasites when prevalence has fallen to very low levels. *Culex quinquefasciatus* transmits more than half of the world's burden of LF (WHO, 2002b). This species of the *Culex* mosquito is anthropophilic, and so has a preference of taking its blood meals from humans. This is unlike the *Culex pipiens pipiens* which are ornithophilic – they are attracted to birds as a source of food.

Culex mosquitoes thrive in dirty water rich in decayed nutrients which is essential for oviposition. Their preference for human blood meal makes them adapt to biting primarily at night but they may also bite during the dusky times of dawn and sunset (Rozendaal, 1997; Williams & Pinto, 2012; <http://www.idph.state.il.us/envhealth/pcmosquitoes.htm> , accessed on 24/10/2016).

2.3.1.3 *Aedes* mosquitoes

Aedes mosquitoes are day biting mosquitoes. Their access to humans for taking blood meals is unlimited. They have a specific inclination for the water environment they select for laying their eggs. They prefer small containers that collect water (Rozendaal, 1997;

<http://www.infectionlandscapes.org/2012/05/lymphatic-filariasis.html>, accessed on 24/10/2016).

The *Aedes* mosquito is originally adapted to forest habitats but due to humans encroaching on forests they have adapted and become as much of an urban mosquito as it is a forest mosquito. They often live in the homes of humans, and are able to complete their whole life cycle here. This mosquito is anthropophilic and so its preferred host is humans. *Aedes aegypti* is a vector of lesser importance for *W. bancrofti* across Southeast Asia while *Aedes polynesiensis* is a very important vector of *W. bancrofti* in the South Pacific Islands of Polynesia (Williams & Pinto, 2012).

2.3.1.4 *Mansonia* mosquitoes

Mansonia mosquitoes are the primary vectors of brugian filariasis. They have been implicated to be vectors of LF in Asia. Studies done in Uganda and Ghana have also shown that they have the ability to carry the L3 stage of the *Wuchereria bancrofti* parasite (Onapa et al., 2007; Ughasi et al., 2012). *Mansonia* breed in stagnant water, especially swamp, marsh or rice fields. *Mansonia* mosquitoes have adopted a unique exploitation of these specific aquatic environments during larval development. The larvae and pupae attach and fix to the aquatic plant stems or roots below the surface of the water, acquiring oxygen directly from these plants

(<http://www.infectionlandscapes.org/2012/05/lymphatic-filariasis.html>, accessed on 24/10/2016)

Mansonia mosquitoes may be indoor or outdoor biting depending on the species involved in transmission. *Mansonia annulata* and *Mansonia uniformis* are both exophagic and avoid entering human dwellings. *Mansonia indiana* also avoids biting indoors even though it is not repelled by houses. *Mansonia annulifera* is endophagic and has a preference for female hosts (Gass et al., 1982).

2.3.2 Vectorial capacity

Vectorial capacity is how competent a given vector is when it comes to the transmission of vector borne diseases. Vectorial capacity is influenced by vector density in relation to host preference, the frequency of successful ingestion of an infected blood meal by a vector, the duration of the latent period and the expected life span of the vectors. These factors determine the ability of vectors to live long enough to develop a parasite to its infective stage after ingesting infected blood (Simonsen, 2008; Nyarko-Osei, 2013).

The ability of a mosquito to ingest and support the development of microfilariae is an important determinant of transmission (Bryan & Southgate, 1988). Vectorial capacity is expressed as $C = maVP^n / -\log_e P$, where:

C = vectorial capacity; the number of infective bites received daily by a single host

m = density of vectors in relation to density of hosts

a = proportion of vectors feeding on a host divided by the length of gonotrophic cycle in days

V = vector competence

P = daily survival of vectors

n = extrinsic incubation period (Norris, 2006).

2.3.3. Vector competence

The vector competence of a mosquito is the ability of the mosquito to acquire a causative agent from a reservoir host and later transmit it to a susceptible host in which the causative agent survives, undergoes essential development, multiplies and eventually causes infection. Vector competence is a component of vectorial capacity.

2.3.4 Vector composition

Vector composition is the number of mosquitoes known to transmit a particular disease in a given geographical area. Adequate knowledge of the vector(s) responsible for the transmission of LF in a particular area enables disease control programmes formulate appropriate strategies used in eliminating the disease from that area.

2.3.5 Vector abundance

Vector abundance is the proportion of vector mosquitoes among the species diversity of a given area. The proportion of vectors in a study area has a direct impact on transmission (Simonsen, 2008). This is especially true in areas where interventions have not been put in place. The absence of interventions leads to high biting rates which can be translated into high infective bites and consequently high Annual Transmission Potential (ATP).

The ability to understand the way filarial parasites are transmitted by mosquitoes is an essential component of planning control measures. To be able to interrupt transmission of filariasis, vector density or microfilaria intensity has to be at a level below which new infection cannot occur. To be able to achieve this, it is important to consider the types of vector-parasite relationships that exist; namely limitation, facilitation and proportionality (Amuzu et al., 2010; de Souza et al., 2012).

These phenomena affect the epidemiology and control of LF and are density dependent processes which are important in the prediction of the effects of the intervention on transmission and considered heterogeneities. Density dependence is a biological term, which indicates that the growth rate of a population depends in a non-linear way on its density (Anderson & May, 1991; Churcher et al., 2005; Duerr et al., 2005).

2.3.5.1 Facilitation

The first of these phenomena is facilitation. This is mainly seen in the anopheline mosquitoes. In facilitation, the number of ingested mf developing to infective stage (L_3) increases as the number of mf ingested increases. This makes vectors highly efficient mainly at high microfilaraemia levels. In areas where mosquito species exhibit facilitation, it should be possible to eliminate lymphatic filariasis by interrupting transmission with MDA alone (Southgate & Bryan, 1992). This is because the GPELF strategy is based on the assumption in situations where microfilariae in circulation are reduced below a certain threshold such that their availability to vectors is decreased. At this threshold known as Webber's critical point, the disease is nowhere near eradication but close to the steady equilibrium where it is possible that transmission can be stopped

and new infections not seen in the population of interest. The population of parasites eventually dies out and the re-establishment of a stable parasite population in the area where transmission has been interrupted and infection eradicated would require the introduction of many parasites either in the humans or the mosquitoes (Pichon, 2002; Amuzu et al., 2010; Ughasi et al., 2012).

This mechanism of facilitation causes instability in the parasite population which explains the patchy distribution of LF endemicity in areas where *W. bancrofti* is transmitted by *Anopheles* mosquitoes. This makes it possible to eliminate LF with MDA alone in these areas. Other interventions are required to ensure the elimination goal is achieved.

2.3.5.2 Limitation

Limitation is the opposite of facilitation. With this phenomenon, vectors of the parasite are able to transmit parasites when mf levels in the blood meal they take up are at very low levels (Boakye et al., 2004). Limitation is a negative feedback mechanism or a negative density dependent system which helps produce stability (Pichon, 2002). Here the number of ingested mf developing to infective stage (L_3) decreases as the number of mf ingested increases (Amuzu et al., 2010).

This phenomenon is exhibited by the culicines. Therefore, for successful elimination of LF in areas where the *Culex* mosquito is responsible for transmission a combination of chemotherapy and vector control is important. In LF endemic areas where limitation occurs, total interruption of transmission is not only hard to reach but also unstable. This

is because the mosquitoes in which this phenomenon occurs are efficient vectors (Pichon, 2002).

2.3.5.3 Proportionality

The third phenomenon is a situation of non-regulated transmission by vectors. This is termed “Proportionality”. The phenomenon here is that there is a linear relationship or a constant percentage of microfilaria taken up by the vector during the uptake of a blood meal that develops into the infective stage. Limitation and facilitation in vectors are deviations from this linear relationship (Duerr et al., 2005; de Souza et al., 2012).



2.4 Lymphatic Filariasis Control Strategies and Tools

The elimination of lymphatic filariasis (LF) is based on the two-pronged approach adapted by the Global Programme for the Elimination of Lymphatic Filariasis (GPELF). The two approaches are (1) interruption of transmission of infection, (2) alleviation and prevention of suffering and disability caused by the disease. This section discusses efforts that have been made in the elimination of the disease.

2.4.1 Mapping

To be able to have a successful control, elimination or eradication programme, it is essential to have access to detailed maps. Before an MDA programme is initiated, geographical mapping of infection is crucial in order to delimit the target areas (Mwase et al., 2014). For mapping to be useful and successful, implementation units (IUs) need to be identified.

This can be done by reviewing existing information on LF in the area of interest, collecting new information through questionnaire administration and carrying out parasitological surveys to detect the presence of the antigen of the parasite. The IUs can then be placed in three categories:

- (i) confirmed filaria-endemic units: areas where antigenemia or microfilaraemia positivity rate is 1% or greater
- (ii) confirmed non-endemic units: areas where the ecological situation is not conducive to transmission and previous surveys indicate antigenemia or microfilaraemia positivity rate is 1%.

(iii) uncertain units contiguous to the confirmed filaria-endemic units and units for which adequate information is not available. These are categorized as doubtful units. It is necessary to carry out surveys in the doubtful units to detect the presence of filarial antigenemia in order to establish boundaries of filaria-endemic areas (WHO, 2011a).

Mapping is carried out using immunochromatographic card test (ICT) based on the WHO recommended strategy (Gyapong & Remme, 2001). For an IU to be eligible for MDA the ICT ratio should be $\geq 1\%$. Mapping is the first stage of the GPELFs LF elimination strategy (Figure 4).

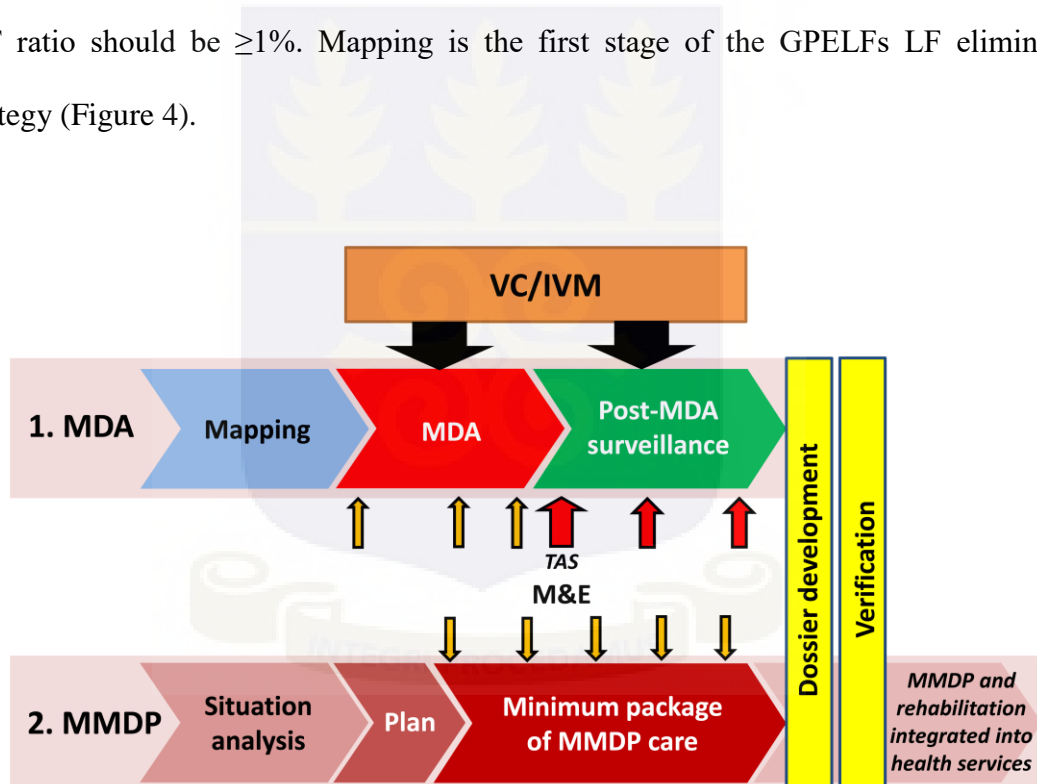


Figure 4: Key programmatic steps towards the elimination of lymphatic filariasis transmission

2.4.2 Mass drug administration (MDA)

In 1997, the World Health Assembly passed a resolution urging member states to strengthen activities towards eliminating lymphatic filariasis by 2020. The passing of this resolution, led to the formation and launch of the Global Alliance to Eliminate Lymphatic Filariasis (GAELF) in 2000 as a partnership to support national elimination programmes in endemic countries. There was great optimism that achieving elimination lies in the availability of safe, single dose, two drug treatment regimens capable of reducing microfilaraemia to near zero levels for one year or more, along with remarkable improvement in techniques for detecting the infection. Some studies informed this decision, some of which were carried out in Ghana.

Some remarkable shifts have occurred in the LF elimination programme over the years with the greatest of these being the shift in focus from diagnosing and treating individuals to diagnosing, treating and preventing the disease in populations (Gyapong et al., 2005). In countries like Surinam, Trinidad and Tobago and China, mass drug administration of diethylcarbamazine (DEC) and DEC fortified salt has led to the interruption of transmission of LF (Zagaria & Savioli, 2002). The effort of the GPELF has brought about substantial decrease in the prevalence of infection and risk of the disease but there are still about 120 million people in endemic countries that remain infected with either *W. bancrofti* or *B. malayi* and some one billion at risk of the disease.

Of the 73 countries endemic for the disease, 17 (23%) no longer require MDA. This includes Malawi which recently announced that they have reached a stage in the elimination programme where MDA is no longer required (Country Leadership and

Collaboration on Neglected Tropical Diseases, 2015). These accomplishments show that ambitious goals can be achieved when all the effort and resources needed for its achievement are made available.

MDA is largely based on single dose combination of ivermectin and albendazole for countries co-endemic with onchocerciasis and albendazole and DEC for other endemic areas for a period of 4-6 years in order to reduce the microfilaria in the blood to levels where its transmission cannot be sustained by the vectors (Goldman et al., 2007). Diethylcarbamazine (DEC) fortified salt is also one of the methods that has been used by control programmes in some endemic countries. This salt when administered for 18 days to one year has been shown to be effective in reducing microfilaria to levels below which transmission can be stopped (Gyapong et al., 2005). MDA has been promoted greatly in the field of public health for three reasons.

The first of these reasons is, the interruption of the transmission cycle between mosquitoes and humans is very important, therefore the mass distribution of the antifilarial drugs have the effect of causing a reduction in the circulation of microfilariae thereby preventing continued transmission. Secondly, it has been proven by countries like Japan and China that LF is an eliminable disease using the antifilarial drugs provided a large enough proportion of the target population consume the drugs. If the drug is administered for a period of 4-6 years, a target population of between about 65-80% is needed to achieve elimination (Mohammed et al., 2006).

This is because it is expected that the prevalence of the disease should reduce due to the ongoing MDA. But, if treatment is more than 6 years then between 70-90% of the population are needed for elimination to be achieved. This is because it will take a much longer time for the prevalence of the disease to go down to the threshold required for elimination.

In Brazil, in order to interrupt transmission, systematic treatment with DEC using two different strategies were carried out: (a) mass screening once a year followed by mass treatment twice a year, (b) annual mass screening followed by selective treatment of persons presenting with microfilaraemia. Both strategies were considered to be successful since they both led to a decline in microfilaria prevalence in Brazil (Schlemper Jr. et al., 2000) but mass treatment seemed to be more effective than selective treatment because transmission blocking is achieved in less time (about 6-7 years) and microfilaraemia eradication takes about 4-7 years less than usual.

The WHO recommended strategy to interrupt transmission is selective screening of a limited number of persons in order to identify affected areas. This is to be followed by mass treatment with anti-filarial drugs (WHO, 1998). This recommendation is based on the considerable time, money and human resources required for more extensive screening and on the ineffectiveness in some areas of alternative approaches, such as mass screening followed by selective treatment of microfilaria-positive individuals. In most countries however, such extensive screening is not economically feasible (Schlemper Jr. et al., 2000).

In a country like China however, *W. bancrofti* and *B. malayi* transmission has been interrupted through mass treatment with DEC salts and tablets. A large majority of the population was screened and all who tested positive for microfilaria were treated with DEC for at least 5 years (Sun, 1995).

In areas where microfilariae prevalence is more than 10%, coverage of 90% or more may be required to achieve elimination (Michael et al., 2004). In spite of whatever coverage levels are required for elimination to be achieved, multiple rounds of treatment are needed because the drugs do not have the ability to kill the adult worms; they rather reduce circulating microfilaria levels until the adult worms finally die off. This may take 6 or more years to occur based on the life cycle of the parasite (Bockarie & Deb, 2010; Parker & Allen, 2013).

The third reason is the pledge by the two pharmaceutical companies GlaxoSmithKline and Merck to donate the relevant drugs until elimination of LF has been achieved (WHO, 2012b). The drug distribution can easily be done through existing health systems in target countries; they have very few side effects and can be consumed by almost everyone. Globally, since the launch of the programme, countries implementing MDA have increased consistently and steadily (World Health Organization, 2010). The results reported by the BBC ('End in sight' for elephantiasis, BBC, 8th October 2008, <http://news.bbc.co.uk/1/hi/health/7659222.stm>) proved that elimination of LF by 2020 was a real possibility. Currently, the number of countries implementing MDA has increased from 12 when the MDA activities began in the year 2000 to 53 out of the 73 endemic countries known to be endemic for the disease.

Twenty-eight countries in Sub-Saharan Africa are currently eligible to use ivermectin and albendazole where onchocerciasis is co-endemic while the remaining nine use the DEC-based therapy. The total population that have undergone MDA has increased from 2.9 million to more than 950 million individuals with 4.4 billion doses of albendazole with either ivermectin or diethylcarbamazine given out in the first eleven years of the GPELFs existence (WHO, 2014). Currently, mass drug administration has been conducted in 60 countries. Fifteen of these countries have reduced infection prevalence, stopped MDA and started surveillance.

Twenty-two countries have achieved 100% geographical coverage in the conduct of MDA while 23 have not yet reached all endemic areas. A remaining 13 countries have not yet started MDA but have been mapped and have been shown to require MDA to eliminate LF. In 18 countries, the endemicity of some implementation units has not been clearly defined and they have been targeted for mapping (WHO, 2014). The cumulative number of treatments delivered since the year 2000 is approximately 5 billion doses to 1 billion people. Taylor et al (2010) reported that 570 million people living in 51 endemic countries have received the one billion drugs which have been delivered with 493.5 million treated in all affected regions in 2013 alone.

The first MDA in Sub-Saharan Africa were implemented in four countries - Ghana, Nigeria, Tanzania and Togo. Since then, MDA implementation has progressed from 4 countries in 2001 to the present 19 countries in the WHO African Region. Nine countries in this region were still undertaking mapping of endemic foci as at 2010; Djibouti is the only country yet to start mapping. In 2006, four countries achieved total coverage of the

entire at-risk population: Comoros - approximately 572,000, Togo - approximately 1.1 million, Ghana-approximately 10.5 million, Burkina Faso-approximately 13.8 million and Tanzania-Zanzibar - approximately 1.2 million ([http://www.aho.afro.who.int/profiles_information/index.php/AFRO:Lymphatic filariasis](http://www.aho.afro.who.int/profiles_information/index.php/AFRO:Lymphatic_filariasis); accessed on 10/03/2017).

The total number of people treated halfway through the GPELFs elimination target; i.e. by 2010, are approximately 82.4 million out of which 23 million are children aged 5-14 years. The number treated so far makes up 69.2% of the target population of 119 million. Globally, more than 4.4 billion treatments have been delivered worldwide since the inception of the GPELF. By the end of 2012, 56 of the 73 endemic countries had implemented MDA. Thirteen of these 56 countries have achieved the target threshold required for interrupting transmission and have stopped MDA (WHO, 2014).

Annual MDA has and still provides an opportunity to improve the health of millions of people (Richards et al., 2011). In the recent past, the move towards integrated preventive chemotherapy programmes to control neglected tropical diseases (NTDs), has brought many national LF elimination programmes to the point of co-implementing the LF elimination programme with programmes to eliminate or control onchocerciasis, schistosomiasis, soil-transmitted helminthiasis (STH) and trachoma (Addiss, 2010; Ichimori et al., 2014). The objective of the MDA is to interrupt transmission by reducing microfilaraemia to levels where transmission cannot be sustained even after MDA has been stopped. The effectiveness of MDA in reducing the prevalence and density of

microfilaria in the blood is directly related to the proportion of the population that ingests the medicines every year.

Studies have demonstrated that a combination of DEC (6mg/kg) and ivermectin (400 µg/kg) is effective in the suppression of microfilaria for over 18 months even though each drug in isolation has been shown to do so with no evidence of activity against adult parasites (Taylor & Turner, 1997). Both drugs are microfilaricidal and whether administered singly or together results in acceptable reduction in mf levels. In areas where onchocerciasis and loiasis occur, combinations of albendazole and ivermectin have also been shown to provide long-term reductions in microfilaraemia. Systematic treatment with antifilarial drugs in many areas appears to have stopped transmission (Shi, 1993; Sun, 1995).

In Ghana, the Filariasis Elimination Programme has been in existence since the year 2000. This was later merged with other NTD control programmes leading to the now existent Neglected Tropical Diseases Control Programme (NTDCP). MDA started in 5 districts from 2001 and scaled up to 82 districts as at 2008. Presently, 96 out of the 216 districts are undertaking MDA activities with some in the post-MDA phase. Currently, the NTDCP has reached total national coverage with all endemic districts covered and the number of treatments delivered ranging between 4 and 10 (NTDCP, unpublished). The programme has given out about 31,053,694 drugs since its inception with approximately 6.8 million people from a target population of 9.8 million treated giving treatment coverage of 69.9%. Several factors have led to the success of the MDA programme even though it has not been without challenges (NTDCP, unpublished).

2.4.3 Alternative intervention strategies

2.4.3.1 Vector control

The principal objective of the Global Program to Eliminate Lymphatic Filariasis (GPELF) is breaking the cycles of transmission of *Wuchereria bancrofti* and *Brugia* spp. through annual mass drug administration (MDAs) of albendazole and ivermectin or diethylcarbamazine and ivermectin to populations at risk of the disease (Bockarie et al., 2009). The rationale behind this strategy is that microfilariae released by gravid female *W. bancrofti* worms gain access to the blood stream where they circulate at night and are available for the nocturnally feeding mosquitoes (Richards et al., 2011).

The ingested microfilariae (mf) go through three larval molts to reach the L₃ stage where they are able to infect humans when the infective mosquito returns to feed on a human. The L₃ then develop into adult male and female worms, mate in the lymphatic system and the female produces microfilariae which complete the life cycle of the parasite (Richards et al., 2011). However, ingestion of these antifilarial drugs breaks the cycle. So, for transmission to be interrupted, vector density or microfilaria intensity has to be at a level below which new infection cannot occur (Bockarie et al., 2009; Gambhir et al., 2010; WHO, 2013).

Significant progress has been made with MDA but questions have been raised regarding the effectiveness of MDA alone to eliminate LF without including vector control since this was once the primary tool that could be used to control filariasis (Sasa, 1976). Even though vector control in the elimination strategy had a supplementary role, it has been realized that without it, it has never been possible to stop transmission of any pathogen

causing vector-borne disease (Prasittisuk, 2002). This is exemplified by the Rockefeller Foundations' antimalaria campaign from 1943 to 1944 where the use of both chemotherapy and vector control was much more successful in the interruption of transmission of malarial parasites rather than using chemotherapy alone (Najera, 2001).

Having a vector control component in the global LF elimination programme is essential for the success of the programme. This is because it will enhance the sustainability of high drug coverage, help interrupt transmission, especially in high risk and peri-urban areas and prevent re-establishment of transmission in vulnerable areas (Prasittisuk, 2002). The choice to use MDA or vector control or both in disease elimination in a particular setting is dependent on the vector-parasite combinations involved and whether the filarial vectors also transmit any other human pathogens (Pichon, 2002).

The filarial parasite is transmitted through the bite of an infective mosquito that contains the third stage infective larvae (L_3 larvae) (M.J. Bockarie et al., 2009). Parasite transmission does not occur with a single bite; about a thousand infective bites are needed to facilitate active infection in humans. In areas where malaria and LF are co-endemic, indoor residual spraying and distribution of long-lasting insecticide treated nets is ongoing to control both diseases. Also in areas where dengue is co-endemic with LF, *Aedes* mosquitoes are the vectors of transmission and integrated control efforts have been put in place to curb the diseases. Vector control has a great potential in becoming an important component of the filariasis elimination strategy (Webber, 1975, 1977, 1979; Kelly-Hope et al., 2013).

Even though extensive work has been done to determine the vector competence of both anopheline and culicine mosquitoes, it is important to improve transmission models to facilitate the effective planning of the global strategy for LF elimination efforts. In areas where *Anopheles* mosquitoes transmit both malarial and filarial parasites, the promotion of Insecticide Treated Nets (ITNs) as part of the Roll Back Malaria (RBM) initiative is seen to have rapidly reduced filarial transmission. Therefore the joint planning of LF elimination and malaria control activities and the enhancement of operational links between the two campaigns will continue to have mutual benefits and be synergistic (Zagaria & Savioli, 2002).

Vector control activities may differ depending on which species is responsible for transmission. Integration of these efforts also has the tendency to yield better results since no single method is sufficient (Prasittisuk, 2002). It is important to have repeated annual rounds of MDA to interrupt the transmission of *W. bancrofti* to humans, but in many situations, this goal can be achieved more rapidly and sustained much longer through the integration of vector control with MDA (Ramzy, 2002).

The ability to identify the mosquitoes responsible for transmission and assessment of their bionomics i.e. distribution, seasonal abundance, feeding and resting habits are very important in the selection of the appropriate method for sampling and control of potential vectors. Having an efficient method for assessing the prevalence of filarial infection in mosquito populations could facilitate the xenomonitoring of elimination programmes (Ramzy, 2002). The ability of a mosquito to ingest microfilariae, support their

development to human-infective third stage larvae is an important determinant of transmission (Gleave et al., 2016).

Overall three processes determine the competence of a vector:

- a. The uptake of microfilaraemia from a microfilaria positive individual
- b. The development of the ingested mf to L₃ stage
- c. Transmission of L₃ to a human host (Kwansa-Bentum, 2005)

Vector control was the primary tool for controlling filariasis in the Pacific before effective antifilarial drugs were available and even thereafter (Burkot et al., 2006). This is mainly because the ministries of health and the departments of health considered MDA labour intensive (Burkot & Ichimori, 2002). In areas where *Anopheles* species is the vector for both malaria and lymphatic filariasis, vector control measures such as indoor residual spraying with DDT was undertaken to control malaria and has also been beneficial in eliminating LF. Two countries where this integrated effort has worked are Papua New Guinea and the Solomon Islands (Webber, 1977, 1979; Bockarie, 1994; Burkot et al., 2006).

Australia eliminated *W. bancrofti* using sanitation campaigns that controlled the major vector *Culex quinquefasciatus* (Boreham & Marks, 1986). In Japan as well, vector control played a significant role in elimination of LF while in India and Zanzibar polystyrene beads were placed in pit latrines or soakage pits which are major breeding sites for *Culex quinquefasciatus* (Maxwell et al., 1990,1999; Curtis et al., 2002). *Culex quinquefasciatus* is globally known to transmit majority of *W. bancrofti* because it typically breeds in stagnant and organically polluted water (Sunish et al., 2007). In areas

where these vectors are responsible for transmission, MDA is unlikely to be sufficient to sustain transmission due the high vectorial capacity of the vector. Vector control therefore would be an important supplement to sustain the interruption of transmission in these areas (Burkot et al., 2006; Sunish et al., 2007).

The goal set by the GPELF is an ambitious one. Mass treatment programmes have had varying degrees of success; in some areas transmission has been interrupted while in others elimination has not been achieved in spite of many years of treatment (Esterre et al., 2000; Vector Control Research Center, 2003; Cano et al., 2015). The influence of choices made strategically in the elimination programme and its success or failure is not understood. However, mathematical models help in understanding the complex transmission dynamics of parasitic diseases. These are useful tools in planning and evaluation of control programmes (Habbema et al., 1992; Goodman, 1994).

Three models have been developed for lymphatic filariasis. The first was a model developed for the evaluation of a specific vector control programme (Rochet, 1990) . The two recent ones known as the “full transmission models” are EPIFIL (Chan et al., 1998; Norman et al., 2000) and LYMFASIM (Plaisier et al., 1998). These models are more general and are both used to predict the long-term impact of mass treatment and assess elimination prospects (Stolk et al., 2006). EPIFIL can be used to simulate the impact of vector control, assuming that control measures reduce mosquito biting rates.

The effects of mass treatment can also be simulated based on the assumption that a proportion of the population is treated with a drug whose specificity is already specified.

This drug may kill part of the present mf and adult worm and also reduce the mf production rate per adult worm. The model is deterministic which implies that the simulation output is always the same with fixed input specifications (Habbema et al., 1992; Habbema et al., 1996).

LYMFASIM simulates that individuals interact through biting mosquitoes and together they form a dynamic population of which the size and age structure may change over time (Stolk et al., 2006). This model also takes into account the limitation in the proportion of engorged microfilariae that develop into L3 larvae into the mosquito and of acquired immunity in human hosts (Michael & Bundy, 1997; Michael et al., 2004).

Like EPIFIL, LYMFASIM also simulates the impact of vector control by assuming that it reduces the mosquito biting rates. Variants of both models developed for *Wuchereria bancrofti* transmitted by *Culex quinquefasciatus* in Pondicherry, India using data from an integrated vector management programme from 1981 to 1985 showed that monthly biting rate was much higher in EPIFIL than in LYMFASIM (Stolk et al., 2006).

Quantification in EPIFIL was based on weekly mosquito catches that had been carried out in several sites while that of LYMFASIM was based on data from a single site where all night mosquito catches had been carried out. Both models accurately mimicked epidemiological data from the study site even though different assumptions were made (Stolk et al., 2006; Changediya & Devdhe, 2013).

Elimination therefore can be achieved within a reasonable time frame and the time period required to achieve this is much shorter than the mean adult worm lifespan due to two main reasons:

- a. Antifilarial drugs have a strong macrofilaricidal or sterilizing effect
- b. Worms do not need to be killed or sterilized to achieve elimination. Prevalence just needs to be brought under a threshold below which acquisition of new worms is lower than the death rate of existing worms so that the worm population will eventually die out (Plaisier et al., 1999; Taylor et al., 2005; Stolk et al., 2006).

In Sub-Saharan Africa where LF is co-endemic with malaria and both are transmitted by the *Anopheles* mosquito, integration of vector control methods for both diseases may be the best means of achieving the goals of elimination and control for both diseases. Naturally, the mosquito does not do transmission of *W. bancrofti* efficiently, due to the density dependent process of facilitation; the increasing development of L₃ larvae is more efficient with increased ingestion of parasites by the mosquito.

This process subsequently reduces the mosquito's survival and makes vector control an attractive option in LF control efforts (Southgate & Bryan, 1992; Pichon, 2002). For example, the use of long lasting insecticide treated nets (LLINs), when introduced in 2007 alongside MDA in northern Uganda, reduced the prevalence of *W. bancrofti* markedly in study communities to below 1% by 2010 (Ashton et al., 2011). A universal coverage of mosquito nets in households was found to be protective against *W. bancrofti* infection.

Other studies have also affirmed that the use of mosquito nets; whether treated or untreated through the efforts of the malaria control programme can reduce LF vector density and infectivity thus bringing about a reduction in transmission of the disease (Bøgh et al. ,1998; Bockarie et al., 2002; Pedersen & Mukoko, 2002; Odermatt et al., 2008; Emukah et al., 2009). Malaria control efforts also rely on indoor residual spraying (IRS). Even though this method has not been used extensively in recent times, it was the most effective control measure used in the 1960s and 70s. Elimination of *W. bancrofti* from the Solomon Islands was made possible through indoor residual spraying (Webber, 1979; Ashton et al., 2011).

In conclusion, the WHO's advocacy for integrated vector management (IVM) will encourage better linkages between LF and malaria in *Anopheles* transmission endemic regions. The significant scale up of ITNs and LLINs and to a lesser extent IRS is promising and it is evident that these malaria control efforts have already had an impact on *W. bancrofti* transmission in many co-endemic areas (Kelly-Hope et al., 2013).

2.4.3.2 Albendazole monotherapy

Apart from onchocerciasis, loiasis is also co-endemic with lymphatic filariasis in the central part of Africa. Treatment of individuals with ivermectin as part of either the onchocerciasis control programme or the LF elimination programme may result in *Loa loa* encephalopathy and eventually death, especially in patients with heavy *Loa loa* infection where eye worm prevalence exceeds 40% (Twum-Danso, 2003; WHO, 2012b). Rapid assessment of *Loa loa* (RAPLOA) by the African Programme for Onchocerciasis Control has provided information on areas at high risk of loiasis while the Mectizan

Expert Committee has published guidelines on MDA in these areas based on benefit-risk assessments.

In March 2012, following a meeting held in Accra, Ghana, WHO has recommended the use of albendazole only in areas where LF is co-endemic with *Loa loa* in association with integrated vector management (WHO, 2012a). A single annual dose of 400mg of albendazole has been shown to result in acceptable reduction in levels of mf; at higher doses the efficacy is greater (Jayakody et al., 1993; Gyapong et al., 2005). The DOLF (death to onchocerciasis and lymphatic filariasis) project is currently conducting community trials to confirm the effectiveness of albendazole alone on lymphatic filariasis.

2.4.3.3 Triple drug therapy

Since the inception of the WHO's Global Filariasis Elimination Programme, there is no known drug that reliably kills or sterilizes adult filarial worms. The focus of the GPELF has been the use of MDA to reduce the source of microfilaria in endemic populations, hence interrupt transmission of LF (Ottesen et al., 2008). Currently, the strategy is to provide repeated, annual single doses of albendazole with either DEC or ivermectin for the lifespan of adult worms which is typically 5–7 years (Tisch et al., 2005).

Mathematical models suggest that the most potent drug combination; diethylcarbamazine and albendazole (DEC+ALB), currently recommended to achieve interruption requires at least 70% compliance of the target population for at least five to seven years in order to achieve elimination (Fenwick, 2006). A single dose of this combination has been shown

in clinical trials to clear microfilaria in approximately 25% of participants. There is the need to have drug regimens that have better activity against microfilaria and prevent new infections that would have to be treated later. This would improve the chances of eliminating LF in endemic areas especially resource poor settings and also better make use of resources available for the elimination programme (Mohammed et al., 2008).

Community studies have shown that MDA with ivermectin and diethylcarbamazine (IVM+DEC) was more effective in reducing mf rates than DEC alone (de Silva et al., 2000). A single dose of ivermectin completely cleared mf in 35% of participants and reduced the geometric mean microfilaria by more than 98% in a year. Two years after single treatment, 20% of participants remained microfilaria negative and geometric microfilaria levels remained reduced by more than 90%. Therefore the addition of ivermectin which is a potent microfilaricide has the ability to clear mf and provide a more lasting effect in endemic areas as compared to the use of DEC and ALB alone (Bethony et al., 2006). Trials conducted in Tanzania also showed that ivermectin in combination with DEC and albendazole was effective in clearing microfilaraemia in study participants six months after treatment (Simonsen et al., 2004).

Triple therapy has also been proven to be effective in Zanzibar where co-administration of ivermectin, albendazole and praziquantel is safe in areas where lymphatic filariasis, soil transmitted helminthiasis and schistosomiasis are co-endemic and where several rounds of treatment with one or two drugs have been implemented in the past (Mohammed et al., 2008). Therefore, it is possible that simultaneous treatment with ivermectin plus diethylcarbamazine plus albendazole (IVM + DEC + ALB) could reduce

the number of rounds of MDA required to reach LF elimination, and this could have a transformative impact on LF- exposed populations (Thomsen et al., 2016) and therefore option needs to be explored into detail.



2.5 Detection of *Wuchereria bancrofti* - Diagnosis

To be able to achieve the GPELF's goals successfully, diagnostic tools are very important because they are needed in decision making as to where to undertake MDA, measurement of the effects of MDA, definition of targets and endpoints for MDA and monitoring endemic population for resurgence of transmission after MDA has been stopped (Weil & Ramzy 2006). The success of MDA depends on interruption of parasite transmission by reducing the prevalence of microfilariae circulating in blood of individuals living in endemic areas (Ottesen et al., 1997). This can only be determined if there are diagnostic tools available to help determine infections in the populations so that MDA can eventually be stopped.

Experience from other disease elimination and eradication programmes has shown that having adequate diagnostic tools for surveillance are critical determinants of the success of the programme (Peeling & Mabey, 2014). The report from the Dahlem workshop, 1997 on eradication of infectious diseases identified 'practical diagnostic tools of sufficient sensitivity and specificity to detect levels of infection that can lead to transmission' as one of the three requirements for disease elimination and eradication (Dowdle, 1998).

Not too long ago, diagnostic tools for filariasis were limited to clinical diagnosis, detection of microfilariae and detection of antibodies to crude native antigen preparations (Weil, 2005). Although useful, these tools were inadequate in answering the basic questions that were crucial to LF elimination programmes. In areas with high prevalence of disease, a diagnostic tool like microscopy is considered sensitive because, parasite

burdens are high and can easily be detected. However, it requires trained technicians, a functioning microscope and quality assurance which is mostly lacking. As disease prevalence and intensity decreases, more sensitive tests are required because microscopy becomes no longer sufficiently sensitive to detect the cases remaining.

As the programme reaches elimination, large numbers of samples need to be tested to ensure interruption of transmission. This requires high throughput tests because as prevalence of a disease decreases, so does the positive predictive value of the diagnostic test and therefore highly specific tests are required to certify elimination (Peeling & Mabey, 2014). Over the years, there have been significant advances in diagnostic tools and it is necessary to study them and determine which will be most useful in defining targets and endpoints for stopping MDA (Weil & Ramzy, 2006).

Recent advances in molecular biological technology have given insights into the structure and function of the filarial parasite and led to the development of more sensitive and specific tools which are less labour intensive and have the ability to detect the parasites at very low intensity (Ramzy, 2002). Antigen and antibody detection assays offer an alternative means of detecting infection. These may be used either on site or in the laboratory. Antigen based tests like the rapid immunochromatographic tests (ICTs) offer an alternative means of detecting infection while the enzyme-linked immunoassays (ELISAs) offer higher sensitivity and high throughput capacity. Molecular assays such as polymerase chain reaction (PCR) are necessary for the detection of the few remaining cases as communities achieve interruption of transmission (Peeling & Mabey, 2014).

Successful MDA campaigns have brought several countries near the point of LF elimination. Current WHO guidelines on how to determine national LF elimination programme endpoints and for establishing post-MDA surveillance are based on the diagnostic tools available. The choice of diagnostic tools for monitoring and evaluating national programmes depends upon the sensitivity and specificity of the tools in assessing potential on-going transmission of parasites that cause LF; as well as their feasibility in terms of being fairly rapid, relatively inexpensive to perform on a large number of samples either at a point of care or in centralized laboratory and requiring minimal technical skills in its use (Gilbert et al., 2001; Gass et al., 2012; Steel et al., 2013).

For the purposes of monitoring interruption of transmission and for the certification of elimination, detection of specific antibodies to a pathogen may be the most sensitive marker of exposure (Peeling & Mabey, 2014). For post-MDA surveillance, especially where children are used as markers of transmission, detection of immunodominant epitopes of a pathogen in the sentinel population is the ideal tool to use. In some cases, it may be necessary to use a combination of tests to achieve the required sensitivity and specificity.

In China, the role of diagnostics as a surveillance tool to support policy decisions related to treatment and control strategies for *Schistosoma japonica* towards elimination is a typical example of the effective use of different types of diagnostics in disease control and elimination programmes (Zhou et al., 2011).

The tools now available to assess the impact of MDA according to the WHO guidelines are:

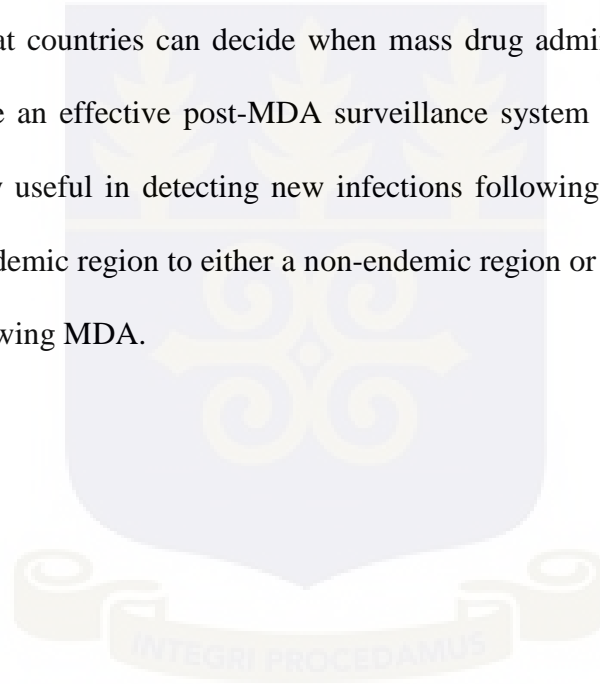
- blood film microscopy (60µl thick) to detect the presence of microfilaraemia;
- ICT to detect circulating antigen to *Wuchereria bancrofti*
- laboratory-based antigen Enzyme Linked Immunosorbent Assay (ELISA): Og4C3
- filarial antibody detection tests Bm14 and Wb123
- polymerase chain reaction (PCR) based techniques to detect parasite DNA in humans and mosquitoes (WHO, 2011a;Rebollo & Bockarie, 2014)

As programmes reduce the prevalence of diseases through mass drug distribution, more sensitive techniques are required to define endpoints. For LF in particular where the programmatic goal is elimination, sensitive surveillance tools are required particularly in those areas where such risks of recrudescence are high. As much as antigen based tests are important in determining the absence of transmission, they have their limitations. The current ICT which is an antigen based test is not able to detect infection prior to development of adult parasites, a process which may take up to 18 months following exposure to infective larvae stage (Steel et al., 2013).

It is therefore necessary to include the use of antibody based assays that can detect exposure to infective larvae allowing for early warning signals of potential parasite transmission. However, reports demonstrate that the ICT assay is equal to or more efficient than traditional parasitological examination to assess the prevalence of *W. bancrofti* infection (Ramzy et al., 1999; Simonsen & Dunyo, 1999; Schuetz et al., 2000).

In Africa and the Americas where LF is co-endemic with other filarial infections, specificity of such assays is necessary to prevent false results (Steel et al., 2013). Unlike PCR, microscopy and antigen assays, antibody detection assays are not able to identify active infection definitively. They also do not have the ability to distinguish between past and current infection but they can be used to estimate infection prevalence rates which is very important in surveillance activities (Kubofcik et al., 2012) .

The standardization of a panel of tools for LF will allow the definition of endpoint parameters so that countries can decide when mass drug administration (MDA) can be stopped and have an effective post-MDA surveillance system (Molyneux, 2009). Also, they will be very useful in detecting new infections following an influx of immigrants from a filarial endemic region to either a non-endemic region or one that has been cleared of infection following MDA.



2.6 Stopping MDA and post-MDA Surveillance

The World Health Assembly (WHA) called for a worldwide effort to eliminate lymphatic filariasis as a public health problem by the year 2020 (www.filariasis.org). For any disease elimination or eradication programme, a set of controls and criteria are needed to verify interruption in transmission and cure of all positive individuals. This then certifies that elimination has been achieved (Richards & Hopkins, 1989). For any disease elimination programme it is necessary to conduct effective monitoring and evaluation throughout the length of the programme.

For LF elimination, monitoring and evaluation is required to: (a) be able to monitor the effectiveness of MDA (b) assess if infection levels have been reduced to a point where transmission is no longer sustainable and recrudescence is unlikely to occur. (c) conduct surveillance activities after MDA is necessary to detect early recrudescence (WHO, 2012b; WHO, 2012c). Transmission dynamics differ depending on the vector species and the type of filarial parasite responsible for infection. The identification of the vector responsible for transmission will affect the threshold below which transmission can no longer be sustained even in the absence of MDA (WHO, 2012a).

A standard methodology Transmission Assessment Surveys (TASs) has been developed by WHO to assess whether MDA has successfully reduced the prevalence of infection to levels equal to or below the threshold required for the different vector species and parasites and to decide whether to stop MDA or not. TAS has three main objectives:

- (a) to provide a simple, robust survey design for documenting that the prevalence of lymphatic filariasis among 6 -7-year-old children is below a predetermined threshold
- (b) to provide the evidence base for programme managers that MDA can be stopped
- (c) to assure national governments that national programmes have achieved their elimination goals (WHO, 2013).

For any implementation unit (IU) to be eligible for TAS, two conditions have to be met:

(a) treatment coverage of the total population should exceed 65%, (b) the prevalence of infection in sentinel and spot-check sites should be below 1% using blood film as the diagnostic test for the presence of microfilaria or antigen prevalence using the ICT card test should be below 2%. Using these two criteria, some areas in an endemic country may be eligible for TAS while others may require more MDA (WHO, 2012b). Countries like Togo and Malawi have stopped treatment and undergone post-MDA surveillance while in countries like Ghana, Benin, Burkina Faso, Nigeria and Tanzania, some IUs have stopped MDA and are currently undergoing post-MDA surveillance (Sodahlon et al., 2013; Gyapong & Boatin, 2016).

The WHO recommends the repetition of TAS at least twice at a 2 or 3-year interval after MDA has been stopped in implementation units. This is done to make available enough proof that recrudescence has not occurred and transmission has been interrupted. For evaluation units that are not able to satisfy the criteria for a successful TAS, two additional rounds of MDA must be implemented and the process of TAS repeated. Togo is currently implementing laboratory-based surveillance in both endemic and non-endemic districts in its post-MDA surveillance programme to detect early recrudescence

(Mathieu et al., 2011). For any country to be considered to be ready for validation, all endemic implementation units (IU) must have successfully passed the 5-year post-MDA surveillance. Elimination is therefore verified at the national level (WHO, 2011b).



2.7 Summary of literature review

The 2020 target of eliminating lymphatic filariasis as a public health problem (Ichimori et al., 2014) is drawing close and governments, donor groups, pharmaceutical companies and non-governmental organizations are working hard at achieving this by giving political support, drug donations, provision of diagnostic tools and morbidity management. But to be able to ensure that this goal is achieved, there is the need to prevent re-infection in treated populations where the prevalence has gone below the 1% and 2% threshold for antigenemia and microfilaraemia in areas where MDA has stopped.

The reviewed literature elaborated on the background of lymphatic filariasis disease, its epidemiology and distribution globally, in Africa and specifically in Ghana. It also gave information on the biology, life cycle and transmission of the parasite *Wuchereria bancrofti* responsible for causing the disease in Sub-Saharan Africa and in Ghana. Lymphatic filariasis is a vector borne disease therefore the vectors responsible for carrying the parasite were described; their capacity, competence, composition and abundance. The strategies for stopping MDA and the conduct of post-MDA surveillance were described.

The post-MDA surveillance strategy is only in humans and does not include the vectors. Also, the focus is only on children born during and after MDA started. There is therefore a gap in knowledge of the status of the older population and the vectors. There is also a gap on best practices suited for each endemic area. The biggest gap is in the assessment of ongoing transmission of filarial parasites in the vector mosquitoes in the conduct of post-MDA surveillance. There is no clear-cut criterion to conduct assessment and to

determine a cut-off point where it can be said that the vectors are no longer capable of causing active or residual transmission.

In summary, this work seeks to fill in some of the gaps and also highlight some of the best practices to enable the 2020 elimination goal of the GPELF be achieved. Finally, it is necessary to continually test available diagnostic tools to determine their usefulness under different conditions.



CHAPTER THREE

GENERAL METHODS

3.1 Study design

The study was a repeated cross-sectional study which was conducted annually for four years (2010-2014) post-MDA. Post- MDA surveillance is necessary in areas that have stopped MDA because there is the need to monitor for recrudescence in the treated population. This study was done in three parts.

The first part of the study was a school-based survey. This investigated whether school-aged children between the ages of 6-10 years carried infection after 10 years of MDA in the study areas. It was necessary to carry out this investigation because children born during and after MDA started in endemic areas are expected to be free of infection if coverage and compliance to MDA is high and prevalence in the area of interest reduces with each round of MDA.

The second was a household survey which involved members of households in selected communities aged 11 years and older. The communities selected for the household survey were the communities in which the schools selected for the school-based survey were located. As per the TAS guidelines, the communities in which the selected schools for the school based surveys are conducted are the communities in which the household surveys should be conducted. This part of the study was done to determine if recrudescence had occurred in the treated population four years after MDA was stopped.

The third part was an entomology survey. This involved collection of mosquito samples from rooms of twenty selected households per community. Mosquitoes were collected from communities where both the school-based and household surveys were conducted. Sampling was done using the Pyrethroid Spray Catch (PSC) method. This was done to assess whether the vectors still carried parasites and were transmitting parasites to humans after MDA was stopped.

3.1.1 Inclusion Criteria

Each district included in the study met the following inclusion criteria:

- a. Completion of four or more effective rounds of Mass Drug Administration (MDA)
- b. Achieved coverage rate $\geq 65\%$ in four or more rounds of annual MDA
- c. Stopped mass drug administration for four years
- d. Microfilaria (mf) prevalence at monitored sentinel and spot check sites of the Neglected Tropical Disease Control Programme was less than 1% in the treated population (Table 3).
- e. Circulating filarial antigen prevalence was less than 2% in the treated population (NTDCP, unpublished).

Table 3: MDA coverage rates and microfilaria prevalence at sentinel and spot-check sites in study areas

District	Coverage									
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Awutu Effutu Senya	87.7	93.1	76.7	82.4	75.9	84.2	79.2	62.2	57.9	*78.4/60.5
Agona East and West	NA	91.7	85.1	87.6	76.0	77.0	88.1	87.9	75.6	*70.0/71.7
	MF Prevalence									
	Baseline (%)	Midterm (%)	Pre 5 th MDA (%)							Pre TAS (%)
Awutu Effutu Senya	23.8	0.2	0.2							0.6
Agona East and West	23.5	6.0	1.3							0.1

*In 2010 the districts had been divided so they were considered as separated areas

NA-Not Applicable

Source: Neglected Tropical Disease Control Programme, Ghana

3.2 Ethical Considerations

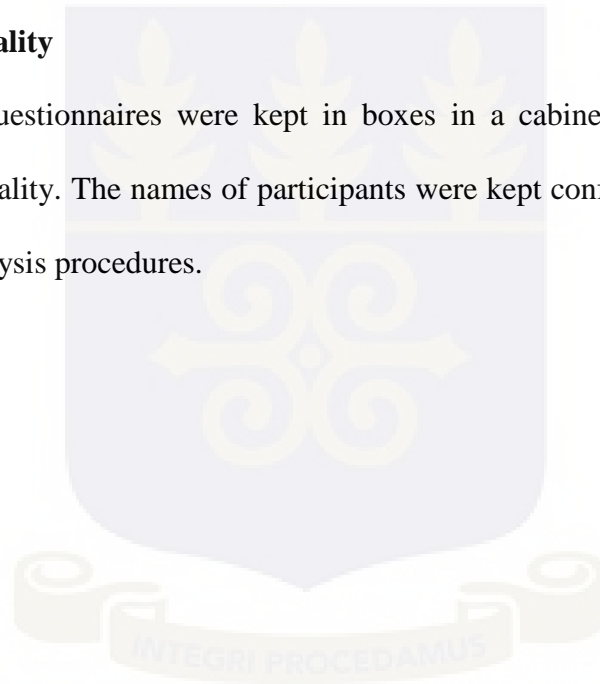
The study proposal was reviewed and approved by the Ethics Review Committee of the Ghana Health Service. The Ghana Health Service Ethics Review Board was chosen because that is the review board the School of Public Health, University of Ghana is affiliated to. Full board approval was sought. Informed consent and assent (Appendices I, II and III) was obtained from all study participants. This was done by explaining fully all the objectives of the study and procedures to all participants either in English or translated into their local dialect which was mainly fante. Persons who agreed to participate were made to sign or thumbprint in the presence of a witness who also signed or thumb-printed the same consent form. Permission was sought from the heads of schools and members of the community to take pictures which were used in this thesis.

Due to mainly poor knowledge of consenting to participate in studies, superstition and cultural beliefs, some participants refused to thumbprint. They were however willing to participate in the study so oral informed consent was obtained from them. This was documented on the consent form. All the wishes of potential and enrolled participants in the study were respected by permitting withdrawal from the study at any time and protecting their privacy by providing unique identification numbers for each participant. The names or identity of participants will not be revealed in any report that subsequently comes out of this study. Participants did not benefit directly but all participants who tested positive for ICT were referred to the nearest Ghana Health Service facility to be treated with ivermectin and albendazole as per WHO recommendations.

Permission was sought from the various regional, municipal and district directors of health services and education for the study to be conducted. The SHEP coordinators for the districts and municipalities were also duly informed to obtain their support and cooperation. Finally, permission was also sought from the municipal and district chief executives of the study areas to provide access to the communities of interest in order to be able to gather all the information relevant to the study. The NTD programme manager and his team provided support throughout the study.

3.2.1 Confidentiality

All completed questionnaires were kept in boxes in a cabinet under lock and key to ensure confidentiality. The names of participants were kept confidential and not revealed in any of the analysis procedures.



3.3 Pre-study activities

3.3.1 Training

Training was organized for all technicians and research assistants involved in data and sample collection for three days. The procedure of data and sample collection were explained to them and the necessary bench aids given to enable them perform their work better. Trainees went through simulations of all the processes to be undertaken on the field. Research assistants and technicians were trained to completely seek informed consent from all participants, complete the structured questionnaire, draw finger prick blood samples and also conduct pyrethrum spray catches.

3.3.2 Mobilization Activities

Fieldwork was undertaken between January and February, 2010, March and June, 2012 and May and July for 2013 and 2014. All stakeholders were engaged in the initial planning and proposal development phase to ensure that they all agreed with the work to be done and also ensure their collaboration and contribution. The Neglected Tropical Disease Control Programme (NTDCP) was involved and gave all the necessary support to the implementation of the study.

Information on school enrolment was obtained from the National School Health Education Programme (NSHEP). The population of the districts and all other district information were obtained from the Ghana Statistical Service (GSS). The head of the Parasitology Department of the Noguchi Memorial Institute for Medical Research (NMIMR) gave permission for all samples to be processed in the molecular biology and

immuno-parasitology laboratories of the department. The department also made available research assistants and technicians to assist in data and sample collection and processing.

All the Regional, Municipal and District directors of Health Services and Education of the selected districts were duly informed for their permission to conduct the study in the districts. These persons were namely, the Regional Directors of Health and Education in the Central Region and the Municipal Directors of Health and Education of Agona West and Effutu, the District Directors of Health and Education of Agona East and Awutu Senya.

The SHEP coordinators for the districts and municipalities were also duly informed to obtain their support and cooperation. Finally, the Municipal and District Chief Executives of the study areas were informed to enable us get access to the communities of interest to be able to gather all the information relevant to the study. Permission was sought from the Neglected Tropical Disease Control Programme Manager and his team and their support solicited for the study.

The entire team comprising the principal investigator, research assistants, technicians and the community based volunteers with the help of the heads of schools and leaders of the communities allotted days on which the different aspects of the study would be carried out ie: school-based survey, household survey and entomological survey. Each member of the research team was given a T-shirt with a lymphoid leg printed in front and “Stop elephantiasis now” printed at the back. This concept was adapted from the LF control programme MDA exercise. These T-shirts were worn by the members of the team to

make them easily identifiable by members of the community and staff of the participating schools. For all three surveys, mobilization activities were done with the help of headteachers, teachers, assembly men and community based volunteers.

For the school-based survey which involved school children aged 6-10years, the heads of the schools and the teachers served as liaison between the parents and guardians and the research team. They helped identify the school children in the target age group and organize meetings with the parents and guardians to fully explain the study to them. In some cases, PTA meetings were organized by the heads of the selected schools for the research to be explained to parents and guardians. Some heads of schools and teachers also consented on behalf of parents and guardians who were either illiterate or due to some traditional and cultural beliefs could/did not thumbprint or sign but gave oral consent.

In the boarding schools as well, written informed consent was given by the heads of the schools. On the day of the survey only children who presented signed or thumb printed consent forms were included. A questionnaire was administered to each participating child after which finger prick blood sample was drawn for testing.

In the household survey, assembly men and community based volunteers (CBVs) served as liaison between the community and the research team. The research team was introduced to the head of the community and opinion leaders and their cooperation and consent was solicited for the study to be carried out. In the communities, the information was passed on to members of the community in three ways.

The first was by use of the town-crier. This was normally done in the evening when all the members of the community had returned from their farms and various places of work or at dawn when the community was quiet and calm thus the voice of the town crier could be heard clearly by all members of the community.

The second was by use of the local public announcement (PA) system; this was also done at dawn when the atmosphere was quiet and all that was to be said would be heard clearly. The third means was by passing on the information to each other mostly after PTA meetings. The most efficient means of passing on the information mainly by observation was through the school children who had been included in the study. The children were able to explicitly describe what was entailed in the study and also convince members of their households and communities to participate if the research team entered their homes.

The entomological survey was conducted in the same manner as the household survey with the help of assembly men and CBVs. Informed consent was sought from all heads of households selected to be part of the study. Pyrethrum spray catches were done in all selected houses.

3.4 Study Sites

The study was conducted in four districts of the Central Region of Ghana; namely, Effutu Municipality, Awutu Senya District, Agona East District and Agona West Municipality. The geographic location of the four study sites are shown in Figure 5. Agriculture, fishing and commerce in this order are the main occupation of the residents of the four study districts and municipalities.

3.4.1. Effutu municipality

Effutu municipality covers a total land area of 95 square kilometers and has a total population of 68,597 which is 3.1% of the Central Region's population. It is one of the 20 administrative districts in the Central Region. The administrative capital is Winneba and it is situated between latitudes $5^{\circ}16'$ and $20.18''N$ and longitudes $0^{\circ}32'$ and $48.32''W$ of the eastern part of Central Region. The municipality is bordered to the west, east and north by the Gomoa East district and to the south by the Gulf of Guinea. The municipality's administrative capital is Winneba, a town renowned for its specialized major institutions of higher learning.

The Effutu municipality lies within the dry-equatorial climatic zone with the area being generally low lying with granite rocks and isolated hills. The vegetation is mainly that of the coastal savannah grassland. Rainfall is low and the dry season is long lasting up to six months per year. Annual rainfall ranges from 400mm to 500mm while temperature ranges between $22^{\circ}C$ and $28^{\circ}C$. Two major rivers are found in the municipality; the Ayensu and the Gyahadze. They enter the sea at Warabeba and Opram respectively.

There are 60 nurseries/kindergartens, 52 primary schools (24 public and 28 private schools), 32 junior high schools, seven senior high schools and one major tertiary institution (University of Education) in the Effutu municipality. The municipality also houses the National Sports College of Winneba where students receive advanced training in their specialized fields and disciplines. More than 80% of the population who are aged 11 years and older are literate with males having a higher proportion of literates as compared to females.

About one-third of the of the municipality's population are in primary school with a higher proportion of females enrolled than males. At the high school level, 13.3% of population is enrolled in junior high school while 6.9% are in senior high school. At the tertiary level however, about 28% of the population are enrolled which can be attributed to the location of the University of Education in the municipality. At this level, the proportion of males is higher than that of females while at the lower levels of education, the proportion of females enrolled is higher than males.

The municipality has one municipal hospital and two private hospitals. There is also a community health nursing school which provides auxiliary services to the communities in the municipality, two maternity and child health/ family planning clinics, one private clinic, two private maternity homes and several CHPS compounds. Health facilities are adequate in the municipality but access to these facilities is impeded by the poor nature of the roads. The five diseases most prevalent in the area are malaria, hypertension, heart disease, upper respiratory tract infection, typhoid and gynecological disorders (Ghana Statistical Service, 2014).

3.4.2 Awutu Senya District

The Awutu Senya district covers a surface area of 244.473square kilometres with Awutu Bereku as its administrative capital. Awutu Senya lies between latitudes 05°20'N and 05°42'N and longitudes 00°25"W and 00°37"W. The district is bordered to the south by the Gulf of Guinea, to the east by the Awutu Senya East Municipality, to the west by the Gomoa East and Agona East districts and to the north-eastern part side by the West Akim district. The Gomoa East District has dotted enclaves within the district. The district has a population of 86,884 which makes up 3.9% of the central region's population.

The topography of the district is mainly isolated undulating highlands with the coastline having lowlands with isolated hills. In the northern part of the district, the vegetation is mainly semi-deciduous while that of the southern part is savannah grassland. There are two major rivers which drain into the sea; Ayensu and Okrudu. Temperature within the district ranges between 22°C and 38°C. The major rainy season starts from April to July while the minor one starts from August to November. The dry season is from November to March.

The district has the highest number of educational institutions in the Central Region with about 700 schools (484 private and 216 public schools). The public schools constitute 73 pre-schools, 78 primary schools, 63 junior high schools and two senior high schools. About 48% of the districts' population aged 11 years and older is literate in English. A total of 31,951 children of school-going age are in school (16,402 males and 15,549 females) while 27,787 have attended school to a certain level in the past (14,436 males and 13,351 females).

In terms of provision of health care services, there are four public health centres, 12 Community-based Health Planning Services (CHPS) zones and 31 registered private health facilities which provide clinical, maternity and eye services. At the community level there are 132 outreach clinics, 189 trained Traditional Birth Attendants (TBAs) and 189 Community-based Disease Surveillance Volunteers (CBDSVs). There is however no district hospital. The top 10 diseases in the district are malaria, acute respiratory tract infection, skin disease, gastrointestinal infection, hypertension, rheumatism, home and occupational accidents, diarrhoeal diseases, road traffic accidents and anaemia in pregnancy (Ghana Statistical Service, 2014).

3.4.3 Agona East District

Agona East district is situated in the eastern corner of the Central Region and is located within latitudes 5° 30'N and 5° 50" N and longitudes 00° 35'W and 00° 55" W. The administrative capital is Nsaba. The district has a total land size of 539.7 square kilometres and a total population of 85,920 making up 3.9% of the region's population. It is bordered to the south by the Agona West municipality and the Gomoa East district, to the north by the Birim South district, to the northeast by the West Akim district, both in the eastern region, to the east by the Awutu Senya district and to the west by Asikuma-Odoben-Brakwa and Ajumako-Enyan-Essiam districts.

The district is located mainly within the wet semi-equatorial climatic zone. The topography of the area is mainly undulating from north to south as well as a diversified relief pattern with an average height of between 75-150 meters and a peak of 350 meters above sea level. It is partly coastal savannah and semi-deciduous forest. The rainfall

pattern in the district is bimodal with the maximum occurring between May and June and September - October. Annual rainfall ranges between 1,000mm -1,400mm.

There are 211 educational institutions in the district. This is made up of 28 private schools and 183 public schools. The public schools are made up of 10 pre-schools, 10 primary schools, 5 junior high schools and 3 senior high schools. The 183 private schools are made up of 50 pre-schools, 70 primary schools, 60 junior high schools and 3 senior high schools. Approximately 75.4 % of the population aged 11 years and above are literate while the remaining 24.6% are non-literate. Of the population aged three years and above in the district, 21.2%t have never attended school, 41.7% are currently in school while the remaining 7.1% have attended school in the past. More females (27.6%) than males (14.2%) have never attended school.

The district is served by two health centres, four clinics, one maternity home and ten Community-based Health Planning Services (CHPS) zones with five functional CHPS compounds. There are 85 outreach points where healthcare can be accessed (Ghana Statistical Service, 2014)

3.4.4 Agona West Municipality

The area lies between latitudes 05° 30'N and 05° 50"N and longitudes 00°35'W and 00° 55"W with Swedru as its municipal capital. Agona East has a total land area of 356.16 square kilometres. The population of the district is 115,358 which is 5.2% of the region's population. It is bordered to the north by Agona East district, to the south by Gomoa

East, to the northwest by Asikuma-Odoben-Brakwa district and to the west by Ajumako-Enyan-Essiam district.

The relief of the municipality is diversified with altitudes varying between 75-150m above sea level while the topography is undulating which slopes from north to south with isolated hillocks in the north-east. The municipality lies in the wet semi-equatorial climate zone making the area fall within the moist tropical and semi-deciduous forest. The Akora is the main river which flows through the municipality. Rainfall pattern is bi-modal with the maximum occurring in May/June and September/October. Annual rainfall ranges between 1000mm-1400mm.

In the municipality, out of the population aged 11 years and older, 77.2% are literate while the remaining 22.8% are non-literate. Males had a higher level of literacy as compared to females. Of the population aged 3 years and older, 14.8% have never attended school, 41.6% are currently in school and the remaining 43.6% have attended school in the past. There are five CHPS zones in the municipality where health care can be accessed (Ghana Statistical Service, 2014).

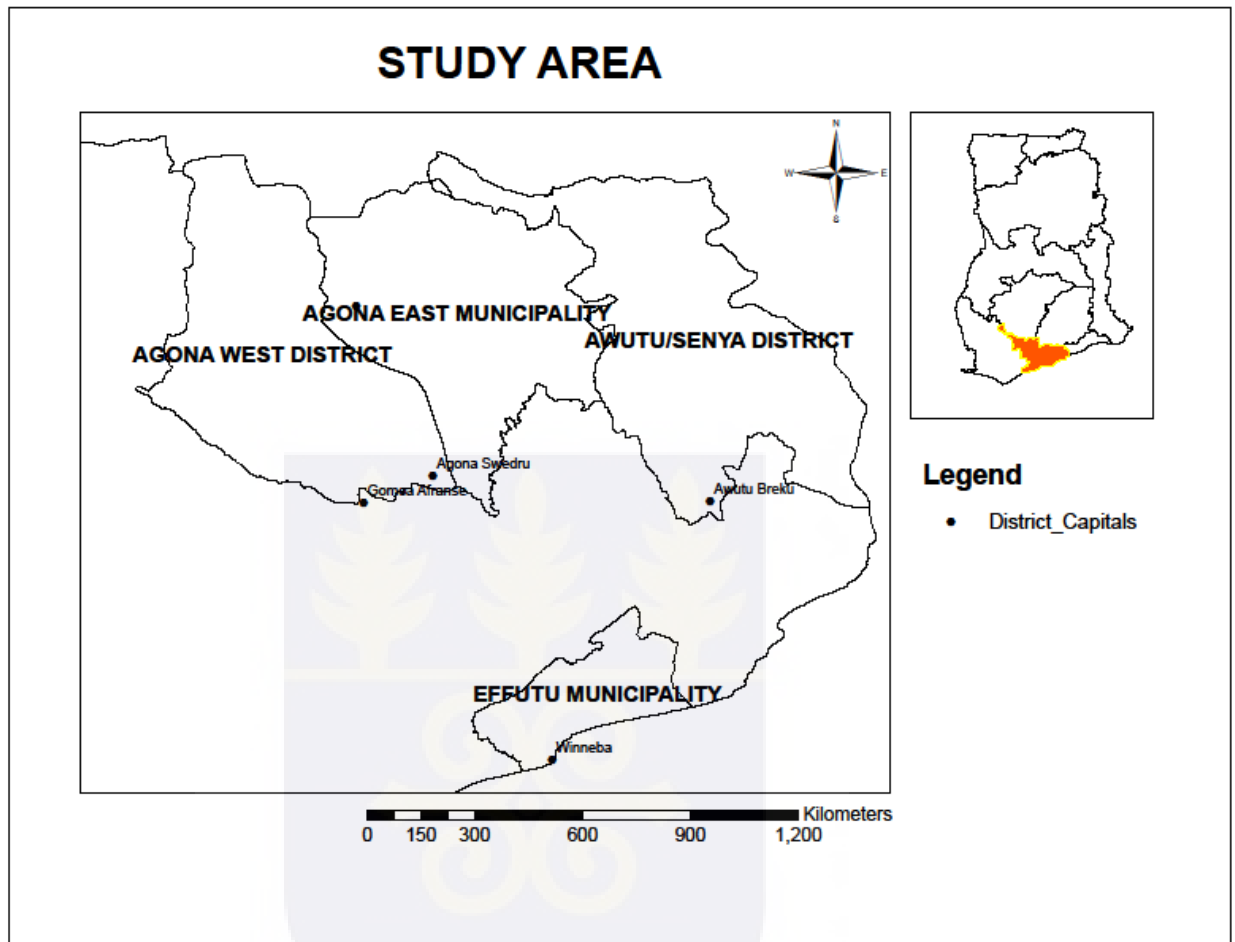


Figure 5: Map showing the location of the study areas

Source: Centre for Remote Sensing and Geographical Information Systems, University of Ghana

3.5 Study Population

Participants for the study included:

- a. School children aged 6-10 years attending the 30 selected schools in the four study districts/municipalities whose parents or guardians provided informed consent.
- b. Members of households in the communities in which the selected schools were located aged 11 years and older who provided informed assent or consent.
- c. Mosquitoes were collected from homes of members of the study communities where at least one room had been slept in overnight

3.5.1 Sample Size Determination

3.5.1.1 School-based survey

The sample size calculation for the school-based survey was done using the Survey Sample Builder. The Survey Sample Builder v 2.1 is an excel tool designed to help programme managers automatically calculate the sample size required for the survey and also determine the appropriate survey design to use in post-MDA surveillance. It also facilitates the random selection of clusters and children or households from a list of randomized numbers.

The information needed to be able to calculate the sample size for post-MDA surveillance using the Survey Sample Builder is as follows:

- a. a comprehensive list of all primary schools in the area of interest
- b. the net primary school enrolment rate
- c. the population of eligible school children in the target grades

- d. the average absentee rate for the schools in the area of interest
- e. the vector responsible for transmission of LF in the area of interest

The input of the information listed above into the SSB software generates;

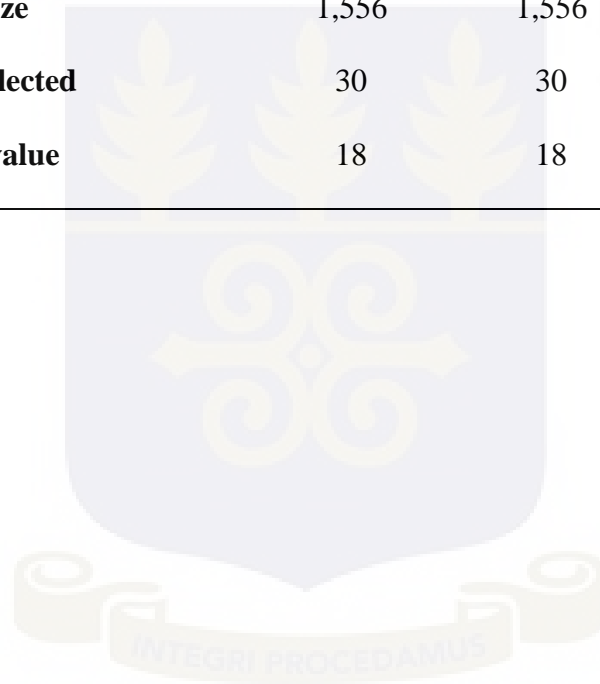
- a. the type of survey to be conducted, i.e. either a cluster survey or systematic sampling
- b. the sample size for the survey
- c. the sampling fraction
- d. the number of schools to be sampled
- e. the sampling interval
- f. the critical cut-off for an area to “pass” TAS

This software is available to LF elimination programmes conducting Transmission Assessment Surveys (TAS) and post MDA surveillance (WHO 2013).

In 2010 and 2012, the sample sizes calculated were 1,556 per year. School children in class one to class three aged between 6-10 years were the target group. The critical cut-off for both years was 18 from 30 schools selected per year. These were calculated using a net primary school enrolment of 80% in 2010 and 85% in 2012. The population of eligible school children provided by the SHEP in 2010 were 14,023 and 31,628 in 2012. The sample sizes calculated for 2013 and 2014 were 1,692 school children per year with a critical cut-off of 20. The net primary school enrolment rate for both years was 85% assuming an absentee rate of 15%. The population of eligible school children was 64,604 per year. The output of the calculation is summarized in Table 4. The sample size for the school surveys were calculated across the four districts.

Table 4: Sample size determination of the school-based survey using survey sample builder

Year	2010	2012	2013	2014
Net primary school enrolment rate	80%	85%	85%	85%
Absentee rate	20%	15%	15%	15%
No. of eligible children	14,023	31,628	64,604	64,604
Target sample size	1,556	1,556	1,692	1,692
No. of schools selected	30	30	30	30
Critical cut-off value	18	18	20	20

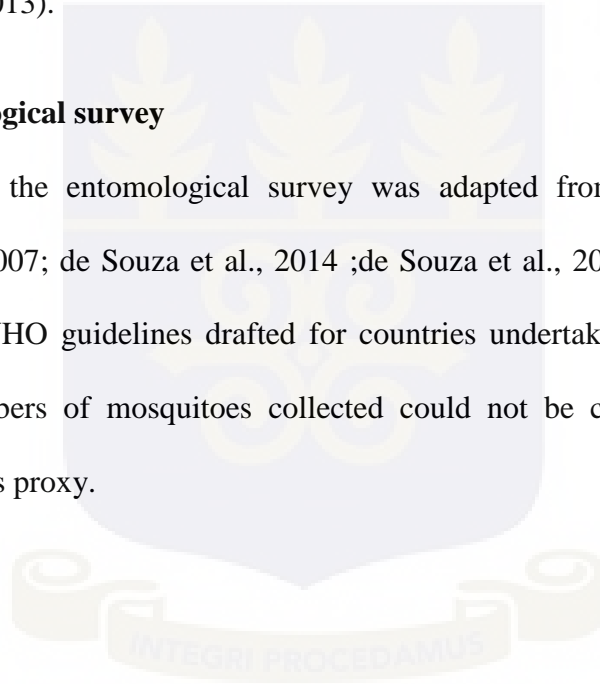


3.5.1.2 Household survey

Sample size for the household survey was adapted from the WHO guidelines drafted for countries undertaking Transmission Assessment Surveys (TAS). According to the guidelines, using members of the community as a sentinel population in areas where the LF elimination programme is taking place, a community-wide sampling of 1000 individuals provides a good representation to LF transmission status in the community and also a good assessment of the shifts in age distribution of infection following MDA (WHO, 2011b, 2013).

3.5.1.3 Entomological survey

Sample size for the entomological survey was adapted from studies conducted by (Boakye et al., 2007; de Souza et al., 2014 ;de Souza et al., 2015& Offei Owusu et al., 2015) and the WHO guidelines drafted for countries undertaking TAS (WHO, 2011b, 2013). The numbers of mosquitoes collected could not be calculated so households sampled served as proxy.



3.6 Sampling

3.6.1 School-based survey

Information on all the schools in the 10 regions of Ghana was provided by the National School Health Education Programme (SHEP) in a database called Education Management Information System (EMIS) created in Microsoft Excel[®]. All primary schools in the four selected districts were selected from the database. School children in primary class 1 to 3 were selected and the total enrolment noted. This number made up the total population of school children within the targeted age group.

The schools were listed and using randomization (RAND) in Microsoft Excel[®], the first 30 numbers were selected. The schools with the corresponding numbers were selected to be included in the study. The next 10 numbers were selected and the schools with the corresponding numbers selected as substitutes/supplementary schools. This was done to cater for any of the 30 schools which could not be located and also in cases where the heads of the schools did not agree to the study being conducted in their schools. These 10 schools were also selected to make up for the total sample size where the number of children sampled in the 30 schools did not make up for the required sample size.

The selected schools were located and the heads of the schools informed of the study and their consent sought to conduct the study in the school. A date was set to bring consent forms to the school, explain the study to school children in the target age group and give the consent forms for the children to be taken home for their parents' endorsement (Appendix I). In some schools, the headteachers organized Parent-Teacher Association (PTA) meetings where the research was fully explained to parents and guardians detailing

the objectives of the study, the procedures to be followed and the risks and benefits of participation. On the consent form, the telephone number of the principal investigator was made available for parents who needed further clarification or explanation to call.

A second date was set for questionnaire administration and blood sampling from all children within the selected age group who returned their signed or thumb printed consent forms. Every child within the target age group who brought back the endorsed consent form was included in the study. However, children who displayed some form of fear or were unwilling to participate even though their parents or guardians had given informed consent were not included in the study. Also, no child was included in the study without informed consent from a parent or guardian. All the eligible children willing to participate with informed consent from their parents or guardians were included in the study. Data collection began with the administration of a questionnaire (Appendix IV) followed by daytime blood sample collection from fingerprick.

3.6.2 Household Survey

For each school visited, the community in which the schools were located was included in the study. Identifying a landmark such as a mosque, community center or a church building as the central point in the community with the help of community volunteers, each community was divided into four quadrants. The closest house to the landmark in each quadrant from where the data collection team was standing was chosen as the first house to be sampled and subsequently every second house after the initial house. In each house entered, the study was fully explained to all persons who were prepared to listen.

In every house, two people who were ready and willing to participate were enrolled in the study after giving informed consent or assent. Individuals aged 11-17 years gave informed assent (Appendix III) while their parent or guardians consented on their behalf. All participants aged 18 years and older gave informed consent (Appendix II). A total of 40 individuals were enrolled from each community. A structured questionnaire was administered followed by blood sample collection. Data collection methods and tools for both the school based and household surveys have been described in detail in chapter four.

3.6.3 Entomological Studies (xenomonitoring)

For each community visited, a landmark such as a community center, mosque or church building was identified with the help of community members assigned leaders of the community to assist in data collection. This landmark was used as the central point of the community. From that point the community was divided into four quadrants. The closest house to the landmark in each quadrant from where the data collection team was standing was chosen as the first house to be sampled. Subsequently every second house after the initial house was selected to be sprayed until the number of five houses per quadrant was obtained. Twenty houses were sampled in each community.

Mosquitoes were collected using the Pyrethrum Spray Catch (PSC) method. In each house, two rooms in which residents had slept in overnight were sprayed. If compound house was entered into, three rooms were randomly selected provided the heads of the households agreed to participate in the study and sprayed. The chiefs and opinion leaders provided four trustworthy young members of the community who led and assisted in the

spraying activities voluntarily. Spraying was done between 5am and 9am. The houses where PSC was done were not necessarily the same houses where blood collection was done. However, in the very small communities, the two often overlapped.

Food, water and all movable furniture were taken outside before spraying a room. Greybaft sheets were spread on the floor and over furniture that were too heavy to be moved. Spray guns were filled with Raid® (SC Johnson) insecticide which contains 0.05% Pynamin Forte, 0.05% Neopynamin, 0.015% Deltamethrin, Solvent and 99.885% Fragrance. Two people, one outside and the other inside the room did the spraying.

The eaves, windows and doors of the rooms were first sprayed from the outside and immediately after, the inside of the rooms were sprayed. The rooms were kept closed for between 10 and 15 minutes after which the greybaft sheets were carefully removed from the room, folding them from the edges so the knocked-down insects would collect in the middle of the sheets. The sheets were moved out of the rooms to the open and the knocked-down mosquitoes separated from the other haematophagous insects (Figure 6).

The mosquitoes were transported to the field laboratory in well labelled Petri dishes lined with wet filter paper to prevent desiccation of the samples for processing (morphological identification and detection of *W. bancrofti* using LAMP and PCR). The Petri dishes were labelled with house number (if any), number of persons that slept in the sprayed room the night before and the number of mosquitoes caught in that room. All testing procedures have been described in detail in chapter five. The houses of ICT positive individuals were included in the xenomonitoring. Therefore, more than one spraying for

mosquito collection was done in these communities where individuals tested positive for ICT.





Figure 6: Mosquito capture using Pyrethrum Spray Catch (PSC) method, 2013 & 2014.



3.7 Sample Collection

A field laboratory was set up where all blood samples collected were transported to in cold boxes with ice packs. Blood samples collected from both the school based and community surveys from the four study districts were stored in Ethylenediaminetetraacetic Acid (EDTA) blood collection tubes. ICT was performed on all blood samples that were sufficient for the test to be conducted on to detect the presence of microfilaria antigen. Filter paper discs were blotted with 10 μ l of blood each (total of 60 μ l), air dried, stored in zip lock bags and transported to NMIMR to be used for Og4C3 and Wb123 ELISAs. All blood testing procedures have been described in detail in chapter four.

Mosquitoes collected using the PSC method were identified morphologically using morphological identification keys (Gillies & De Meillon, 1968; Gillies & Coetzee, 1987). They were packed singly into 1.5ml eppendorf tubes and transported to the NMIMR entomology laboratory in zip lock bags containing silica gel. The presence of *W. bancrofti* was detected using two molecular methods (LAMP and PCR) as described in chapter five.

3.8 Data Quality Checks

All field workers used in data and sample collection were trained in sample collection techniques and the use of the PDAs to ensure that all samples were well collected and interviews well conducted. Blood samples were kept in ice chests with ice packs and transported to the laboratory for analysis to detect the presence of *W. bancrofti* antigens and antibodies. The blood smear results were examined twice by well trained technicians to eliminate errors in accuracy associated with the individual's ability to identify microfilariae on the slide.

Questionnaires were designed with consistency checks to prevent errors at both the data collection and entry stages. In the study, all questionnaires were checked by the principal investigator before the information on it was entered into the database designed in Epi Info TM 7. All questionnaires were checked daily for completeness and accuracy. Incomplete questionnaires were taken back to the field to be completed within 24 hours. For all laboratory work, positive and negative controls were included in the assays as quality control measures. In addition to the positive and negative controls, internal controls were included in the assays which were used to determine cut-offs. Three days of training involving all data collectors was carried out before the start of data collection.

3.9 Limitations of the study

There were a few limitations to the study. The first of these was the limited shelf life of the ICT cards. ICT cards must be used within a stipulated time period and also require a cold chain for effective functionality and therefore must always be kept at optimum temperature. This could not be eliminated totally but to minimize this limitation, maintaining the cold chain was ensured at all times. A thermometer was kept in the 4°C freezer and checked twice a day to ensure that the right temperature was maintained. ICT cards were only brought out of the cold chain when they are ready to be used and kept in cold boxes with ice packs to maintain the optimal temperature.

The second limitation was the design issues associated with transmission assessment surveys. TAS is done in the same evaluation unit but uses different clusters at survey period. This did not make it possible to follow the same cluster over the period of the study.

Although community mobilization was done to ensure participation of all members of the community, the younger age groups were more inclined to participate in the study as compared to the older age groups. This resulted in the data being skewed towards the younger age groups, particularly the 20-29-year-olds.

The final limitation was the subjectivity of the LAMP assay. The results were read visually under UV light and sometimes it was difficult to distinguish between a low positive and a negative sample.

CHAPTER FOUR

VERIFICATION OF THE ABSENCE OF LYMPHATIC FILARIASIS TRANSMISSION IN HUMANS

ABSTRACT

Lymphatic filariasis is one of the 17 NTDs that have been targeted for elimination by the WHO. Mass drug administration is the recommended strategy. After stoppage of MDA in areas that have achieved elimination, transmission assessment survey (TAS) is the primary tool recommended by the WHO for post-MDA surveillance to monitor for early recrudescence. In Ghana, four districts, Awutu Senya, Effutu, Agona East and West have stopped MDA after 9 rounds. There is the need for post-MDA surveillance to monitor for early recrudescence. The aim of this study was to monitor for early recrudescence of LF post-MDA in Ghana using periodic surveys. This study was in two parts; school-based survey and household survey. From all study areas, a total of 1,600 children aged 6-10 years and 1,200 aged 11-60 years were selected annually for 4 years to participate in a four-year repeated cross-sectional study. Daytime finger-prick blood samples were collected from all consenting participants and tested using ICT, OG4C3 and Wb123 ELISA. Night time blood was collected for blood smear. Results obtained show that prevalence of LF in humans remained lower than the 1% threshold required to stop MDA and fairly stable [2010=0.07%, 2012=0%, 2013=0.12%, 2014= 0.12%] among 6-10-year-old children tested and the general population [2010=1.1%, 2012=1.0%, 2013=0.17%, 2014=0.60%]. Both surveys revealed very low antigen, antibody and microfilaria prevalence four years after last MDA. The results from this study show that the earliest time for recrudescence to occur in an endemic area that has stopped MDA goes beyond

four years post-MDA irrespective of when MDA started in all four districts and parasitemia levels at pre-MDA.



4.1 INTRODUCTION

One of the goals of the Global Lymphatic Filariasis Elimination Programme is reduction of microfilaria prevalence to levels where transmission is no longer sustainable. To be able to achieve this goal albendazole and ivermectin/diethylcarbamazine are administered to persons living in endemic areas annually (Turner, 1977; Aboagye-Antwi et al., 2015). Regular mass administration of these drugs has the tendency to reduce microfilarial loads thereby interrupting transmission of the filarial parasite but, has little macrofilaricidal effect (Ottesen et al. 2008). There is the need therefore to continue with mass drug administration (MDA) until adult worms die naturally or cease to produce microfilaria. The time estimated for this to occur is about five years (Michael et al., 2004; Parker & Allen, 2013).

The conduct of continued annual treatment reduces the density of parasites circulating in the blood of infected persons. This leads to a reduction in the prevalence of infection to such low levels that transmission can no longer be sustained and therefore the occurrence of new infections eventually ceases. A reduction of infection below target thresholds implies that MDA is no longer required (WHO, 2016b). What then is required is surveillance to ensure that any cases of recrudescence are detected early and dealt with accordingly to prevent a surge in transmission (WHO, 2011a).

MDA has variable impact depending on the mf prevalence pre-MDA. The WHO technical advisory committee report on lymphatic filariasis (LF) in 2005 stated that in areas like Papua New Guinea, transmission has been completely interrupted after four rounds of MDA in one site using a combination of diethylcarbamazine (DEC) and

albendazole (Lammie et al., 2002; Melrose, 2002). Countries like Ghana and Mali have recorded significant reduction in transmission by *Anopheles gambiae* and *An. funestus* after three rounds of MDA using a combination of ivermectin and albendazole (Dreyer et al., 2000; Dreyer & Piessens, 2000). Areas like Pondicherry in India have achieved low levels of transmission after nine rounds of MDA with DEC only (WHO, 2005a).

In resource constrained settings, maintaining high MDA coverage over several years is challenging but commitment and consistency has made it possible in areas like Ghana and India to reach the “Stop MDA” target. This process however has to be approached with caution especially in areas where co-infection with other filarial parasites like *Loa loa* is present. This is because serious adverse events are experienced by the ‘treatable’ population which may sometimes led to death of affected persons (WHO, 2000; Kolaczinski et al., 2007; Padgett & Jacobsen, 2008; Ashton et al., 2011).

Surveillance is an important component of all elimination programmes. Effective surveillance requires diagnostic tools or techniques which are sensitive and specific, rapid and easy-to-use. The ability of these diagnostic tools to detect recrudescence is also very important (Murdoch et al., 2002). Surveillance may be done routinely or periodically depending on the disease of interest. Earlier guidelines proposed by the WHO for post-MDA surveillance were cumbersome, resource-intensive and conservative based on actual field implementation.

This led to the design of new guidelines that were more logistically feasible and adaptable to varying demographic and epidemiologic conditions called Transmission Assessment Surveys (TAS) (Deming & Lee, 2009; Chu et al., 2013). Transmission

Assessment Surveys are designed to help LF elimination programmes determine whether prevalence in endemic areas have gone below the critical threshold of infection whereby further transmission will not occur. Its design is flexible and can be modified to best fit the local situation of an area (WHO, 2011b). TAS provides useful information to national LF elimination programmes regarding the decision to stop MDA, where MDA should be stopped and can be used as a post-MDA surveillance method to detect early recrudescence in an area that has stopped MDA.

Generally post-MDA surveillance can be implemented in two ways. It can be done as periodic surveys or ongoing surveillance. Repetition of TAS is best when conducting periodic post-MDA surveillance. This is necessary to evaluate whether recrudescence has occurred. The best option is to conduct these surveys roughly 2-3 years after the previous survey using a similar TAS design throughout. It is necessary to compare antigen-positive cases to the critical cut-off value rather than the differences between the first and second surveys. If the results obtained are greater than the critical cut-off value, this could mean recrudescence has occurred.

The population groups of interest that should be surveyed are children born after the start of MDA activities aged between 6 and 10 years and the older population in the areas where MDA has stopped. Ongoing surveillance should cover entire endemic areas. The population groups of interest that should be surveyed are military recruits, university students, blood donors and hospitalized patients (WHO, 2008). Clinical laboratories in hospitals could be made use of to obtain blood samples from these groups of persons and tested for the presence of microfilaraemia, antigenaemia or antibodies (WHO, 2011b).

For assessment of progress towards the endpoints of LF, the diagnostic tool approved for surveillance in areas that have stopped MDA is the immunochromatographic card test (ICT). It is the value obtained from the conduct of tests using this tool that the critical cut-off value is measured against. This tool especially, is used to assess cohorts of children born since the LF elimination programme began and also monitor transmission and infection in older children and adults who are eligible to be treated in the MDA programme (WHO, 2005b; Baker et al., 2010). Egypt has successfully used this approach to determine the absence of transmission in endemic areas after multiple rounds of MDA (Ramzy et al., 2006). The ICT card is also being used in many other countries which have reached the post-MDA surveillance phase including Ghana.

Alternative approaches to endpoint assessment and surveillance have been included and even though they are not rapid and field applicable they provide extensive information on the post-MDA surveillance phase of the evaluation unit (EU) being assessed. These approaches include detection of exposure antibodies in children using Enzyme-Linked Immunosorbent Assays (ELISA) (Njenga et al., 2007) and polymerase chain reaction (PCR) methods for monitoring filarial parasites in both humans and mosquito vectors (Ottesen, 2006; Bockarie, 2007).

In Ghana, the MDA programme has covered all at-risk populations and completed more than 6 rounds since its inception in all endemic areas. In line with the GPELFs goals, the neglected Tropical Disease Control Programme (NTDCP) in Ghana aims at reducing LF to less than 1% in the entire endemic population and obtaining an antigen prevalence of 0% among children by 2015 (Abudu et al., 2015). After 9 and 10 rounds of MDA in the

Awutu Senya, Effutu, Agona West and Agona East districts/municipalities, LF prevalence levels have decreased from a baseline of almost 24% to 0.6% in Awutu Effutu and 0.1% in the Agona East and West district/municipality (Table 3) (NTDCP, unpublished).

The WHO criteria for stopping MDA and conducting post-MDA surveillance requires that prevalence rates be below 1% for an endemic area to consider stopping MDA and begin surveillance activities. However, surveillance needs to be continuous to ensure that the disease does not resurge within the population of interest. Also monitoring is needed to determine changes in LF infection and transmission to ensure that the fall in infection is sustained and early recrudescence is detected.

In 2006, Zanzibar was the first country in Africa to complete five rounds of MDA and achieve 100% geographic coverage with effective treatment coverage of over 65% in each round. Due to the lack of continuous surveillance in 2012 when TAS was conducted it was shown that transmission had not been interrupted therefore MDA had to be resumed in 2013 (Rebollo et al., 2015). In the past, diagnosis of LF infection was based on detecting microfilaraemia. However, it is now clear that this approach is not sensitive enough to detect many infections of low density often found in children and those where adult worms are present but produce no microfilariae (Ramzy et al., 1997; Witt & Ottesen, 2001). This may have been the reason for Zanzibar's resurgence of infection. The development of new and improved diagnostic techniques has led to the appreciation of the extent and importance of LF infection globally. The availability of filarial specific antigen assays which provide more sensitive diagnosis especially in early or low-density

infection have brought about a change in diagnosis of LF and many other filarial infections (Witt & Ottesen, 2001).

There are at least seven diagnostic tools currently available to detect LF exposure and infection. Five of these tools were evaluated by Offei Owusu et al., (2015) and the results obtained showed that for post-MDA monitoring activities, the usage of a combination of tests and comparison of the results obtained is recommended to ensure accuracy of the results obtained. In terms of convenience, comparable accuracy, ability to standardize easily and easy accessibility as a point of care test, ICT was the recommended diagnostic test while for specificity and a test being less tedious even though not field-based, PCR was recommended. Therefore, PCR can be used together with ICT to ensure that results obtained are as close to accurate as possible while all the other tests can be used as confirmatory tests.

Parasite infection rates and intensity in both humans and vector species decrease after several rounds of MDA. Humans may however remain antigenemia positive and sometimes microfilaria positive even after the interruption of transmission. It is therefore necessary to assess whether recrudescence has occurred four years after stopping MDA. Demonstrating the absence of recrudescence in these areas in Ghana, will be the first step in the verification process.

The approach will be a prototype which can be adapted by other districts classified as no longer endemic thus shrinking the LF map district by district. The main objective of this aspect of the study was therefore to conduct post-MDA surveillance using the Transmission Assessment Survey (TAS) protocol to verify the absence of transmission of

LF in the four selected previously endemic districts/municipalities and also monitor for early recrudescence using four diagnostic tools. This protocol was modified to suit the study areas of interest. Data for this study were collected in two parts. The first set of data were collected as part of multicountry transmission assessment surveys (TAS) conducted in 2010 and 2012 while the second part collected in 2013 and 2014 as a follow-up to the multicountry TAS. All four years of data collected were analysed to give a full description of LF status in the areas of interest.



4.2 MATERIALS AND METHODS

The study was conducted in four districts/municipalities; Effutu Municipality, Awutu Senya District, Agona East District and Agona West Municipality. It was a longitudinal study involving data collection in 2010, 2012, 2013 and 2014. Each period of study included a school-based survey and a household survey.

4.2.1 Data collection methods and tools

4.2.1.1 Questionnaire administration

Study IDs were assigned to all participants. Questions asked covered issues on demography (age, sex, place of residence, marital status, occupation and educational status), drug treatment, bed net use, presence/absence of lymphoedema, compliance to MDA, frequency of participation in MDA and reasons for non-compliance to MDA. All the information was captured on a structured questionnaire. The questionnaires were administered by members of the data collection team who had been trained in questionnaire administration. Questions were asked in the local languages which were mainly fante and twi.

4.2.1.2 Blood collection

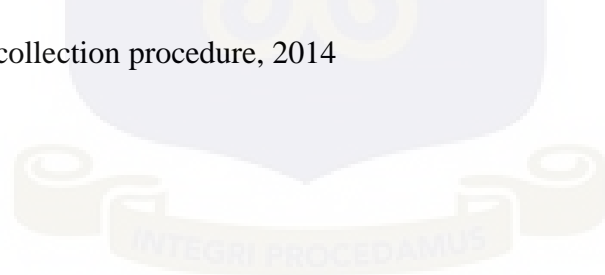
Daytime blood samples were drawn from all study participants. This was done by cleaning the surface of the tip of the finger to be pricked with 70% ethanol swab then allowing it to dry for a few seconds. The inner side of the tip of the finger was pricked using a sterile and self-extracting lancet which was discarded into an available safety/sharps container box after use. The first drop of blood was wiped off with sterile cotton wool and discarded into the safety box made available. About 450 μ l of blood i.e. about 10 drops (one drop is approximately equivalent to 40 μ l) was collected into an

EDTA coated tube and inverted a few times to prevent clotting. The blood samples collected were kept in sealed zip lock bags in cold boxes with ice packs to maintain them at optimum temperature and transported to the field laboratory for testing. All blood samples were labelled with unique IDs. Dry cotton wool was then placed at the blood collection site to help stop the blood flow and facilitate clotting (Figure 7).





Figure 7: Blood collection procedure, 2014



4.2.2 Laboratory testing

4.2.2.1 Immunochromatography card test (ICT)

To assess the presence of circulating filarial antigen (CFA), 100µl of the blood collected by fingerprick into EDTA tubes was tested using the ICT. The ICT cards were stored in a refrigerator with temperatures between 2°C and 8°C. The temperature was monitored daily with a thermometer to ensure that storage conditions were optimal. The number of cards required for each day were removed in the morning and placed in cold boxes with ice packs to keep them cold and at optimal temperature. Before use, each ICT card was removed from its pouch, labelled with the participant's ID and laid flat on the work bench.

The blood samples in the EDTA tubes were shaken thoroughly to mix and 100µl pipetted onto the white pad area of the card. The adhesive liner was then removed and the card closed after 30 seconds to one minute when the blood samples had flowed into the pink area and it was completely wet. The start time was then recorded on the front of the card while the timer was set. At exactly 10 minutes, the results were read and the appropriate result (positive, negative, invalid) circled on the card to create a permanent record.

A test was considered positive when both test and control lines could be seen through the visualization window. Any line (light or dark) which appeared in the test position indicated that the test result was positive. A test was considered negative when only the control line could be seen through the visualization window. A test was invalid if both control and positive lines were not seen in the visualization window or only the positive line could be seen.

All invalid tests were repeated. The exact 10 minutes stop time was also recorded on the front of the card. All the ICT card tests were conducted according to the manufacturer's instructions and done by trained laboratory personnel. For quality control purposes, a positive control was tested on each batch of cards to ensure they were in good working condition.

4.2.2.2 Dried blood spots and ELISA (Og4C3 and Wb123)

Round filter paper manufactured by Tropicbio Limited[®] was used for dried blood spots. Each filter paper had six protruding ears. Each filter paper was labelled with the study participant's ID, and held in place by a pencil pushed into styrofoam boards. Each ear was blotted with 10µl of participant's blood. Each filter paper had approximately 60µl of blood blotted onto it. The blood spots were air dried overnight and each filter paper sample packed into airtight zip lock bags and kept in a -20°C freezer provided by the district health management team after each day's work.

All samples collected and processed were transported to NMIMR at the end of field activities and stored at -20°C for further testing. Four dried bloodspots were used to perform Enzyme Linked Immunosorbent Assays (ELISA); three (3 x 10µl) were used to measure quantitative filarial antigen levels by the Og4C3 ELISA while the fourth bloodspot (1x10µl) was used to determine the presence of the third stage of the microfilaria (L₃ stage) by Wb123 ELISA.

4.2.2.2.1 Og4C3 ELISA

Filter paper samples were put in 1.5ml eppendorf microcentrifuge tubes. As per the instructions of the manufacturer in the kit, 200µl of sample diluent was added to each tube and the blood samples were left to elute overnight in a refrigerator at temperatures between 2°C and 8°C. The next morning, all tubes were placed in a pre-heated water bath at 100°C for five minutes. After heating the samples in the water bath, they were centrifuged at 2,000g for 15 minutes to get the supernatant which contained the heat stable antigen. All reagents were brought to room temperature as per manufacturer's instructions. A plate map was drawn with each well indicating the sample ID and matched with the test plate (Table 5).

All samples were tested in duplicate with kit provided standards. Fifty microliter (50µl) aliquots of boiled sample supernatant were added to each test well according to the plate map. Up to 40 samples were tested per plate. Fifty microliter (50µl) aliquots of standard antigens (those were not boiled) and conjugate control (this was the sample diluent alone) were added in duplicate on each plate. The plates were kept moist by placing them in a zip lock bag lined with wet tissue and sealed tightly. They were placed in an incubator pre-set to 37°C for 60 minutes.

After 60 minutes, the plates were removed and washed with pre-prepared 1X wash buffer. The plates were washed three times, inverted and tapped gently on tissue to remove residual buffer after each wash. One percent (1%) hydrogen peroxide (H₂O₂) solution was prepared by adding 400µl of 30% hydrogen peroxide to 12ml of 1X wash buffer. Fifty microliters (50µl) of the 1% hydrogen peroxide solution was then added to

each test well excluding the wells containing the standards and controls and the plate incubated for 10 minutes at room temperature.

The plates were then washed three times, inverted and tapped gently on laboratory tissue to remove residual buffer from the wells. Fifty microliters (50 μ l) of anti-Onchocerca antibody was diluted in 6ml of antibody diluent, mixed thoroughly and 50 μ l of the solution added to each well in each test plate. The plate was then placed back on the wet tissue in the zip lock bag, sealed and placed in an incubator pre-set at 37°C for 45 minutes.

After 45 minutes, the plate was washed three times, inverted and tapped gently as before. Fifty microliters (50 μ l) of anti-rabbit HRPO conjugate was diluted in 6 ml of antibody diluent and 50 μ l of the solution added to each well. The plate was then placed back on the wet tissue in the zip lock bag and sealed and placed in the incubator at 37°C for 45 minutes. After 45 minutes, the plate was washed as before to remove residual buffer. The substrate was prepared by diluting 300 μ l of TMB substrate concentrate in 5700 μ l of substrate buffer and mixed thoroughly. Fifty microliters (50 μ l) was added to each well and incubated for 15 minutes in the dark at room temperature. The reaction was then stopped by adding 50 μ l of stopping solution to each well.

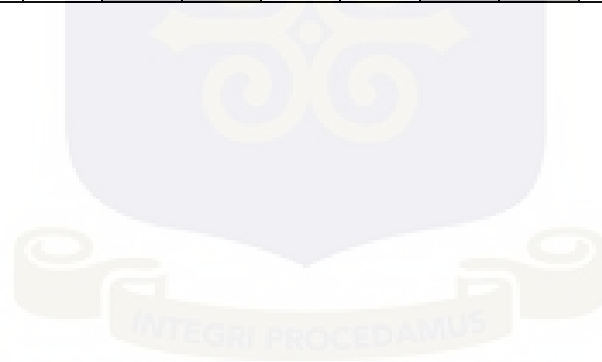
A colour change from blue to yellow indicated a stop in the reaction. Each plate was then read using a spectrophotometer at 450nm wavelength immediately the reaction was stopped. Any sample with an optical density value greater than or equal to Standard 3 (Table 5) was considered as positive. All positive test samples were repeated to confirm the results. By way of quality control, each test kit had seven standards/ controls which

were run alongside the test samples and double distilled water as the negative control.

The results obtained were compared to the standards and the negative control.

Table 5: Sample plate map for Og4C3 ELISA assay

Test Samples											Standards	
	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	9	9	17	17	25	25	33	33	Standard 1	Standard 1
B	2	2	10	10	18	18	26	26	34	34	Standard 2	Standard 2
C	3	3	11	11	19	19	27	27	35	35	Standard 3	Standard 3
D	4	4	12	12	20	20	28	28	36	36	Standard 4	Standard 4
E	5	5	13	13	21	21	29	29	37	37	Standard 5	Standard 5
F	6	6	14	14	22	22	30	30	38	38	Standard 6	Standard 6
G	7	7	15	15	23	23	31	31	39	39	Standard 7	Standard 7
H	8	8	16	16	24	24	32	32	40	40	Standard 8	Standard 8



4.2.2.2.2 Wb123 ELISA

Microcentrifuge tubes (1.5ml) were labelled with sample identification numbers. A dry blood spot with a corresponding identifying number was placed in the tubes and 250 μ l of sample dilution buffer provided in the reagent kit was added onto each blood spot. The samples were then incubated overnight at 4°C on a gently rocking platform. All kit reagents and samples were brought to room temperature before use on the day of testing. Wash buffer solution at 1X concentration was prepared by mixing 120ml of 10X wash buffer provided in each kit with 1,080ml of double distilled water until all crystals were dissolved. This solution was stored at room temperature for use.

The microtiter assay plates were numbered with a permanent marker and taped at the top and bottom edges to prevent the detachable wells from falling out during the wash steps. The controls which come as part of the kit (Kit positive, Kit negative, Kit Low positive) and in-house controls (IHC) supplied by the Centres for Disease Control and Prevention (CDC), Atlanta (IHC H3, IHC H19) were mixed well by vortexing and diluted in sample buffer as shown below (Table 6). One hundred microliters (100 μ l) of the samples and controls were added per well in duplicate. The plate map format is shown in Table 7.

The filled plates were covered with a plate cover and incubated at 37°C for 30 \pm 2 minutes. During the incubation time, the ELISA spectrophotometer was turned on to allow the machine to warm up and the protocol set to read the plate at 450nm. After incubation, the plate was washed 6 times by filling all the wells with 1X wash buffer using a squirt bottle, inverting and tapping gently on laboratory tissue to remove residual buffer. A 1:100 dilution of IgG4-HRP was prepared by adding 120 μ l of IgG4 to 12ml of

conjugate diluent (for 1 plate). This was done in the dark, away from light because the conjugate is light sensitive. One hundred microliters of the dilute IgG4-HRP was added to each well using a multichannel pipette. The plate was covered again and incubated at 37°C for 30 ± 2 minutes.

After incubation, the plate was washed 6 times as before. One hundred microliters (100µl) of liquid TMB substrate was added to each well and the plate incubated at room temperature in the dark for 10 ± 1 minute. After incubation, 50µl of stop solution was added to each well in the same sequence as the TMB. The plate was then incubated at room temperature for 1 minute without a plate cover. Any bubbles remaining were removed gently by applying air with an empty and dry squirt bottle. The plate was then read at 450nm with the micro plate reader. A Levy Jennings chart was drawn for each set of controls for each batch of test kits as a way of quality control

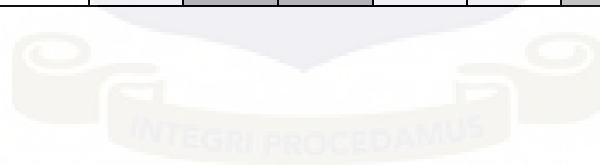
Table 6: Control Dilution Volumes for Wb123 assay

Control	Dilution	One Plate		Two Plates	
		Control volume (µl)	Buffer volume (µl)	Control volume (µl)	Buffer volume (µl)
Kit Positive	1:50	5	245	10	490
Kit Negative	1:50	5	245	10	490
Kit Low Positive	1:50	5	245	10	490
IHC H3	1:75	5	370	7	518
IHC H19	1:75	5	370	7	518

Suggested volumes per plate (2 wells at 100µl per well needed)

Table 7: Sample plate map for Wb123 ELISA assay

	Controls		Test Samples									
	1	2	3	4	5	6	7	8	9	10	11	12
A	Kit Pos.	Kit Pos.	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 27	Sample 27	Sample 35	Sample 35
B	Kit Neg.	Kit Neg.	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 28	Sample 28	Sample 36	Sample 36
C	Kit Low Pos.	Kit Low Pos.	Sample 5	Sample 5	Sample 13	Sample 13	Sample 21	Sample 21	Sample 29	Sample 29	Sample 37	Sample 37
D	H3@ 1:75	H3@ 1:75	Sample 6	Sample 6	Sample 14	Sample 14	Sample 22	Sample 22	Sample 30	Sample 30	Sample 38	Sample 38
E	H19 @ 1:75	H10 @ 1:75	Sample 7	Sample 7	Sample 15	Sample 15	Sample 23	Sample 23	Sample 31	Sample 31	Sample 39	Sample 39
F	Buffer only	Buffer only	Sample 8	Sample 8	Sample 16	Sample 16	Sample 24	Sample 24	Sample 32	Sample 32	Sample 40	Sample 40
G	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 25	Sample 25	Sample 33	Sample 33	Sample 41	Sample 41
H	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 26	Sample 26	Sample 34	Sample 34	Sample 42	Sample 42



4.2.2.3 Blood Smear

All participants (both school-aged children and adults) who tested positive for the ICT were sampled to test for the presence of microfilaria. Sixty microliters (60 μ l) (approximately equivalent to 3 or 4 drops) of night blood sample was collected from each participant between 9pm and 2am by trained laboratory technicians. Sample collection was done in the homes of all consenting participants. The procedure was the same as described for blood sample collection in section 4.2.1.2.

To determine the presence of microfilaria, 20 μ l each of blood samples collected was streaked onto a clean microscope glass slide to make 3 lines of 20 μ l each. The slides were then placed in slide racks and allowed to air dry overnight. The slides were then loaded onto staining racks and the blood de-haemoglobinized for approximately 15-20 minutes in tap water and air dried. The blood smears were then fixed in absolute methanol for about a minute or two; air dried and stained with 10% Giemsa solution for 45 minutes. The slides were air dried again.

The slides were examined under a light microscope using the 10X objective lens first to locate the microfilaria, followed by using the 40X lens to identify the filarial species present. The examined slides were then kept in slide boxes for independent confirmation by a senior laboratory technician. Blood testing procedures are shown in Figure 8D.

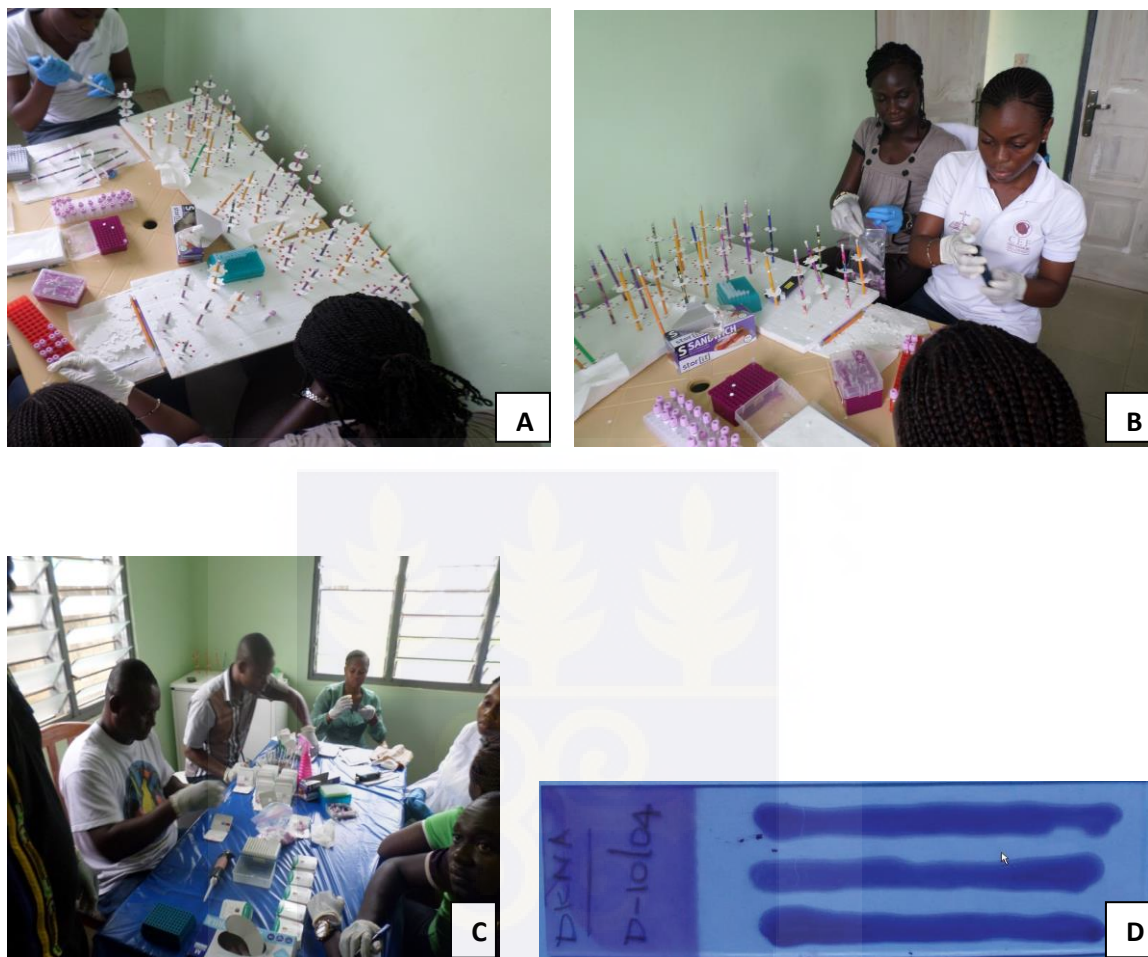


Figure 8 : Pictures showing the different testing procedures performed on sampled blood, 2013 & 2014

A & B: Filter paper blotting for ELISA (Og4C3 and Wb123)

C: ICT Card test waiting to be read

D: Blood smear to detect the presence of microfilaria

4.2.3 Description of variables

This section categorizes the dependent and independent variables that were to be included in the analysis. The outcome/dependent variables were prevalence of infection in the population and sensitivity and specificity determination of the diagnostic tools used for monitoring and surveillance. The independent variables were broadly grouped as demographic, practice and clinical status (Table 8).

Table 8: Definition of variables and their scale of measurement

Variables	Operational Definition	Type of Variable	Scale of Measurement
Demographic			
Age	Age in years of study participants	Independent	Continuous
Sex	This was defined as either male or female	Independent	Binary - Male - Female
Practice			
Bednet use	This was defined as sleeping in a bednet or not	Independent	Binary - Users - Non-users
Bednet use recall over 7 nights	How many times the study participant recalled sleeping in a bednet over a period of 7 nights	Independent	Categorical - Once - Twice - Thrice - Four times - Five times - Six times - Seven times
MDA participation	This was defined as participated in MDA or not	Independent	Binary - Participants - Non-participants
Clinical Status			
Hydrocoele	The presence or absence of hydrocoele	Independent	Binary - Present - Absent
Lymphoedema	The presence or absence of lymphoedema	Independent	Binary - Present - Absent

4.2.4 Statistical Analysis

All data collected were entered into Epi Info™ 7.1. Cleaning of data was done by removing duplicate information and all tests that had indeterminate and invalid results. All invalid and indeterminate results were retested to confirm the results obtained making it plausible to remove any data with these results. Blood samples that were not enough blood to conduct at least one test were excluded from the analysis. Graph Pad Prism 7 was used in analysing the data Mega Stat (Microsoft Corporation), and Microsoft Excel data analysis softwares were used in analysing the data.

Background data analyzed included age, sex, bednet use, bednet use recall, participation in MDA and disease status of study participants. Data was analyzed using percentages, proportions and chi-square to determine association between the different diagnostic tests; bivariate analysis was done to determine the association between age and prevalence of disease. Simple proportions were used to determine the prevalence of disease and positivity of tests. In order to determine the validity of the different tests, sensitivity, specificity, positive and negative predictive values of the different tests were calculated. Significance for analysis was set at $p < 0.05$.

$$\text{Sensitivity} = \frac{\text{True positives}}{\text{True positives} + \text{False negatives}}$$

$$\text{Specificity} = \frac{\text{True negatives}}{\text{True negatives} + \text{False positives}}$$

$$\text{Positive Predictive Value} = \frac{\text{True positives}}{\text{Total positive test results}}$$

$$\text{Negative Predictive Value} = \frac{\text{True negatives}}{\text{Total negative results}}$$

4.3 RESULTS

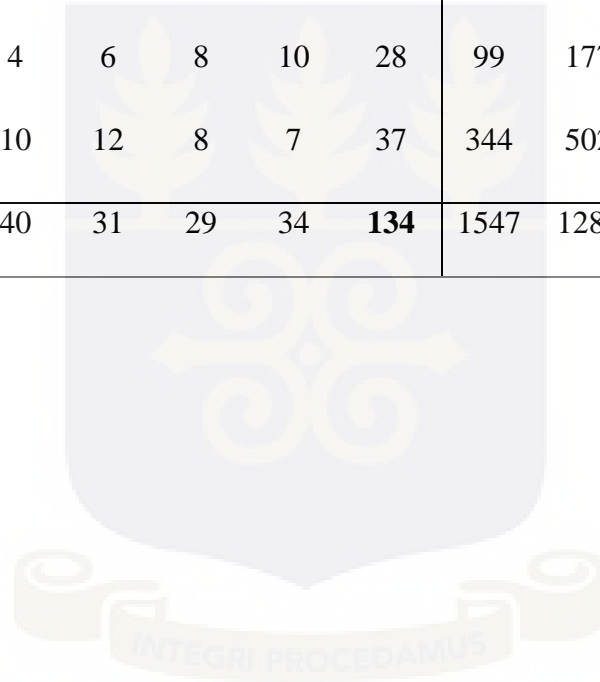
4.3.1 School-based survey

School-age children between 6 and 10 years were recruited into the study from 134 primary schools between 2010 and 2014. A total of 6,761 school children were recruited into the study. However, 92% (6,244/6761) of the total school children recruited had their blood samples collected and tested, and gave valid test results for one or more diagnostic test (Table 9). This number comprised 3,024 boys (48%; 3024/6244) and 3220 girls (52%; 3220/6244) (Figure 9). For both sexes, the highest number of school children recruited and tested were from the Awutu Senya district (males: 1382/6244; females: 1533/6244) while the lowest numbers were recruited from the Effutu municipality (male: 44/6244; females: 204/6244) (Figure 9). Each sample had valid results for at least one test. Analyses done were based on the 6,244 school children with valid test results for at least one diagnostic test.

Out of the school children tested, 24% (1512/6244) were six years old, 29% (1799/6244) were seven years old, 17% (1042/6244) were eight years old, 14% (894/6244) were nine years old and 16% (997/6244) were ten years old (Figure 10). MDA participation was assessed for the years 2010, 2013 and 2014 only. No data were collected on MDA participation in 2012 (Table 9). The number of children that reported participation in the MDA programme prior to the conduct of the study in 2010, 2013 and 2014 differed significantly ($\chi^2= 264.6$, $p<0.001$), at 15% (236/1547), 21% (353/1338) and 3% (46/1718) respectively.

Table 9: Distribution of schools and participants per district in the school-based survey

District/Year	Number of Schools Surveyed					Number of school children tested with valid test results				
	2010	2012	2013	2014	Total	2010	2012	2013	2014	Total
Awutu	23	12	11	16	62	875	594	676	770	2915
Senya										
Effutu	3	1	2	1	7	229	15	81	89	414
Agona East	4	6	8	10	28	99	177	472	278	1026
Agona West	10	12	8	7	37	344	502	462	581	1889
Total	40	31	29	34	134	1547	1288	1691	1718	6244



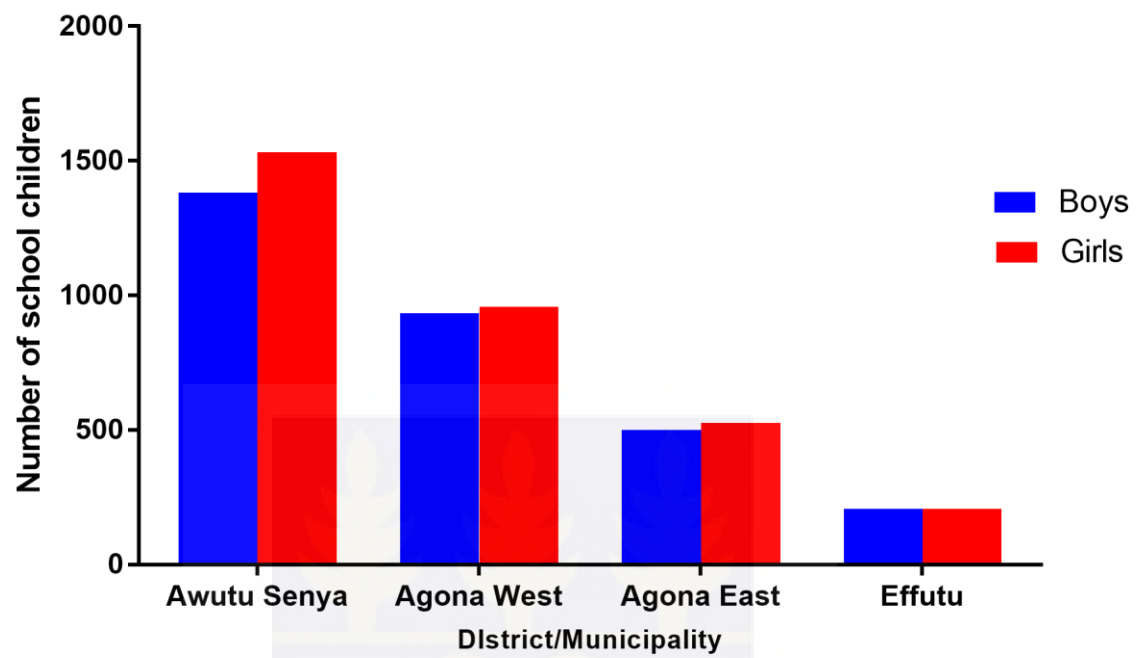


Figure 9: Distribution of participants in school-based survey by sex and district

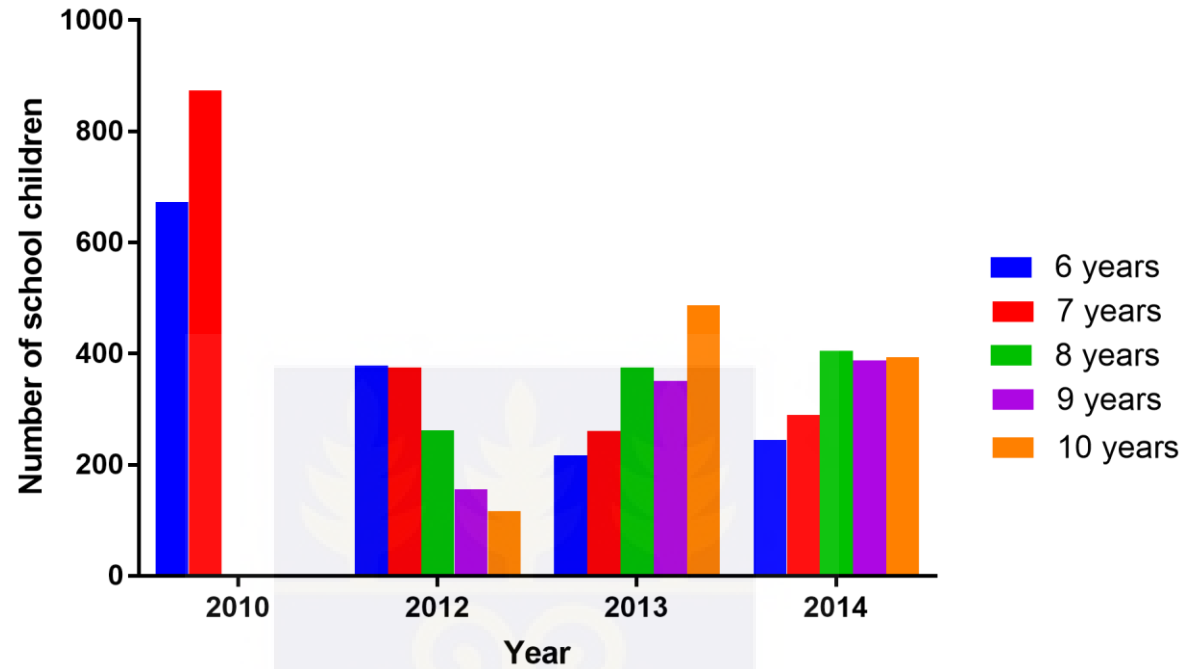


Figure 10: Age distribution of participants in school-based survey by year



Table 10: MDA participation and bednet usage among study participants by year and district

Year	District	MDA participation		Bednet Use		Bednet use recall over 7 nights			
		Participants	Non-participants	Users	Non-Users	0	1-3	4-6	7
2010	Awutu Senya	124	751	215	517	NA	NA	NA	NA
	Effutu	56	173	46	109	NA	NA	NA	NA
	Agona East	26	73	40	43	NA	NA	NA	NA
	Agona West	30	314	63	265	NA	NA	NA	NA
	Total	236	1311	364	934	NA	NA	NA	NA
2013	Awutu Senya	130	546	335	341	456	34	11	175
	Effutu	19	62	67	14	23	5	0	53
	Agona East	99	373	252	220	314	13	8	137
	Agona West	105	357	266	196	298	26	10	128
	Total	353	1338	920	771	1091	78	29	493
2014	Awutu Senya	30	740	451	319	515	41	53	161
	Effutu	3	86	64	25	47	7	2	33
	Agona East	4	274	182	96	171	24	24	74
	Agona West	8	573	345	236	395	28	16	142
	Total	46	1673	1042	676	1128	100	95	410

NA: Not applicable

Information on bed net use showed that 28% (364/1,298) of respondents used bed nets the night before the survey in 2010. This number increased to 54% (919/1,691) in 2013 and in 2014 was 61% (1,042/1,718) (Table 10). No data were collected on bednet use in 2012. The rest of the recruited population either did not have bednets so did not use any at all or had bednets but did not use them. The increase in bed net ownership was statistically significant for the 3 years ($\chi^2= 224.0, p< 0.001$). Bed net use was positively correlated with number of children sampled over the three years ($R^2 0.98, p=0.07$).

Information collected on recall of bednet use for seven nights prior to the conduct of the study was collected only in 2013 and 2014 showed that an equal proportion of school children reported not to have used a bednet all 7 nights prior to the study (2013: 64.5% (1091/1691); 2014:65.1% (1128/1733)) for both years. No visible signs of lymphoedema or hydrocele were recorded on any of the school children.

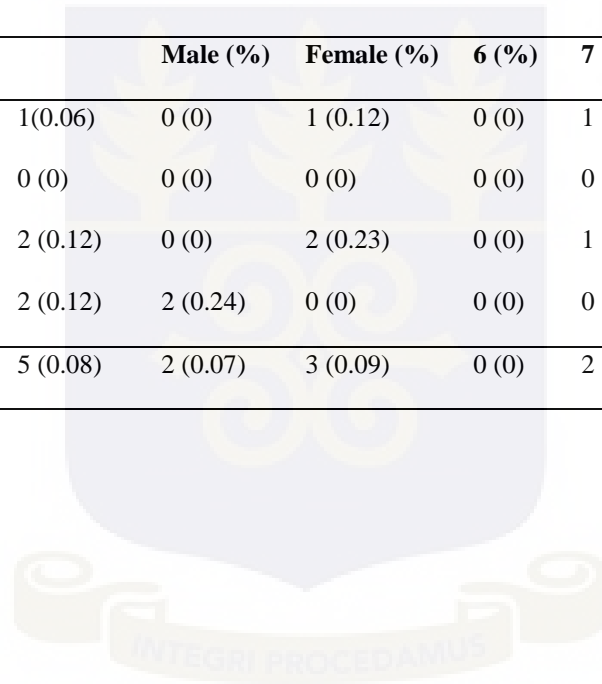
4.3.2 Results of diagnostic tests

All blood samples collected from study participants were tested using at least one of four diagnostic tests; ICT, Og4C3, blood smear and Wb123. Testing with Og4C3 and Wb123 was done only in 2013 and 2014 as further confirmation of the absence of LF in the study areas and also to detect early recrudescence. The overall circulating filarial antigen (CFA) prevalence using ICT was 0.08% (5/6,227); comprising two males (0.07%, 2/3013) and three females (0.09%, 3/3214). The ICT positive children were aged between seven and nine years (Table 11). None of the 6 and 10-year olds tested positive for ICT. Yearly, ICT prevalence rates were, 2010: 0.06%, (1/1547); 2012: 0% (0/1288); 2013: 0.12% (2/1674) and 2014: 0.12% (2/11718) ($\chi^2= 0.71, p=0.40$). None of the ICT positive

children tested positive for blood smear. There was also no statistical difference in ICT positivity over the four-year period ($\chi^2=1.68, p=0.64$).

Table 11: ICT results in school-based survey

Year	Total	ICT (%)	Sex		Age (years)				
			Male (%)	Female (%)	6 (%)	7 (%)	8 (%)	9 (%)	10 (%)
2010	1547	1(0.06)	0 (0)	1 (0.12)	0 (0)	1 (0.12)	0 (0)	0 (0)	0 (0)
2012	1288	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2013	1674	2 (0.12)	0 (0)	2 (0.23)	0 (0)	1 (0.38)	1 (0.27)	0 (0)	0 (0)]
2014	1718	2 (0.12)	2 (0.24)	0 (0)	0 (0)	0 (0)	1 (0.25)	1 (0.26)	0 (0)
Total	6227	5 (0.08)	2 (0.07)	3 (0.09)	0 (0)	2 (0.11)	2 (0.19)	1 (0.11)	0 (0)



Og4C3 and Wb123 ELISA assays were performed on samples collected in 2013 and 2014 years only. Overall Og4C3 positivity was 0.09% (3/3162) while yearly positivity was 0% (0/1531) in 2013 and 0.18% (3/1631) in 2014. The positive individuals comprised 2 males (0.13%; 2/1512) and 1 female (0.06%; 1/1650) from 3 districts; Agona West, Awutu Senya and Effutu. Two were 6 years old (0.46%; 2/435) and the third was 7 years old (0.20%; 1/510) (Table 12).

Table 12: Og4C3 results obtained in school-based survey

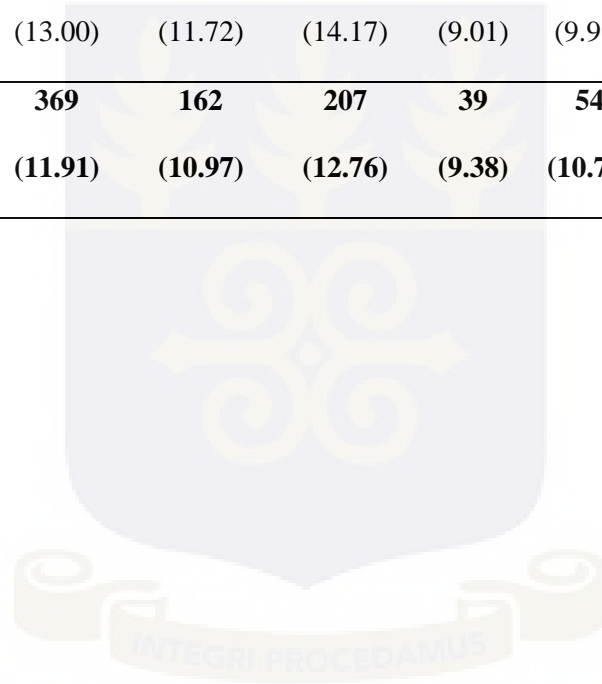
Year	Total	Og4C3 (%)	Sex		Age (years)				
			Male (%)	Female (%)	6 (%)	7 (%)	8 (%)	9 (%)	10 (%)
2013	1531	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2014	1631	3 (0.18)	2 (0.26)	1 (0.12)	2 (0.85)	1 (0.36)	0 (0)	0 (0)	0 (0)
Total	3162	3 (0.09)	2 (0.13)	1 (0.12)	2 (0.46)	1 (0.20)	0 (0)	0 (0)	0 (0)

Results for the Wb123 ELISA assay was categorized as negative, low positive and positive. For the purpose of comparison of results of Wb123 tests results with the results of the other tests used in this study, all Wb123 “Low positive” and “positive” samples were categorised as “positive” in further analyses. In 2013, results of the 1,446 samples tested showed that 10.65% (154/1446) of the samples collected tested positive, while the remaining 89.35% (1,292/1,446) tested negative. Meanwhile, out of 1,653 samples tested using Wb123 in 2014, the results showed that 13% (215/1653) tested positive and the remaining 87% (1438/1653) tested negative.

Overall, Wb123 positivity was 11.91% (369/3099) with positivity in 2014 being higher than that for 2013. Wb123, positivity was higher in females than in males (males: 10.97% (162/1477); females: 12.76% (207/1622) and, positivity increased steadily across the ages with the highest positivity in the 10-year olds, 12.83% (103/803). Wb123 positivity was generally higher when compared to the results of the antigen tests (ICT and Og4C3) (Table 13). None of the samples that tested positive for Wb123 tested positive for blood smear microscopy.

Table 13: Wb123 results obtained in school-based survey

Year	Total	Wb123 (%)	Sex		Age (years)				
			Male (%)	Female (%)	6 (%)	7 (%)	8 (%)	9 (%)	10 (%)
2013	1446	154 (10.65)	70 (10.12)	84 (11.14)	18 (9.83)	26 (11.66)	30 (9.49)	29 (9.51)	51 (12.17)
2014	1653	215 (13.00)	92 (11.72)	123 (14.17)	21 (9.01)	28 (9.96)	50 (12.85)	63 (17.26)	52 (13.54)
Total	3099	369 (11.91)	162 (10.97)	207 (12.76)	39 (9.38)	54 (10.71)	80 (11.35)	92 (13.73)	103 (12.83)



Comparison of positivity between Og4C3 and Wb123 in 2013 and 2014 showed that there was a statistically significant difference in a sample testing positive for either test in both years ($p < 0.0001$). ICT, Og4C3 and Wb123 positivity for 2013 and 2014 also showed statistically significant difference in a sample testing positive for either of the three tests ($\chi^2 = 794.5$, $p < 0.0001$). ICT positivity by district showed that three ICT positive children were from the Agona East district; one from Awutu Senya district and one from Agona West municipality.

All children from the Effutu municipality tested negative for ICT. Samples that tested positive for ICT were further tested with blood smear, Og4C3 and Wb123 to detect the presence of microfilariae, antigens and antibodies. Assessment of bednet use and ICT positivity in school children showed that two ICT positive children slept in bednets, 2 had bednets in their home but did not sleep in them while the last one did not have or use a bednet in his/her home.

A cross-comparison of the test results obtained showed that of the 6,227 samples tested with ICT, five were positive. All five samples tested negative for blood smear and Og4C3 while one out of the five was positive for Wb123. The child that tested positive for both ICT and Wb123 ELISA assay was from the Awutu Senya district. Comparison of blood smear test results with the three other tests showed negative results for all.

Comparison of Og4C3 with ICT, blood smear and Wb123 showed that out of the 3,162 samples tested, three tested positive for the Og4C3 ELISA assay. None of the positive samples was positive for any other test conducted. For Wb123, 3,099 samples were

tested, 369 tested positive. Out of the 369 Wb123 positive samples, one was positive for ICT (Table 14). Four diagnostic tests were used in testing all samples collected in the four-year study period. Each one has a different level of sensitivity and specificity at low levels of infection. This accounts for the discordance in test results recorded in 6.0% (376/6,244) of the samples obtained (Table 15).

Table 14: Cross-comparison of test results for school-based survey

Test	Index n	ICT	Blood Smear	Og4C3	Wb123
ICT	6227	5/6227	0/5	0/5	1/5
Blood Smear	5	5/5	0/5	0/5	0/5
Og4C3	3162	0/3162	0/3162	3/3162	0/3159
Wb123	3099	1/3099	0/3099	0/3099	369/3099

Table 15: Discordant test results for school-based survey

ICT Result	Blood Smear Result	Og4C3 Result	Wb123 Result	n
POS	NEG	ND	ND	1
POS	NEG	NEG	NEG	3
POS	NEG	NEG	POS	1
NEG	ND	POS	NEG	3
NEG	ND	NEG	POS	368
Total Discordant Results				376
POS – Positive NEG - Negative ND- Not done				

4.3.3 Household survey

Blood samples were collected from 4,492 individuals aged 11 years and older from 53 communities in the four study districts for this part of the study. Blood samples collected from each of these participants had valid test results for one or more of the four diagnostic tests used. The highest number of participants was from the Awutu Senya district (42%; 1902/4492) and the lowest from the Effutu municipality (7%; 327/4492) (Table 16). Females comprised 68% (3041/4492) of participants while the remaining 32% (1451/4492) were males (Figure 11). Individuals between the ages of 20-29 were the highest number of participants recruited into the study (31%; 1368/4492) followed by those within the ages of 30-39 (24%; 1092/4492) (Figure 12).

Table 16: Distribution of participants in the household survey

District	Number of Participants				
	2010	2012	2013	2014	Total (%)
Awutu Senya	449	454	489	510	1902 (42.3)
Effutu	507	317	300	255	1379 (30.7)
Agona East	151	177	229	327	884 (19.7)
Agona West	83	40	165	39	327 (7.3)
Total	1190	988	1183	1131	4492

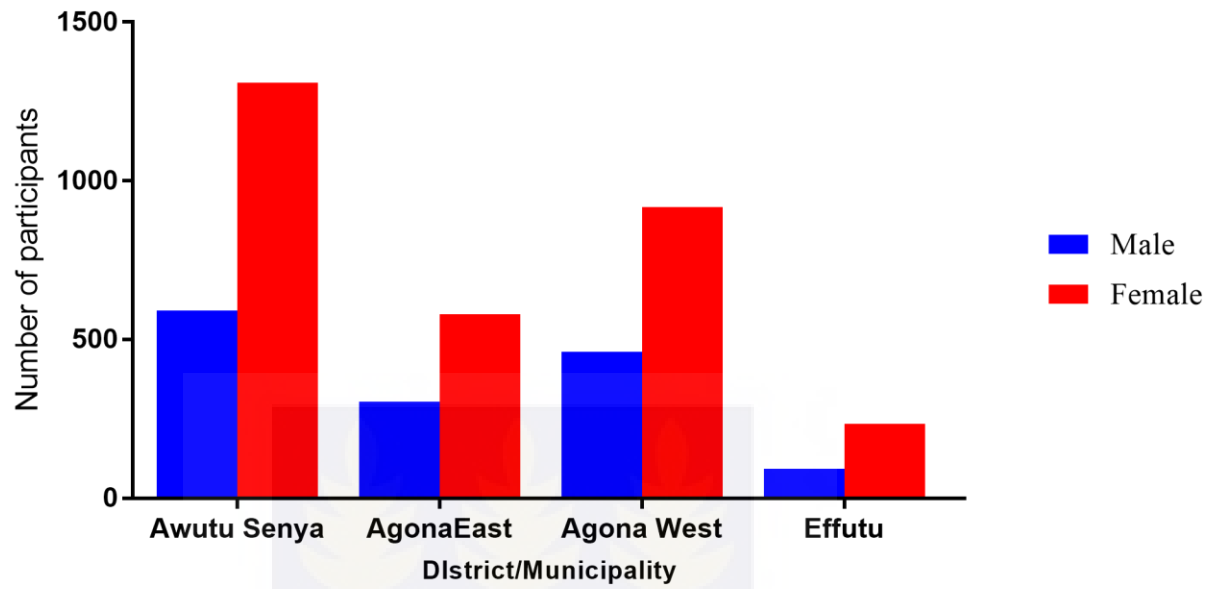
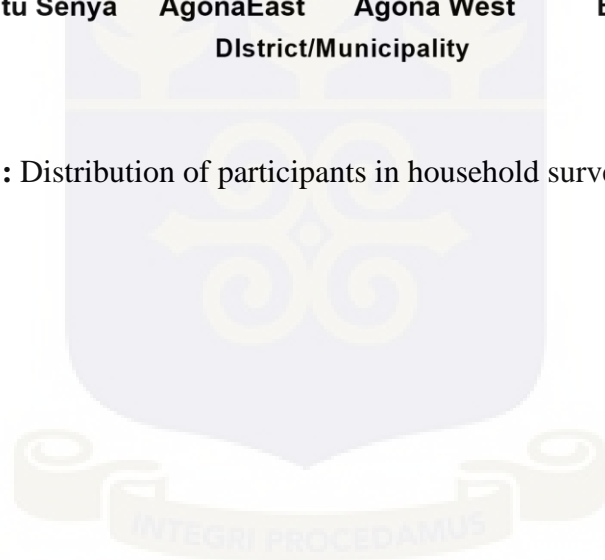


Figure 11: Distribution of participants in household survey by sex and district



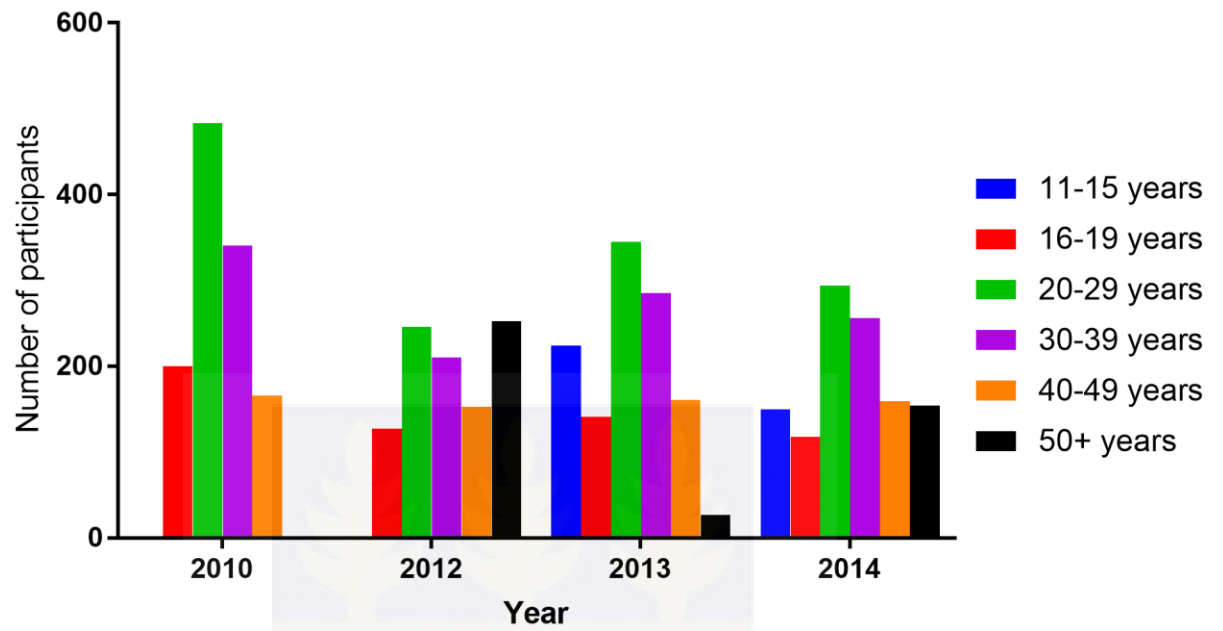
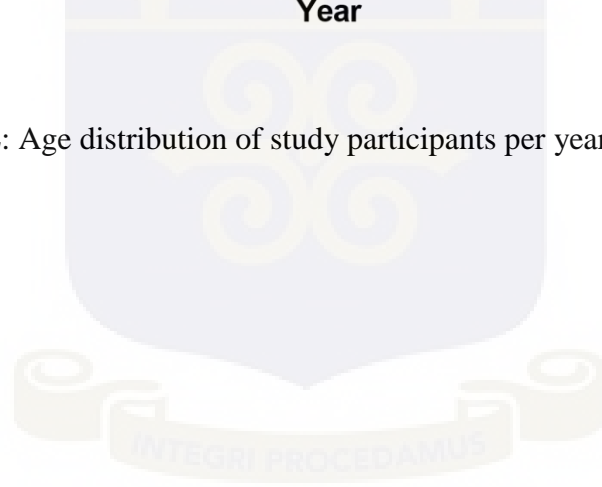


Figure 12: Age distribution of study participants per year in household survey



Yearly response to MDA participation showed that in 2010, 52% (614/1190) of participants reported to have participated in MDA. In 2013, 60% (704/1183) of recruited participants took part in the MDA while in 2014, 56% (635/1131) reported to have participated. MDA participation increased significantly between 2010 and 2013 ($p=0.0001$) and 2010 and 2014 ($p=0.03$) but was similar in 2013 and 2014 ($p=0.11$).

Out of the total respondents, 44% (1555/3499) used bed nets at night (Table 17). This usage varied over the three-year period (2010; 24%, 2013; 58% and 2014; 52%; $\chi^2=308.4$, $p<0.0001$). In 2012, no data were collected on bednet use. Bednet use in the total population was higher in females (32%; 1132/3499) than in males (12%; 423/3499) and was significantly different ($\chi^2=13.8$, $p=0.001$)

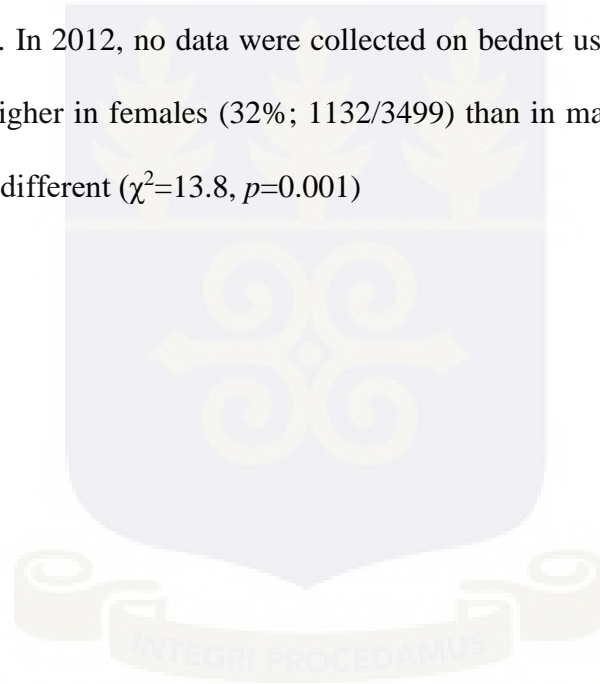


Table 17: MDA participation and bednet usage of study participants by year and district

Year	District	MDA participation		Bednet Use		Bednet use recall over 7 nights			
		Participants	Non-participants	Users	Non-users	0	1-3	4-6	7
2010	Awutu Senya	234	215	96	350	NA	NA	NA	NA
	Effutu	26	57	21	62	NA	NA	NA	NA
	Agona East	106	45	54	97	NA	NA	NA	NA
	Agona West	248	259	115	390	NA	NA	NA	NA
Total		614	576	286	899	NA	NA	NA	NA
2013	Awutu Senya	219	270	250	239	373	17	9	90
	Effutu	135	30	118	47	80	2	1	82
	Agona East	144	85	151	78	142	8	4	75
	Agona West	206	94	166	134	196	11	5	88
Total		704	479	685	498	791	38	19	335
2014	Awutu Senya	232	278	262	248	370	24	13	103
	Effutu	22	17	18	21	34	2	0	3
	Agona East	231	96	171	156	234	13	10	70
	Agona West	150	105	133	122	167	6	7	74
Total		635	496	584	547	805	45	30	250

4.3.4 Results of diagnostic tests

At least one diagnostic test/assay was performed on all the samples collected. Og4C3 and Wb123 ELISA assays were conducted only in 2013 and 2014. Overall prevalence of infection over the assessment period (all four years inclusive) was 0.72% (32/4460) for ICT, 6.25% (2/32) for blood smear, 0.33% (7/2122) for Og4C3 and 9.6% (203/2122) for Wb123. Testing for antigenemia prevalence using ICT over the four years was statistically significant ($\chi^2=209.1$, $p<0.0001$). ICT positive individuals were aged between 16 and 54 years with the highest number of positives between the age group 20-29 years (Figure 13).

Yearly ICT positivity was statistically significant over the four years; 2010 (1.09%; 13/1190), 2012 (1.01%; 10/988), 2013 (0.17%; 2/1173) and 2014 (0.63%; 7/1109) ($\chi^2=8.595$, $p=0.035$) (Table 18) but this was not positively correlated with the number of participants recruited and sampled ($R^2=0.049$, $p=0.077$). More females tested positive for ICT as compared to males with the highest numbers of positives obtained in 2010 and 2012 (Table 18).

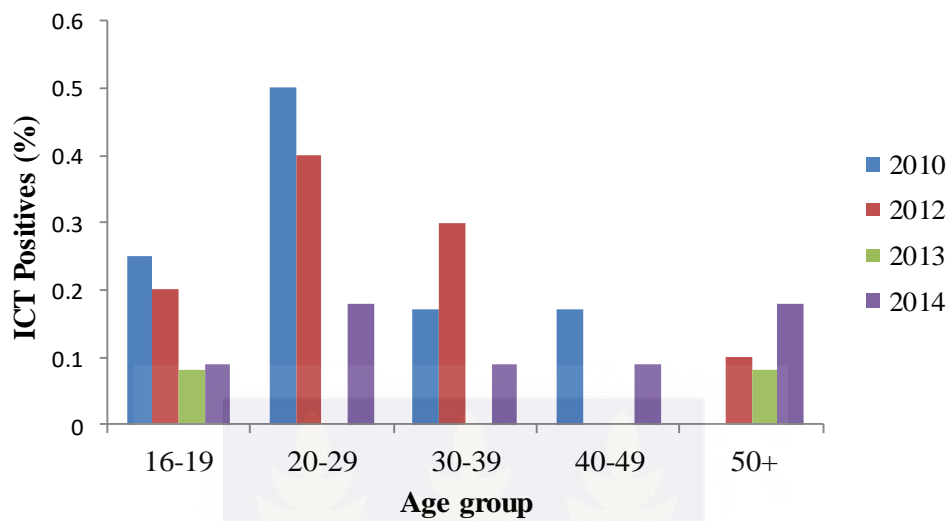


Figure 13: Distribution of ICT positive participants by age in the household survey

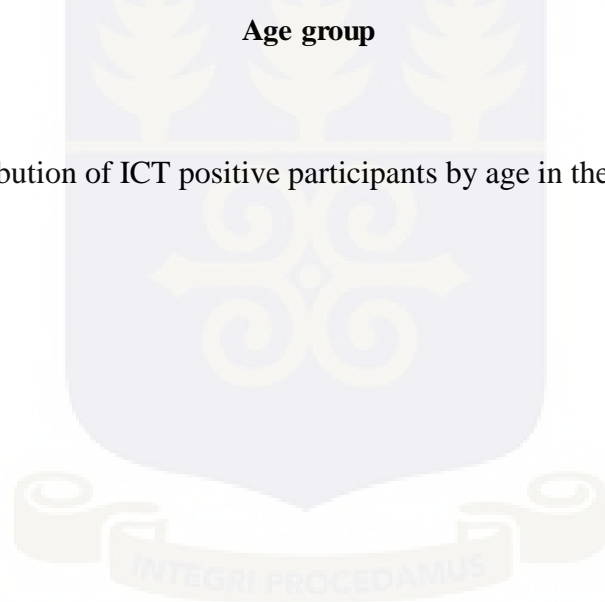


Table 18: ICT results obtained in household survey

Year	Total	ICT+ (%)	Sex		Age group (years)					
			Male (%)	Female (%)	11-15 (%)	16-19 (%)	20-29 (%)	30-39 (%)	40-49 (%)	50+ (%)
2010	1190	13 (1.09)	5 (1.41)	8 (0.96)	0 (0)	3 (1.50)	6 (1.24)	2 (0.59)	2 (1.20)	0 (0)
2012	988	10 (1.01)	2 (0.63)	8 (1.19)	0 (0)	2 (1.57)	4 (1.63)	3 (1.43)	0 (0)	1 (0.40)
2013	1173	2 (0.17)	0 (0)	2 (0.24)	0 (0)	1 (0.71)	0 (0)	0 (0)	0 (0)	1 (3.70)
2014	1109	7 (0.63)	2 (0.48)	5 (0.73)	0 (0)	1 (0.86)	2 (0.69)	1 (0.40)	1 (0.64)	2 (1.33)
Total	4460	32 (0.72)	9 (0.63)	23 (0.76)	0 (0)	7 (1.20)	12 (0.88)	6 (0.88)	3 (0.47)	4 (0.93)

Og4C3 and Wb123 tests were conducted in 2013 and 2014 only. Og4C3 positivity for 2013 was zero while in 2014 it was 0.65% (7/1069). More females tested positive as compared to males. Participants between the ages of 20-29 had a highest number of positive results (Table 19)

Table 19: Og4C3 results obtained in household survey

Year	Total	Og4C3 (%)	Sex		Age group (years)					
			Male (%)	Female (%)	11-15 (%)	16-19 (%)	20-29 (%)	30-39 (%)	40-49 (%)	50+ (%)
2013	1053	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2014	1069	7 (0.65)	2 (0.49)	5 (.76)	1 (0.69)	1 (0.91)	3 (1.09)	0 (0)	2 (1.32)	0 (0)
Total	2122	7 (0.33)	2 (0.29)	5 (0.35)	1 (0.29)	1 (0.42)	3 (0.52)	0 (0)	2 (0.68)	0 (0)

Wb123 results were interpreted as negative, low positive and positive. For the purpose of analyses in this study “low positive” and “positive” were classified as “positive”. Out of 1,043 samples tested using the Wb123 antibody test in 2013 8.53% (89/1043) tested positive while 10.57% (114/1079) tested positive in 2014. The study showed that more females tested positive as compared to males. Participants aged between 20 and 29 years had the highest number of participants that tested positive for Wb123 (Table 20)

Table 20: Wb123 results in household survey

Year	Total	Wb123 (%)	Sex		Age group (years)					
			Male (%)	Female (%)	11-15 (%)	16-19 (%)	20-29 (%)	30-39 (%)	40-49 (%)	50+ (%)
2013	1043	89(8.53)	25 (8.36)	64 (8.60)	14 (7.07)	12 (9.38)	33 (10.48)	12 (5.04)	18 (12.50)	0 (0)
2014	1079	114(10.57)	53 (12.71)	61 (9.21)	19 (13.2)	13 (11.71)	28 (10.04)	19 (7.85)	19 (12.18)	16 (10.81)
Total	2122	203(9.57)	78 (10.89)	125 (8.89)	33 (9.68)	25 (10.46)	61 (10.2)	31 (6.4)	37 (12.33)	16 (9.52)

There was no apparent difference between a sample testing positive for either Og4C3 or Wb123, but the results of both tests were found to be statistically significant ($p=0.016$; CI: 0 - 0.5719). In 2010 and 2012, ICT and blood smear were the two tests performed on blood samples collected. Bednet usage among ICT positive individuals was relatively low. Forty percent (9/22) of ICT positive individuals used bednets while the remaining 60% (13/22) did not use bednets. Bednet use information was not collected in 2012 so the ICT positive individuals were not included in the analysis.

In 2010, 13 samples tested positive for ICT out of which 12 were tested using blood smear. All 12 samples tested negative using blood smear. The 13th positive individual was lost to follow-up. In 2012, 10 samples tested ICT positive. All 10 samples were tested using blood smear out of which two tested positive. The two ICT positive individuals were from the Agona East district. In 2013 and 2014, all four diagnostic tests were utilized; ICT, blood smear, Og4C3 and Wb123. Only the ICT positive samples were tested using all 4 tests.

All other samples were tested using ICT, Og4C3 and Wb123. ICT positivity analysed by district showed that 37.5% (12/32) of the ICT positives were found in the Agona West municipality, 18.8% (6/32) in the Agona East district, 31.3% (10/32) in the Awutu Senya district and 12.5% (4/32) in the Effutu municipality (Figure 14).

All the tests performed have different levels of sensitivity and specificity therefore there was some level of discordance in the results obtained, i.e. the results obtained were not consistently positive or negative for all samples tested. 52.4% (2353/4492) of the samples

collected had discordant results. The highest discordance was seen in ICT, Og4C3 and Wb123 testing negative while blood smear was not done (Table 21).

A cross-comparison of test results show that 32 samples tested positive for ICT out of which 30 were tested using blood smear. Two of the 30 ICT positive samples were positive for blood smear. None of the samples however was positive for Og4C3 or Wb123 (Table 22). A comparison of the positivity of ICT, Og4C3 and Wb123 for 2013 and 2014 showed significant differences in positivity ($\chi^2=386.5$, $p<0.0001$) even though the number of positives and the total number of samples analysed were not positively correlated ($p>0.99$).

Comparison of ICT and Og4C3 did not show any statistical difference ($p=0.80$) but the odds of a sample testing ICT positive was 1.2 times more than Og4C3 (OR=1.2 CI: 0.5-3.3). Comparison of the results of ICT and Wb123 tests showed statistical significance ($P<0.0001$). Also, the odds of a sample testing positive for ICT was 0.96 times less than that for Wb123 (OR=0.037, CI: 0.02-0.07).

Finally, a comparison between Og4C3 and Wb123 was also significant ($p<0.0001$) therefore the odds of testing Og4C3 positive was 0.97 times less than that of Wb123 (OR=0.029, CI: 0.14-0.061). All four diagnostic tests were used in testing all samples collected in the four years. The differences in positivity of results for the different tests indicated the ability of the different tests to detect positivity at different levels of infection just as indicated in the school-based survey. Positivity of all tests was low in the

school survey as compared to the household survey making the purpose of conducting TAS plausible (Table 23).

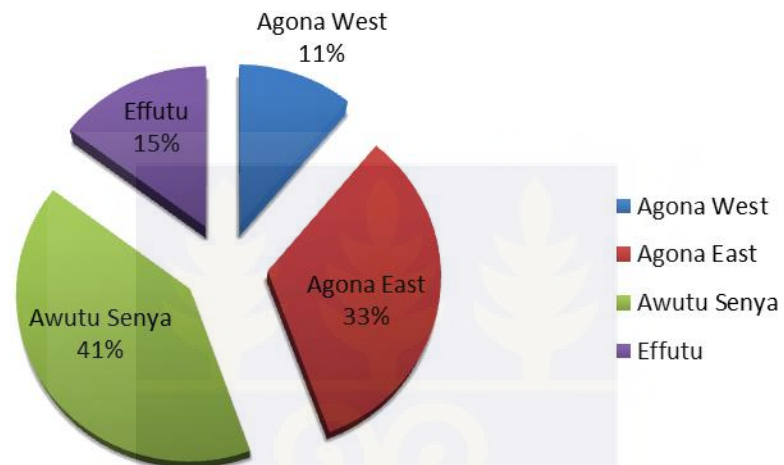


Figure 14: ICT positivity by district

Table 21: Discordant results of test results in household survey

ICT Result	Blood Smear Result	Og4C3 Result	Wb123 Result	n
POS	NEG	ND	ND	20
POS	POS	ND	ND	2
POS	NEG	NEG	NEG	8
POS	NEG	NEG	ND	1
NEG	ND	NEG	POS	186
NEG	ND	NEG	NEG	1,831
NEG	ND	NEG	ND	97
NEG	ND	ND	POS	17
NEG	ND	ND	NEG	84
NEG	ND	ND	ND	92
NEG	ND	POS	NEG	8
NEG	ND	POS	ND	3
NEG	ND	POS	POS	2
INV	ND	NEG	NEG	1
INV	ND	NEG	ND	1

Total Discordant Results = 2353

POS - POSITIVE NEG - NEGATIVE ND - Not Done INV - Invalid

Table 22: Cross-comparison of test results in household survey

Test	Index	ICT	Blood Smear	Og4C3	Wb123
ICT	4460	32/4460	2/30	0/30	0/32
Blood Smear	2	2/2	0/2	0/2	0/2
Og4C3	2122	0/2122	0/2122	7/2122	1/2122
Wb123	2122	0/2122	0/2122	1/2122	203/2122



Table 23: Comparison of results of school and household surveys

Year	School survey						Household Survey				
	Critical	Total	ICT	Blood	Og4C3	Wb123	Total	ICT (Ag)	Blood	Og4C3	Wb123
	Cut-off		(%)	Smear	(%)	(%)		(%)	Smear (%)	(%)	(%)
Value			(%)								
2010	18	1547	1(0.06)	0 (0)	NA	NA	1190	13(1.09)	0 (0)	NA	NA
2012	18	1288	0 (0)	0 (0)	NA	NA	988	10 (1.01)	2 (0.2)	NA	NA
2013	20	1691	2(0.12)	0 (0)	0 (0)	154 (10.6)	1183	2 (0.17)	0 (0)	0 (0)	89 (8.5)
2014	20	1718	2(0.12)	0 (0)	3(0.17)	215 (13.0)	1131	7 (0.62)	0 (0)	5 (0.44)	114 (11.8)
NA – Not Applicable											

4.3.5 Sensitivity and Specificity of diagnostic tests

The tests were compared for their validity (specificity, sensitivity positive and negative predictive values) using blood smear as the gold standard. ICT had the greatest sensitivity (94.1, CI: 0.809-0.986) but the lowest specificity (0.6, CI: 0.004-0.009) (Table 24).

Table 24: Sensitivity, specificity and predictive values of diagnostic tests

Diagnostic test	Sensitivity (CI)	Specificity (CI)	PPV (CI)	NPV (CI)
ICT/Blood Smear	94.1 (0.809-0.986)	0.6 (0.004-0.009)	0.7 (0.005-0.010)	93.3 (0.787-0.988)
Og4C3/ Blood smear	22.2 (0.395-0.547)	98.7 (0.971-0.991)	6.7 (0.119-0.213)	99.7 (0.993-0.998)
Wb123/ Blood smear	0.98 (0.002-0.035)	98.6 (0.979-0.990)	6.7 (0.119-0.213)	90.9 (0.91-0.916)

All values are in percentages

4.4 DISCUSSION

Lymphatic filariasis was highly endemic in the northern and southern parts of Ghana when MDA commenced in 2001. The national microfilaraemia prevalence was 3.0% with the middle belt being relatively free of the disease (Gyapong et al.1996). In 2010, Ghana was one of the few endemic countries in Africa to have completed more than five rounds of MDA using the combination of albendazole and ivermectin with 100% geographic coverage and achieving effective treatment coverage of over 65% during each round.

The question that remained was whether transmission had truly been interrupted in these areas. To answer this question, based on WHO recommendations a post-MDA surveillance using the Transmission Assessment Surveys methodology was conducted in the four districts in the Central Region of Ghana which had stopped MDA to verify the absence of LF transmission; namely Awutu Senya, Effutu, Agona East and Agona West. This is in line with the GPELFs on-going efforts to reduce the LF risk to the barest minimum.

This study was conducted between 2010 and 2014. It was done in two parts. The first part was conducted in 2010 and 2012 as part of a multicountry study with a grant from the Bill and Melinda Gates foundation. Two diagnostic tests were used in these years, ICT and blood smear. The second part was conducted in 2013 and 2014 to provide a more detailed post-MDA picture of the LF status of the study areas. Data from all four years were analysed and discussed in this thesis.

School-based and household surveys were conducted in all four districts in all four years. Test results from a total of 6,244 school age children and 4,492 members of the communities in which the schools are located, were analysed over a period of four years. The results presented here are based on the calculated TAS cut-offs; 18 for the first 2 years (2010 and 2012) and 20 for the next two years (2013 and 2014).

In the school-based survey, statistically significant differences were observed in the number of school children who participated in MDA ($p < 0.0001$). Bed net use was positively correlated with number of children sampled in the three years bednet information was collected (2010, 2013 and 2014) ($R^2 = 0.98$, $p = 0.07$). None of the children recruited into the study showed any visible signs of lymphoedema or hydrocele. In the household study persons aged between 20 and 29 years formed the largest group of participants recruited into the study.

Response to participation in the last MDA increased significantly between 2010 and 2013 ($p = 0.0001$) and 2010 and 2014 ($p = 0.03$) but was similar in 2013 and 2014 ($p = 0.11$). Bednet usage varied over the three-year period that data was collected (2010; 24%, 2013; 58% and 2014; 52%; $\chi^2 = 784.3$, $p < 0.0001$). However, a lot more females owned and used bednets as compared to males and these numbers were statistically significant (females: 32%; 1132/3499; males: 12%; 423/3499; $\chi^2 = 13.8$, $p = 0.001$).

Overall *W. bancrofti* microfilaraemia, antigenemia and antibody prevalence rates for both the school and household surveys were low and similar. In the school-based survey, overall ICT prevalence was 0.08%, blood smear was zero, Og4C3 was 0.09% while

Wb123 was 11.91%. While in the household survey overall ICT prevalence was 0.7%, blood smear was 0.04%, Og4C3 was 0.3% and Wb123 was 9.6%.

MDA participation in this study among the school children was low. Some of the older children may have participated but the younger ones would not have done so due to their ages at the time of conduct of the MDA thus accounting for the low levels of participation. In the household survey, coverage was low as compared to what was reported by the control programme. It is necessary to have high coverage rates because MDA has a profound effect on variables related to infection; microfilaraemia (mf) and circulating filarial antigenemia (CFA) prevalence rates and, transmission i.e. antifilarial antibodies in young children and mosquito infection rates (Ramzy et al., 2006). However, MDA activities that go beyond the recommended 4-6 rounds is thought to be enough to reduce mf prevalence to levels where transmission is no longer possible (Ottesen et al., 2011). In areas with low prevalence however, six effective rounds of MDA or less may be useful in reducing transmission.

Currently the targets set by the WHO for MDA programmes require the reduction of microfilaraemia prevalence rates to less than 1% and the reduction of infection rates in children born after the start of MDA to less than 0.1% (Ramzy et al., 2006). Achievement of these targets is indicated by testing for microfilaraemia or antigen (WHO, 2011a). The areas in this study have undertaken 9 and 10 rounds of MDA and coverage rates as indicated by the NTDCP are higher than the recommended 65% for 4-6 years (Table 3). Even though the MDA coverage reported by the study was low, consistent MDA for 9 to

10 years has led to the reduction in microfilaria and antigenemia prevalence in the endemic population.

Repeated rounds of MDA in community-based studies with single dose DEC alone or in combination with albendazole or ivermectin has been shown to reduce the burden of LF in many endemic areas (Bockarie et al. 2002; Simonsen et al. 2004; De Rochars et al. 2005). The data presented in this study suggests that after 9 to 10 rounds of MDA with the minimum coverage of 65%, *Wuchereria bancrofti* infection has been reduced to very low levels and active transmission is not likely to occur four years after stopping MDA. This is because of the near absence of microfilaraemia carriers in the household survey two (2) out of 4492 individuals surveyed testing positive for blood smear and the very low antigen prevalence recorded in both the school-based and household surveys.

Current WHO guidelines for post-MDA surveillance rely on the detection of adult-specific circulating filarial antigen by use of lateral flow immunochromatography (WHO, 2011a). This makes ICT and Og4C3 very useful tools to use when there is the need to detect active filarial infection. The ICT is useful in mapping the distribution of LF to determine areas where MDA is required, assessing the impact of MDA to determine when to stop and to ensure strong surveillance procedures post-MDA to monitor for possible resurgence (WHO, 2011a; Rebollo & Bockarie, 2014) while Og4C3 is used as a confirmatory tool in the laboratory.

The low prevalence recorded by both the ICT and Og4C3, continue to show that both tools are good and appropriate tools to use in post-MDA surveillance. Gass et al., (2012), Chu et al., (2013) and Offei Owusu et al., (2015) have all shown in work done in post-MDA surveillance areas that ICT is the primary tool in making decisions about stopping MDA and post-MDA surveillance while Og4C3 serves as a satisfactory laboratory-based alternative. Both tests are however, not able to detect infection prior to the development of adult parasites. A third stage larva (L₃)-specific antibody test is needed to detect antibodies that may be present in human earlier than the circulating filarial antigen especially when children 6-10 years are targeted for post-MDA surveillance.

The data from this study suggests that Wb123 was able to detect 11.9% (369/3099) of school children to be positive for *W. bancrofti*. Out of this number 7.4% (229/3099) tested low positive while 4.5% (140/3099) were positive. In the household survey, 9.6% (203/2122) of individuals tested were positive. Out of this number 6.5% (137/2122) tested low positive and 3.1% (66/2122) were positive.

Sensitivity of the Wb123 test was very low 0.98% while specificity was very high 98.6%. The high specificity was also demonstrated when Wb123 was performed as an IgG4 ELISA or a lateral flow strip with minimal cross reactivity in individuals carrying other filarial infections (Steel et al. 2013). The negative predictive value (NPV) of 90.9% was also very high as expected in relation to the positive predictive value (PPV) which was 6.7%. The test can therefore be said to be highly specific and has the ability to detect a true negative individual. This removes the issue of false negative which may turn out to be positive in an area where MDA has been stopped and ICT may not be able to detect

antigenemia till about 18 months after infection. This makes it possible to detect recrudescence when both ICT and Wb123 are applied as post-MDA surveillance tools.

The results from this study showed the absence of microfilaria in blood smears performed on all but two participants who tested positive for filarial antigenemia using the ICT card test. The presence of the two mf positives was not enough to continue transmission of the disease due to the inefficient transmission ability of the mosquito vector, *Anopheles gambiae* (Amuzu et al., 2010). Follow-up of the mf positive individuals which is not reported in this study showed clearance of mf a year after treatment.

Computer simulations have shown that the number of treatment rounds needed to eliminate lymphatic filariasis are dependent on treatment coverage, drug efficacy and baseline endemicity (Stolk et al., 2003; Michael et al., 2004). Treatment coverage in these study areas was good even though baseline endemicity was high. Consistent and regular rounds of MDA have brought about a reduction in the prevalence of LF to levels below which transmission would not occur therefore making the decision to stop MDA and conduct post-MDA surveillance to monitor for recrudescence a good one.

Filariasis vectors inefficiently transmit the parasites thereby making vector control a particularly attractive tool in filariasis control. This is because the parasite does not multiply in the mosquito vector and only continuous exposure to infective bites of many infected mosquitoes causes and maintains human infection (de Souza et al., 2012). Therefore, effective control of vectors can lead to interruption of transmission.

One of the available vector control measures is the use of bednets. This has received a lot of attention because it is a familiar concept mainly utilized by the malaria control programs in many African countries (Bockarie et al., 2009). It is also simple to implement and very effective.

Insecticide treated mosquito nets have been shown to reduce morbidity and mortality caused by mosquito borne diseases in various epidemiological settings and also reduce morbidity caused by the LF parasite (Bockarie et al., 2002). Therefore, it is expected that with the increased use of bed nets there will be an effective reduction in mosquito-human contact making transmission ineffective.

Bed net use was found to have increased marginally over the periods in which information was collected in both school and household surveys. Even though the numbers were not high, more than 50% of participants used bed nets possibly contributing to the low prevalence rate of *W. bancrofti* infection observed. In areas where both malaria and LF are transmitted by the same *Anopheles* vectors, the use of bed nets, treated with insecticides or not, has been shown to be more effective against filariasis than malaria (Bockarie et al., 2002).

In the school-based survey, out of the five children who tested positive for ICT, 2 had bednets and used them, 2 had bednets but did not use them while the fifth did not have a bednet so did not use any. Antigen positivity did not translate to microfilaria positivity. This could therefore be explained as a previous infection which has been cleared by participation in MDA. The bednet use which may or may not be regular can then be said

to have contributed to infection not recurring. The ICT positive children reported to have participated in MDA and therefore if the vector was not able to transmit the parasite efficiently, then infection is not likely to recur among the children with or without the use of bednets.

In the conduct of post-MDA surveillance for LF it is necessary to have a cut-off point at which the conclusion can be drawn that LF is no longer prevalent in the area of interest. According to the WHO guidelines set for LF, the cut off depends on the population in the area of interest. TAS is conducted for two reasons. The first is to determine whether LF prevalence is below thresholds under which recrudescence is unlikely to occur (WHO, 2012) and the second is to use it as a post-MDA surveillance tool to detect early recrudescence.

Based on mathematical models, antigen prevalence should be below 2% in areas where *Anopheles* mosquitoes are responsible for transmission (Kastner et al., 2015). Overall antigen prevalence for both school-based and household surveys were below 2% (0.08% for the school-based survey and 0.7% for the household survey). This makes the study areas no longer endemic for the disease. But, it is necessary to follow the WHO guidelines for elimination (WHO, 2011a) which includes the conduct of post-MDA surveillance 5-6 years after MDA has been stopped to evaluate whether recrudescence has occurred.

The target population for post-MDA surveillance is children born after the start of MDA. This is because they are expected to be free of LF in the absence of local transmission. If

the number of antigen positive individuals is less than the cut-off value corresponding to the antigen prevalence threshold for a given sample size, recrudescence is said not to have occurred (WHO, 2012). In areas that have stopped MDA, it is recommended that they undertake two post-MDA surveys following the TAS criteria 2-3 years following the last round of MDA and 2-3 years apart to fulfil post-treatment surveillance for verification of transmission elimination (Noland et al., 2015). This current study was conducted according to the WHO TAS specifications.

In the first year (2010), the cut off value for antigen prevalence among the school-aged children was 1.16%; i.e 18/1556 children needed to test positive for ICT to make the decision that recrudescence had occurred and MDA needed to be continued. However, only one child tested positive for ICT giving an antigen prevalence rate of 0.06%. Two years later (2012), the cut off remained the same but none of the children tested positive for ICT giving zero prevalence. In the third and fourth years (2013 and 2014), cut-off values for ICT antigen prevalence was 1.18% (20/1692) each due to the increase in the number of school going children in the selected areas.

Two children each tested positive in both years giving an antigen prevalence of 0.12% each for each year which was well below the target threshold. All ICT positives for all four years were well below the cut-off required to continue MDA as per WHO guidelines for post-MDA surveillance.

For active infection to occur in an individual, there have to be about a thousand infective mosquito bites (Appawu et al., 2001). In this area of interest where children tested positive, the children had been infected previously but due to the MDA activities that

have taken place in the area, there were not enough infected individuals in the population to enable effective and active transmission. The *W. bancrofti* parasites therefore were not able to thrive to ensure active transmission. However, the antigens of the parasites take about three to four years to clear from the blood stream, thus accounting for the presence of the antigen in the children (Nutman & Kumaraswami, 2001).

In all the 4 years antigen prevalence was well below the cut off values of 18 and 20 after stopping MDA. This means that the area has “passed” TAS and post MDA surveillance has shown that recrudescence has not occurred four years after stopping MDA. Antigen prevalence is also significantly less than 2%, the level below which active transmission will not occur. When the samples were tested using Og4C3, only 3 (0.17%) of those collected in 2014 tested positive giving an even lower antigen prevalence.

Two successful post-MDA surveys satisfy WHO guidelines for post-treatment surveillance (WHO, 2011b), however due to domestic population movements as well as the threat of on-going transmission in neighbouring districts there was the need to conduct two more post-MDA surveillance to ensure consistency of results. This makes the area of interest eligible for the next stage of the LF elimination programme. But surveillance, both active and passive needs to be continued to ensure that recrudescence does not occur. Microfilaria prevalence is an indicator in assessing the impact of MDA.

Microfilaria prevalence assessed in the sentinel and spot check sites in the study areas were 0.6% and 0.1% respectively (Table 3). After 9 to 10 rounds of MDA, mf prevalence recorded among school children in these sites were zero as per the results obtained. For

the household survey, which included mainly adults, antigenemia prevalence was also below the cut-off points calculated by the Survey Sample Builder. The overall prevalence was 0.7% which is also very much below the 2% needed to continue MDA. Microfilaraemia prevalence was also below the 1% needed to continue MDA.

Overall, sensitivity of the ICT was highest with the lowest specificity while Wb123 had very low sensitivity and very high specificity. Individuals that test negative for blood smear could test positive for antigen tests. The negativity could be as result of the poor sensitivity of the finger prick samples for the detection of mf (Lammie et al.,1994). This poor sensitivity may also be due to residual adult worm antigens from past resolved infections which may be found in circulation in the blood for up to three years after treatment (Eberhard et al., 1997).

Studies that have measured MDA antibody levels in areas that MDA has been stopped and post-MDA surveillance are being undertaken have suggested the Wb123 test as a good tool to detect early infection because antigens may take up to 18 months to be detected. Thus making detection of early recrudescence possible (Steel et al., 2012, 2013). In LF elimination, the sensitivity of a test should increase at the expense of the specificity because if a case is missed transmission could continue. This is clearly depicted by the ICT and Wb123 tests. These two tests can therefore be said to complement each other.

It is necessary therefore to make use of these two tests in areas where MDA has been stopped and post-MDA surveillance activities are being undertaken or areas that the

decision needs to be taken to stop MDA. Blood collection at night to detect nocturnally circulating microfilaria is not convenient in areas where LF intensity and prevalence has declined thus making ICT and Wb123 suitable to use.



4.5 CONCLUSION

Overall the microfilaria and circulating filarial antigen prevalence in the four study areas over the four-year study period was too low to attribute to recrudescence. The decision to stop MDA was a good one but surveillance needs to continue to detect any unlikely recrudescence as early as possible. Bed net ownership was good but its use needs to be encouraged especially now that MDA has been stopped.

For a district like the Awutu Senya which has big industrial towns and a lot of peri-urban areas, it is necessary to encourage bednet use to prevent mosquito bites. MDA has the potential to interrupt transmission of LF provided it is continuous and the directly observed treatment therapy (DOT) system is applied.

In testing for the presence of the *Wuchereria bancrofti* parasite, more than one diagnostic test should be used to determine the absence of the disease. A rapid and field applicable test like ICT is necessary to obtain results as soon as possible. This is because it is necessary that a rapid test is available to determine the presence of infection at any point in time to make monitoring, evaluation and surveillance effective and facilitate the detection of any unlikely recrudescence. It can also be used during the day making it convenient to test for the presence of the *W. bancrofti* parasite as compared to the blood smear for which blood samples can only be collected at night.

Although some tools are difficult to use under field conditions, others lack sensitivity when the intensity of infection is low (Gounoue-Kamkumo et al., 2015). Therefore, a

combination of field applicable and non-field applicable tools is necessary to give accurate information on intensity infection in endemic areas.



CHAPTER FIVE

DETECTION OF TRANSMISSION OF FILARIAL PARASITES

USING MOLECULAR XENOMONITORING

ABSTRACT

Post-MDA monitoring of parasite transmission is an important component of any lymphatic filariasis control programme, especially following the implementation of the Global Programme for the Elimination of Lymphatic Filariasis (GPELF) in 2000. This is required to assess the efficacy of MDA, when to stop MDA and to certify the elimination of the disease. Monitoring of the transmission pattern in insects is ideal since the mosquito vectors have the ability to offer real time estimates of transmission. The aim of this aspect of the study was to provide evidence of break in transmission in vectors of lymphatic filariasis in the study areas. A total of 7,268 mosquitoes were collected using the pyrethrum spray catch method in two annual sample collections from 49 communities in the four selected districts that have stopped MDA in Ghana. A total of 4,419 (60.8%) *An. gambiae* (comprising of *An. gambiae s.l* - 4,268 (63.7%), *An. funestus* - 146 (2.0%) and *An. pharoensis* - 5 (0.07%)), 2,430 (33.4%) *Culex* species and 59 (0.8%) *Mansonia* species were collected, identified and tested. Mosquitoes were pooled into batches of 20 according to community and tested for the presence of *W. bancrofti* using LAMP. All LAMP positive samples were confirmed with PCR. Three pools tested positive for the presence of *W. bancrofti* (2 *Anopheles* and 1 *Culex*) for 2013 while 2 pools of *Anopheles* tested positive *W. bancrofti* in 2014. Infection rates for *Anopheles* and *Culex* mosquitoes for 2013 were found to be 0.97 and 0.86, while that for 2014 were 1.35 and 0.00

respectively. Surveillance in humans has shown low levels of mf prevalence but in areas where there is residual infection; xenomonitoring offers real time estimates of transmission in areas where very low levels of microfilaraemia may not be detected in humans. Therefore, the detection of infection in mosquito vectors is an indication that there may be positive individuals in the area of interest.



5.1 INTRODUCTION

Lymphatic filariasis (LF) is one of the diseases that do not require an intermediate host in its transmission. This makes it quite easy to interrupt transmission using mass drug administration (MDA) and/or vector control. In the GPELF's efforts to eliminate LF, the methods mainly used to measure the impact of MDA in treated populations are based on parasite detection, parasite antigen or anti-parasite antibody detection in humans (Bockarie, 2007, Rebollo & Bockarie, 2014).

Even though intensity of microfilaraemia and antigenemia may decrease after MDA, the indicators of infection may persist in humans long after transmission has been interrupted (Rebollo & Bockarie, 2013). Testing the human population for circulating filarial antigen (CFA) provides information on the prevalence of *W. bancrofti* infection, while antibody testing provides a sensitive indicator of the levels of *W. bancrofti* exposure within the population (Weil & Ramzy, 2006; Schmaedick et al., 2014) but this does not indicate whether the vectors are no longer transmitting the parasite.

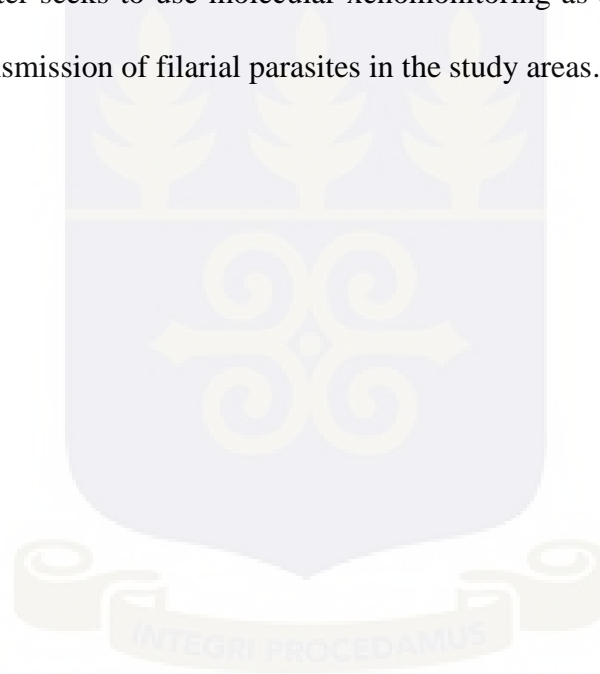
Monitoring the transmission patterns in mosquito vectors is ideal since these have the ability to offer real time estimates of transmission (Goodman et al., 2003). Microfilariae may be easier to detect in humans since it is easier to sample humans as compared to mosquitoes (Boakye et al., 2007). However, very low levels of microfilariae may not be easily detectable in humans using the available diagnostic methods. Detection of infection in mosquito vectors is therefore an indication that there may be individuals harbouring the *W. bancrofti* parasite in the area of interest (Boakye et al., 2007).

Entomological surveillance, also known as xenomonitoring, is a term used to describe the detection of filarial parasites in mosquitoes. Xenomonitoring indirectly suggests the potential for on-going filariasis transmission (Goodman et al., 2003). It is however important to note that xenomonitoring can best be implemented in areas where there are vectors which rest indoors after blood-feeding, since the detection of parasites in such vectors serve as a good estimate of microfilariae (mf) prevalence in a household and in an endemic area (WHO, 2002a, 2013). This makes xenomonitoring a suitable tool for the whole of the Americas, Africa and India. In these areas the vectors of lymphatic filariasis are endophilic (*Anopheles gambiae s.l.*, *Culex quinquefasciatus*, or *Mansonia* species).

After successful implementation of MDA programmes, mosquito infection rates reduce to levels where the traditional dissection method cannot be accurately used to assess the presence of microfilaria. It therefore becomes necessary to employ methods which are more sensitive and can detect persistent filarial parasites at low levels of prevalence (Farid et al., 2007). Detection of parasite DNA or RNA using PCR-based techniques allows testing of pools of mosquitoes which is more efficient and more sensitive than the traditional method of dissection.

This is especially true when large numbers of mosquitoes need to be examined to detect evidence of *W. bancrofti* in areas where the prevalence is low (Plichart et al., 2006; Farid et al., 2007; Chambers et al., 2009). Molecular techniques such as Polymerase Chain Reaction (PCR) (Bockarie, 2007) or more sophisticated methods like Loop-Mediated Isothermal Amplification (LAMP) (Takagi et al., 2011) are among the most sensitive and rapid methods employed in xenomonitoring activities.

Molecular xenomonitoring (MX) has been proposed as a sensitive tool for assessing the endemicity of lymphatic filariasis and is also useful in evaluating the progress and success of control programmes (Weil & Ramzy, 2006;Rebollo & Bockarie, 2014). It can also be used to estimate relative prevalence of *W. bancrofti* infection in areas with both low and high filariasis prevalence rates (Farid et al., 2007). Xenomonitoring results however should be validated against human blood surveys using ICT tests, other antibody or antigen based tests and night blood films in different epidemiological settings (WHO, 2002). This chapter seeks to use molecular xenomonitoring as a post-MDA surveillance tool to assess transmission of filarial parasites in the study areas.



5.2 MATERIALS AND METHODS

Mosquitoes were captured using the Pyrethrum Spray Catch (PSC) method as described in chapter three (section 3.9). All captured mosquitoes were tested for the presence of *W. bancrofti* using the methods described below.

5.2.1 Morphological Identification

Mosquitoes collected from the study communities were identified using morphological identification keys (Gillies & De Meillon, 1968; Danilov, 1982; Gillies & Coetzee, 1987) (Appendix 6), and grouped into Anophelines (*Anopheles* mosquitoes) and Culicines (*Aedes*, *Culex* and *Mansonia*). *Anopheles* mosquitoes were further identified using the patterns on their wing and hind legs. All identification was done under a dissection microscope (Olympus SZ60). Each mosquito sample was placed in a well-labelled 1.5ml eppendorf tube, sealed and packed into zip lock bags containing silica gel to keep them dry. Samples were transported to Noguchi Memorial Institute for Medical Research (NMIMR) for molecular analyses.

5.2.2 Pooling and DNA extraction

In the laboratory, mosquitoes were pooled by species and community. Mosquitoes were pooled into groups of 20 mosquitoes per tube. However, if the number remaining was less than 20, the mosquitoes were put in a tube and considered as one pool. The maximum pool size was 20 mosquitoes while the minimum was one mosquito. DNA extraction from each pool was done using the DNeasy Blood and Tissue Kit (QIAGEN). Each pool of mosquitoes was put into a sterile 1.5 ml eppendorf tube and placed on ice. One hundred and eighty microliters (180µl) of phosphate buffered saline (PBS) was

added to each pool and homogenized using a sterile plastic pestle, briefly centrifuged using an Eppendorf Centrifuge (5415D) and placed back on the ice. Twenty microliters (20 μ l) of proteinase K was added to each homogenate to take apart the histone proteins that is wound around the mosquito DNA, making the DNA strand accessible in solution. The homogenates were mixed thoroughly by vortexing using a Standard Mini Vortexer, (VWR Scientific Products) and centrifuged briefly for 15 seconds. The homogenates were then incubated in a water bath at 55°C for 1.5 hours.

After incubation, homogenates were vortexed for 15 seconds, 200 μ l of buffer AL was added to adjust buffering conditions and provide ideal DNA binding conditions and incubated again at 70°C for 10 minutes. The mixture was then briefly centrifuged to collect all drops of homogenate on the lid and sides of the tube and 200 μ l of absolute ethanol (96% - 99%) was added. The mixture was then vortexed thoroughly to mix the solution, and then centrifuged at 14,000 revolutions per minute (rpm) (20,000xg) for 5 minutes to collect all debris at the bottom of the tube. The supernatant was transferred onto a DNeasy Mini spin column and centrifuged at 8,000rpm for one minute. The flow-through and the collection tube were discarded into 10% bleach solution. The mini columns were placed in new 2 ml collection tubes, 500 μ l of buffer AW1 was added to the columns and centrifuged at 8,000rpm (\geq 6,000xg) for 1 minute. The flow-through and collection tubes were again discarded into 10% bleach solution. The DNeasy Mini spin columns were again placed in new 2ml collection tubes and 500 μ l of buffer AW2 was added.

The buffers (AW1 and AW2) were used to wash and remove any contaminants and enzyme inhibitors so that the deoxyribonucleic acid (DNA) obtained is pure. The solution was centrifuged at 14,000rpm (20,000xg) for 3 minutes to dry the DNeasy membrane. The flow-through and collection tubes were again discarded into 10% bleach solution. The DNeasy Mini spin columns were each finally transferred into labelled 1.5ml microcentrifuge tubes. Fifty microliters (50µl) of buffer AE was added to each column and left to incubate at room temperature for 2 minutes. The tubes were centrifuged at 8,000rpm ($\geq 6,000xg$) for 2 minutes and purified DNA was eluted. A second elution was done by adding 100µl of buffer AE again to the DNeasy Mini spin columns and incubated at room temperature for 2 minutes. The solution was centrifuged at 10,000rpm for 2 minutes to elute more of the purified DNA. The eluted pure DNA was then stored at -40°C for use.

5.2.3 Identification of *Wuchereria bancrofti* in pooled mosquitoes using Loop-Mediated Isothermal Amplification (LAMP)

The LAMP reaction was performed according to the procedures outlined by Takagi et al., 2009, 2011. A set of four primers designed for *W. bancrofti* detection (Takagi et al., 2011) were used in the LAMP assay (Figure 15). The total volume of the LAMP reaction mixture was 25µl (Table 25). To avoid transfer of aerosols between samples, all reagents and DNA samples were aliquoted with sterile aerosol-barrier pipette tips. *Wuchereria bancrofti* genomic DNA sample and 18.2 milliQ water were used as positive and negative controls respectively. The incubation and enzyme inactivation steps were done in a thermal cycler at 62°C for 70 minutes and 90°C for 10 minutes, respectively. Progression

of the LAMP reaction caused the reaction by-product (pyrophosphate ions) to bind to magnesium ions and form white precipitates of magnesium pyrophosphate. The addition of the fluorescent dye to the reaction mixture changes the colour of the white precipitates of magnesium pyrophosphate to green which can easily be viewed with the naked eye or causes it to fluoresce under UV light. After incubation and enzyme inactivation, the intensity of fluorescence was inspected under UV light and compared to the positive and negative controls (Figure 16).



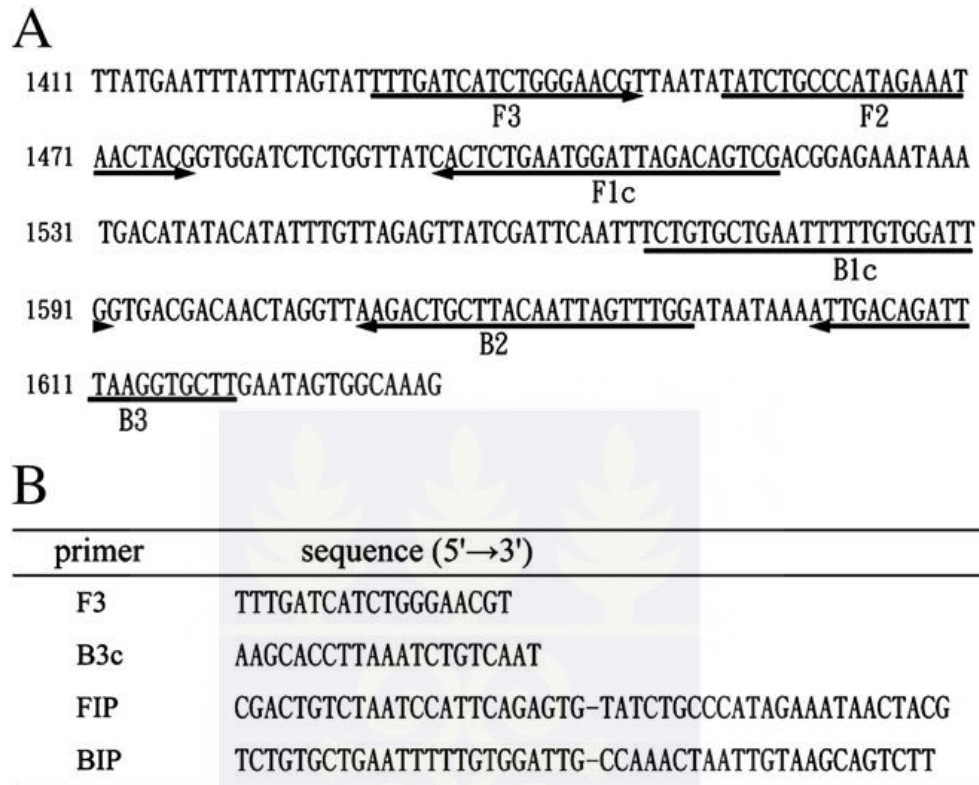


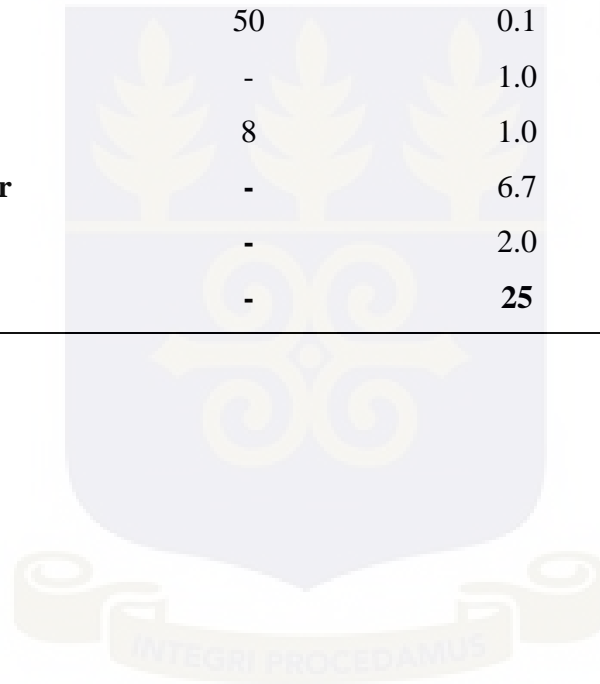
Figure 15: The target sequence on *W. bancrofti* DNA and the primer set used for amplification in LAMP.

(A) Locations and names of target sequences on *W. bancrofti* nuclear scaffold/matrix attachment region (GenBank accession no. AY297458). Numbers on the left margin indicate the nucleotide position.

(B) Sequences of four primers, F3, B3c, FIP (F1c-F2) and BIP (B1c-B2) used in the LAMP assay

Table 25: Constituents of LAMP reaction mix

Reagents	Initial concentration (μM)	Aliquoted volume (μl)	Final concentration (μM)
Reaction buffer	2	12.5	0.04
FIP	50	0.8	1.6
BIP	50	0.8	1.6
F3	50	0.1	0.2
B3c	50	0.1	0.2
Fluorescent Dye	-	1.0	-
Bst polymerase	8	1.0	0.13
18.2 milliQ water	-	6.7	-
DNA	-	2.0	-
Total	-	25	-



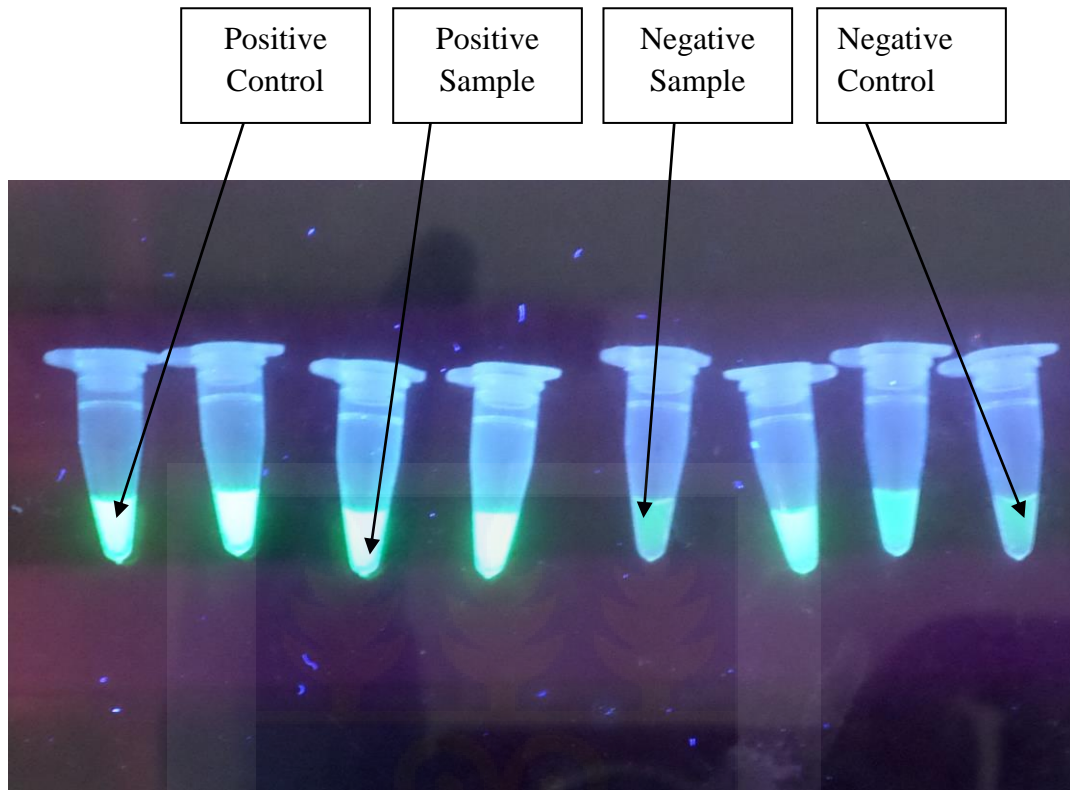
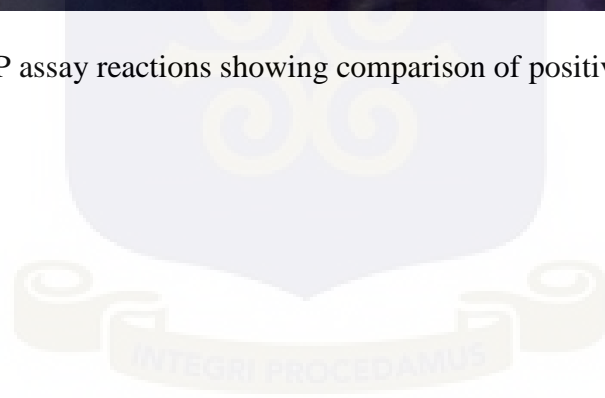


Figure 16: LAMP assay reactions showing comparison of positive and negative controls under UV light



5.2.4 Polymerase chain reaction (PCR)

Positive LAMP samples were crosschecked with conventional PCR by amplifying the *Ssp I* repeats for the detection of the LF parasite, which results in a band size of 188bp (Derua et al., 2012). Two primers were used, NV-1 (5'-CGTGATGGCATCAAAGTAGCG-3') and NV-2 (5'-CCCTCACTTACCATAAGACAAC-3'). The reaction contained 0.2µM each of NV-1 and NV-2 primers, Go Taq (comprised of 1X Green Go Taq (Flexi Buffer), 25mM MgCl₂, 0.2mM of each dNTP and 1.25 units of Go Taq DNA polymerase), 5µl DNA template and topped up to 20µl with double-distilled water (ddH₂O).

PCR amplification was done using a thermocycler set at the following cycling conditions: heating at 95°C for 10 minutes, 40 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute and, extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. Ten microliters (10µl) of the PCR products were analysed by electrophoresis on a 2% agarose gel stained with ethidium bromide. The gel was observed and photographed under an ultraviolet illuminator (TOYOBO Transilluminator Model TM-20- TOYOBO FAS-III monitor system).

5.2.5 Data analyses

In this study, the transmission indices of relevance were the biting rate, infection rate, minimum infection rate and maximum likelihood of infection. These indices help in determining the transmission status of an endemic area hence very useful for xenomonitoring studies in post-MDA surveillance areas. All data collected were recorded

on entomological survey data entry sheets and entered into a database created in Microsoft Excel. This was exported and analysed as described below.

The variables involved in the analyses were:

- (i) the number of rooms sprayed
- (ii) the number of mosquitoes collected in each room
- (iii) the number of different mosquito species collected per room sprayed
- (iv) the number of people sleeping in each room.

Data tables were tested for normality using the Shapiro Wilk normality test in GraphPad Prism 7[®]. Where the data was normally distributed; parametric tests were used in the analysis while non-parametric tests were used in the analysis of data that were not normally distributed. Statistical analyses were performed using GraphPad Prism 7[®], Microsoft Excel[®], The Mosquito Surveillance Software “PoolInRate, version 4” designed for West Nile Virus surveillance by the Centres for Disease Control and Prevention (CDC), Atlanta, USA and the Poolscreen Software, “Poolscreen v 2.0.3” (Katholi & Barker, 2010).

The transmission indices of interest are defined below.

a. Biting Rate

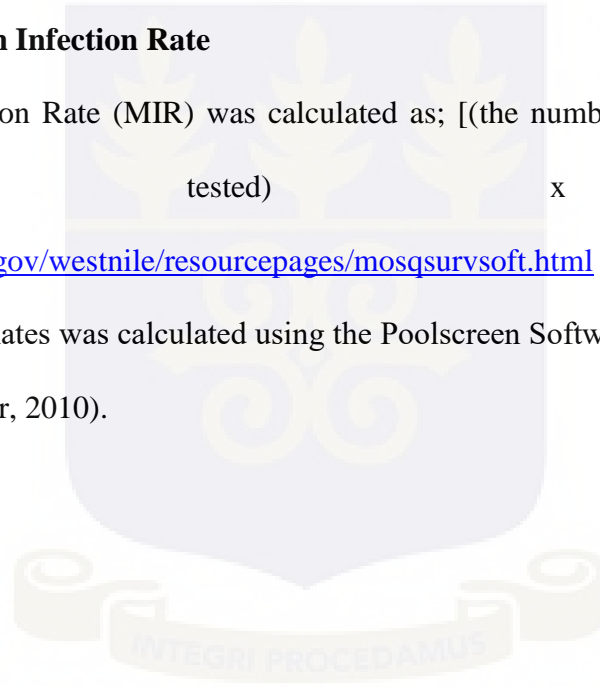
The biting rate (BR) was calculated as the estimated number of vector mosquitoes biting one person during one sampling night. It was expressed as the number of vector mosquitoes caught divided by the product of the number of persons sleeping in the room during sampling and the number of catch nights. The monthly and annual biting rates were estimated by multiplying by 30 days and 365 days, respectively.

b. Infection Rate

Infection rate (IR) was expressed as the number of mosquitoes with any stage of the *W. bancrofti* parasite divided by the total number of mosquitoes examined for this parasite. Infection rate was calculated using the Mosquito Surveillance Software “PoolInRate, version 4” designed for West Nile Virus surveillance by the Centres for Disease Control and Prevention (CDC), Atlanta, USA.

c. Minimum Infection Rate

Minimum Infection Rate (MIR) was calculated as; [(the number of positive pools/total specimens tested) x 1,000] (<http://www.cdc.gov/westnile/resourcepages/mosqsurvsoft.html>). Maximum likelihood of infection estimates was calculated using the Poolscreen Software, “Poolscreen v 2.0.3” (Katholi & Barker, 2010).



5.3 RESULTS

5.3.1 Distribution of mosquitoes

A total of 7,268 mosquitoes of interest in lymphatic filariasis transmission were collected in 2013 and 2014 from 49 communities in the selected study districts. This number included communities that were sampled twice due to the presence of ICT positive individuals found in the school and household surveys (Chapter 4). Generally, collections per district were higher in 2014 than in 2013 except in the Effutu municipality where more mosquitoes were collected in 2013 (81.1%; 1215/1499) than in 2014 (18.9%; 284/1499) (Table 27). Collection for 2013 made up 44% of total collections for both years and this comprised of 60.3% (1941/3217) *Anopheles gambiae s.l.*, 1.3% (43/3217) *Anopheles funestus*, 0.06% (2/3217) *Anopheles pharoensis*, 37.9% (1218/3217) *Culex* species and 0.4% (13/3217) *Mansonia* species.

Collections for 2014 made up the remaining 56% of mosquitoes collected. This comprised of 66.3% (2687/4051) *Anopheles gambiae s.l.*, 2.5% (103/4051) *Anopheles funestus*, 0.07% (3/4051) *Anopheles pharoensis*, 29.9% (1212/4051) *Culex* species and 1.1% (46/4051) *Mansonia* species (Table 26). More mosquitoes were collected in 2014 (4,051) than in 2013 (3,217) but, the average number of mosquitoes collected each year was similar (2013=37.17, 2014=36.37) (Figures 17 and 18). Statistical analysis showed that the number of communities sprayed per year did not influence the number of mosquitoes collected (Student's t-test: $p=0.95$).

Table 26: Distribution of mosquito species collected in 2013 and 2014

Mosquito Species	Year		Total (%)
	2013 (%)	2014 (%)	
<i>Anopheles gambiae</i>	1941 (60.3)	2687 (66.3)	4628 (63.7)
<i>s.l.</i>			
<i>Anopheles funestus</i>	43 (1.3)	103 (2.5)	146 (2.0)
<i>Anopheles pharoensis</i>	2 (0.06)	3 (0.07)	5 (0.07)
<i>Culex</i>	1218 (37.9)	1212 (29.9)	2430 (33.4)
<i>Mansonia</i>	13 (0.4)	46 (1.1)	59 (0.8)
Total	3217 (44.3)	4051 (55.7)	7268

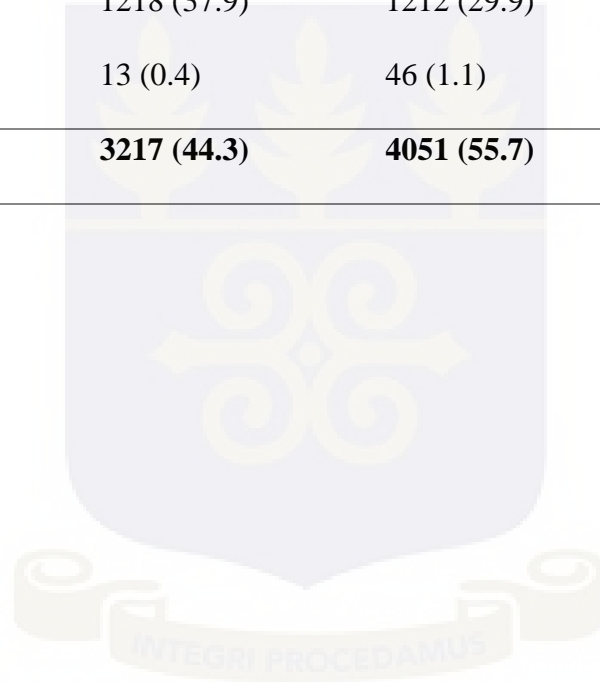


Table 27: Distribution of mosquito species collected per district

District	Mosquito species	Year		Total
		2013	2014	
Agona East		2013	2014	
	<i>An. gambiae s.l.</i>	552	1055	1607
	<i>An. funestus</i>	33	65	98
	<i>An. pharoensis</i>	0	2	2
	<i>Culex</i>	73	86	159
	<i>Mansonia</i>	12	9	21
Subtotal		670	1217	1887
Agona West	<i>An. gambiae s.l.</i>	95	452	547
	<i>An. funestus</i>	0	3	3
	<i>An. pharoensis</i>	0	0	0
	<i>Culex</i>	87	277	364
	<i>Mansonia</i>	0	7	7
	Subtotal		182	739
Awutu Senya	<i>An. gambiae s.l.</i>	516	1064	1580
	<i>An. funestus</i>	4	35	39
	<i>An. pharoensis</i>	1	1	2
	<i>Culex</i>	629	681	1310
	<i>Mansonia</i>	0	30	30
	Subtotal		1150	1811
Effutu	<i>An. gambiae s.l.</i>	778	116	894
	<i>An. funestus</i>	6	0	6
	<i>An. pharoensis</i>	1	0	1
	<i>Culex</i>	429	168	597
	<i>Mansonia</i>	1	0	1
	Subtotal		1215	284
Total		3217	4051	7268

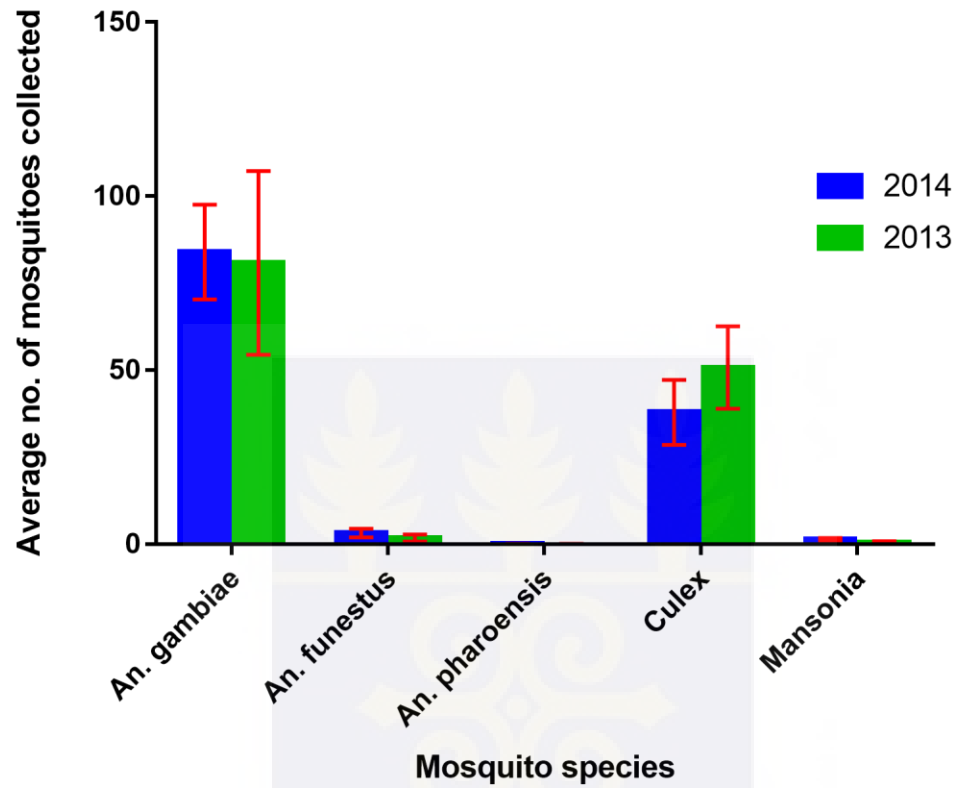


Figure 17: Average number of mosquito species caught from study areas per year.

Error bars represent the standard error of the mean.

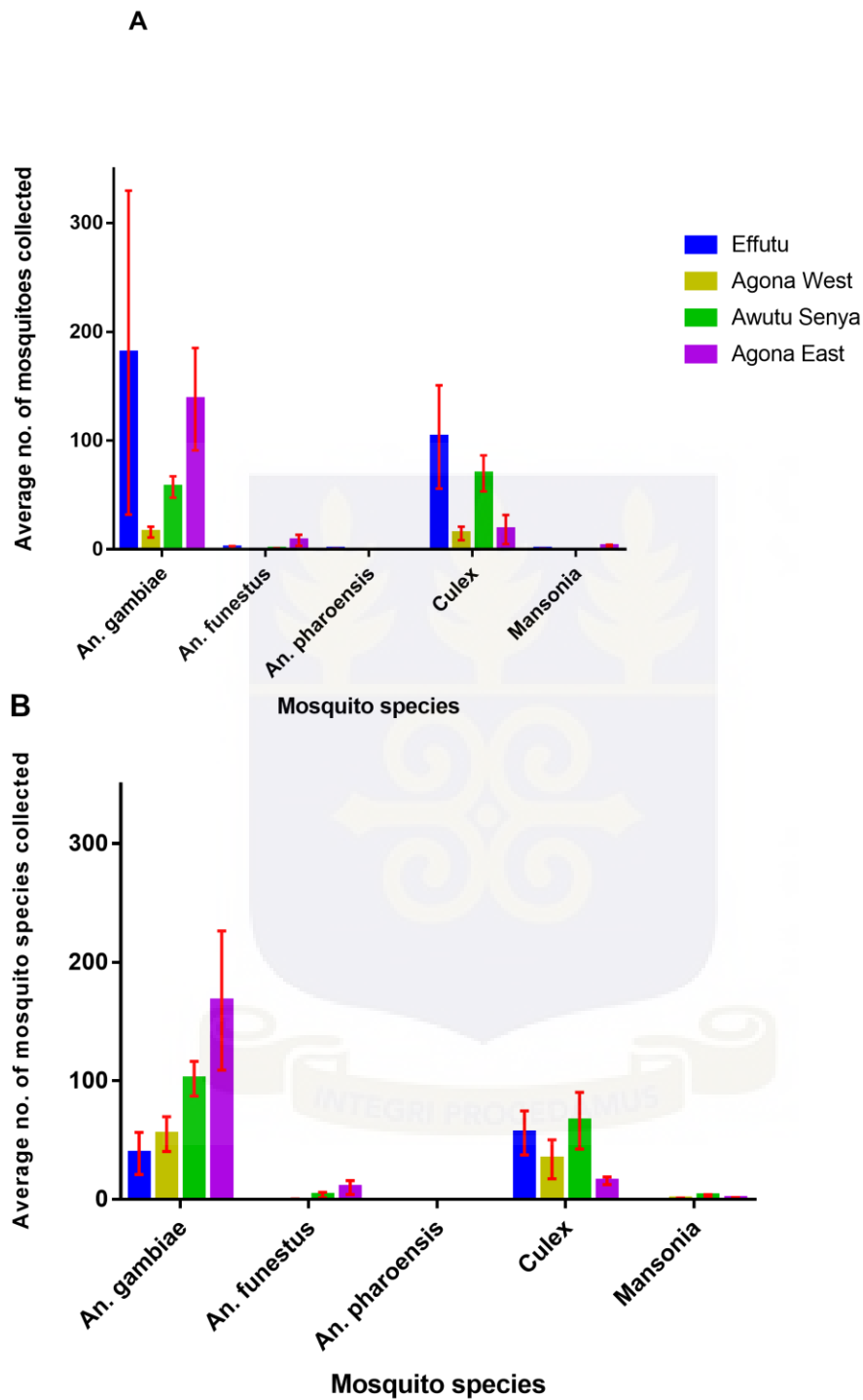


Figure 18: The average number of mosquito species collected per district in (A) 2013 and (B) 2014.

Error bars represent the standard error of the mean.

The number of *Anopheles gambiae s.l* collected each year was comparatively higher than all the other species and there was a statistically significant difference in the number of *Anopheles gambiae s.l* collected per district as compared to the other mosquito species in both 2013 and 2014 (2013: $p < 0.0001$; 2014: $p < 0.0001$). To determine whether the high number of *Anopheles gambiae s.l* collected was attributable to some districts, the numbers of *Anopheles gambiae s.l* collected in each district were compared statistically.

The results obtained show that there was a statistical difference in the *Anopheles gambiae s.l* collected for some districts while for others there was no statistical difference. *Anopheles gambiae s.l* numbers were significantly different in Effutu than in Awutu Senya in 2013 ($p = 0.006$) while in 2014 a difference was observed between Agona East and Agona West ($p < 0.0001$) and Agona East and Effutu ($p = 0.001$) (Table 28). Within districts, the various mosquito species collected were compared between the two years. Statistically significant differences were seen in *Anopheles gambiae s.l* and *Mansonia* species collected from Awutu Senya in the two years (*Anopheles gambiae s.l.*, $p = 0.02$, *Mansonia*, $p = 0.01$) (Student's T-test). Comparison with the other species did not show any statistical differences.

Follow-up mosquito collections were done in 33 index houses and surrounding houses in five communities where ICT positive individuals were found in both years. A total of 395 mosquitoes were collected. In 2013, 71 mosquitoes were collected from 6 houses in the Effutu municipality in 2013. This comprised 55 (77%) *Anopheles gambiae s.l* and 16 (22%) *Culex* species. In 2014, 324 mosquitoes were collected from the Awutu Senya, Agona East and Agona West districts. *Anopheles gambiae s.l* was the most abundant

species collected (82%; 266/324). *Culex* was the second most abundant species (9.9%; 17/324), followed by *Anopheles funestus* (5.2%; 3/324), *Mansonia* (1.9%; 32/324) and *Anopheles pharoensis* (0.9%; 6/324) (Table 29). All mosquitoes collected in the entire survey were pooled, tested and transmission indices calculated.



Table 28: Test of significance of the distribution of *Anopheles gambiae s.l.* per district/municipality

Year	2013	2014
Comparative districts/municipalities	<i>p</i>-value	<i>p</i>-value
Agona East vrs Agona West	0.013	< 0.0001
Agona East vrs Awutu Senya	0.128	0.015
Agona East vrs Effutu	0.752	< 0.0001
Agona West vrs Awutu Senya	0.567	0.067
Agona West vrs Effutu	< 0.0001	0.929
Awutu Senya vrs Effutu	0.006	0.079

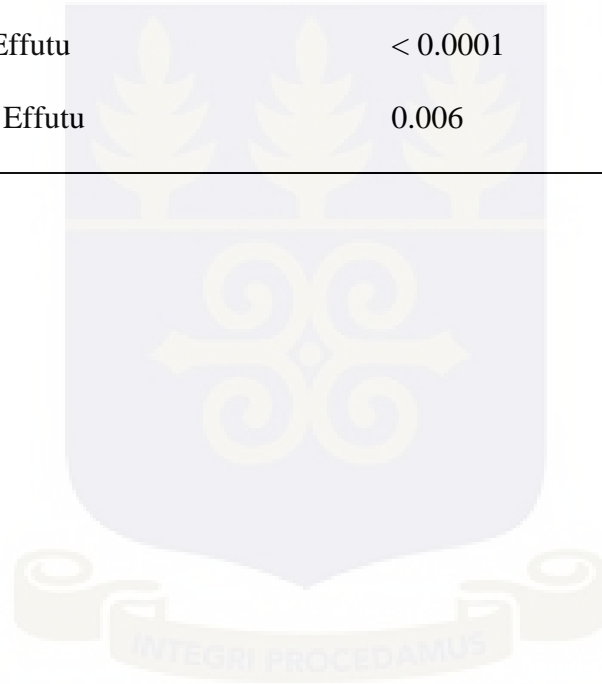
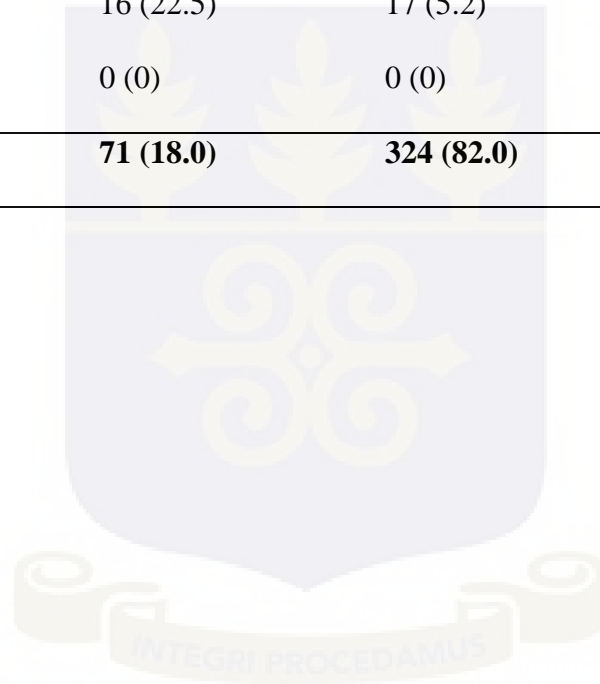


Table 29: Distribution of mosquito species in follow-up collection in 2013 and 2014

Mosquito Species	Year		Total (%)
	2013 (%)	2014 (%)	
<i>Anopheles gambiae</i>	55 (77.5)	266 (82.1)	321 (81.3)
<i>s.l.</i>			
<i>Anopheles funestus</i>	0 (0)	3 (5.2)	3 (0.8)
<i>Anopheles pharoensis</i>	0 (0)	32 (9.9)	32 (8.1)
<i>Culex</i>	16 (22.5)	17 (5.2)	33 (8.4)
<i>Mansonia</i>	0 (0)	0 (0)	0 (0)
Total	71 (18.0)	324 (82.0)	395



5.3.2 Transmission indices

5.3.2.1 LAMP and PCR assays

A total of 425 pools of mosquitoes were screened for the presence of *W. bancrofti* using LAMP. Six pools (two pools of *Anopheles gambiae s.l* and one pool of *Culex* species in 2013, and three pools of *Anopheles gambiae s.l* in 2014 tested positive for the presence of *W. bancrofti*. The pools found to be positive in 2013 were from Ofaakor in the Awutu Senya district and Kwanyako in the Agona East district. The pools found to be positive in 2014 were from Bawjiase in the Awutu Senya district and Wawase in the Agona West municipality. When screened using conventional PCR, 66.7% (4/6) of the LAMP-positive pools gave positive results for the presence of *W. bancrofti* (Table 30).

5.3.2.2 Biting rate

Biting rate (BR) of the mosquitoes collected was calculated. Biting rates in all districts were low and similar for both years (2013 = 0.057; 2014 = 0.058). The cumulative monthly and annual biting rates were similarly low for both years (Table 31).

Table 30: Infection rates and maximum likelihood of infection of pooled mosquitoes

Test	Pools tested	Positive pools	Negative Pools	Species	Year							
					2013				2014			
					Infection rate (%)	95% CI	MLI (%)	95% CI	Infection rate (%)	95% CI	MLI (%)	95% CI
LAMP	425	6	419	<i>An. gambiae</i>	0.97	0.17-3.17	9.8×10^{-4}	1.2×10^{-4} - 3.4×10^{-3}	1.35	0.36-3.65	1.4×10^{-3}	2.6×10^{-4} - 3.9×10^{-3}
PCR	6	4	2	<i>Culex</i>	0.86	0.0-4.15	8.6×10^{-4}	2.7×10^{-5} - 4.4×10^{-4}	0.00	0.00-3.32	0.00	0.00- 1.7×10^{-3}

MLI – Maximum Likelihood of Infection



Table 31: Biting rates of mosquitoes per district for 2013 and 2014

Year		2013					2014					
Districts	Mosquitoes	No. of Sleepers	No. of Catch Nights	BR	MBR	ABR	Mosquitoes	No. of Sleepers	No. of Catch Nights	BR	MBR	ABR
Agona East	587	246	6	0.398	11.94	145.27	1123	373	8	0.376	11.28	137.24
Agona West	95	169	7	0.080	2.4	29.2	452	320	9	0.157	4.71	57.31
Awutu Senya	584	600	10	0.097	2.91	35.41	1096	586	12	0.156	4.68	56.94
Effutu	680	217	5	0.627	18.81	228.86	116	223	3	0.173	5.19	63.15
Total	1946	1232	28	0.057	1.69	20.81	2787	1502	32	0.058	1.74	21.16

5.3.2.3 Infection rate

Using the PoolInRate mosquito surveillance software, the cumulative infection rates calculated for mosquitoes of the *Anopheles gambiae* complex were 0.97 (CI: 0.17-3.17) in 2013 and 1.3 (CI: 0.36-3.65) in 2014 per 1,000 mosquitoes, while that for *Culex* species was 0.86 (CI: 0.05-4.15) in 2013 per 1,000 mosquitoes. None of the *Culex* pools tested positive in 2014. Yearly infection rates combining both *Anopheles gambiae* complex and *Culex* were 2013: 0.36 (CI: 0.02-1.74; 2014: 0.79 (CI: 0.14-2.59). The maximum likelihood of infection for both years for both *Anopheles* and *Culex* were low (Table 30). The maximum likelihood of infection estimated per district was also low for both years (Tables 32 and 33).

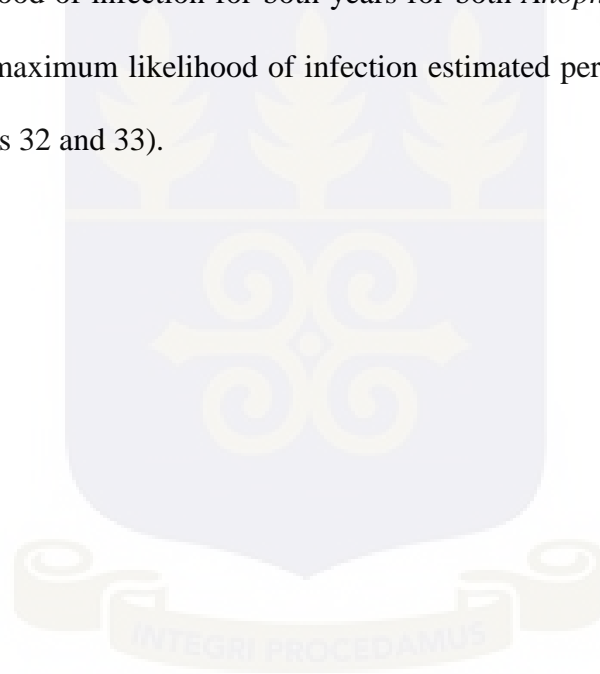


Table 32: Calculated Maximum Likelihood of Infection per district (2013)

Year		2013			
EA	Mosquito Species	Total No. of Mosquitoes	No. of Pools	No. of Pools positive for <i>W. bancrofti</i>	Max. Likelihood of Infection (%) (CI)
Agona East	<i>Anopheles</i>	600	44	2	0.003 (4×10^{-3} - 1×10^{-2})
	<i>Culex</i>	74	7	0	0.00 (0.00-0.03)
	<i>Mansonia</i>	10	3	0	0.00 (0.00-0.17)
Agona West	<i>Anopheles</i>	74	8	0	0.00 (0.00-0.03)
	<i>Culex</i>	74	7	0	0.00 (0.00-0.003)
Awutu Senya	<i>Anopheles</i>	650	49	0	0.00 (0.00-0.003)
	<i>Culex</i>	779	45	1	0.001 (4×10^{-5} - 7×10^{-4})
Effutu	<i>Anopheles</i>	720	38	0	0.00 (0.00-0.003)
	<i>Culex</i>	315	17	0	0.00 (0.00-0.006)
	<i>Mansonia</i>	1	1	0	0.00 (0.00-0.85)

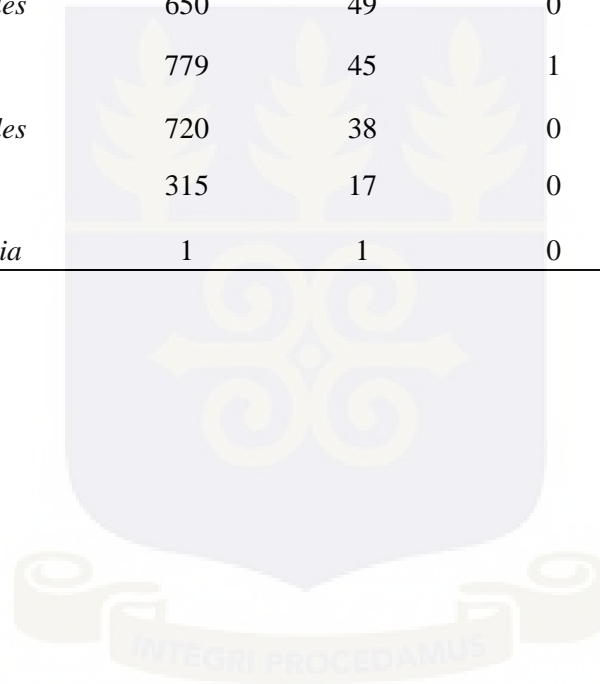
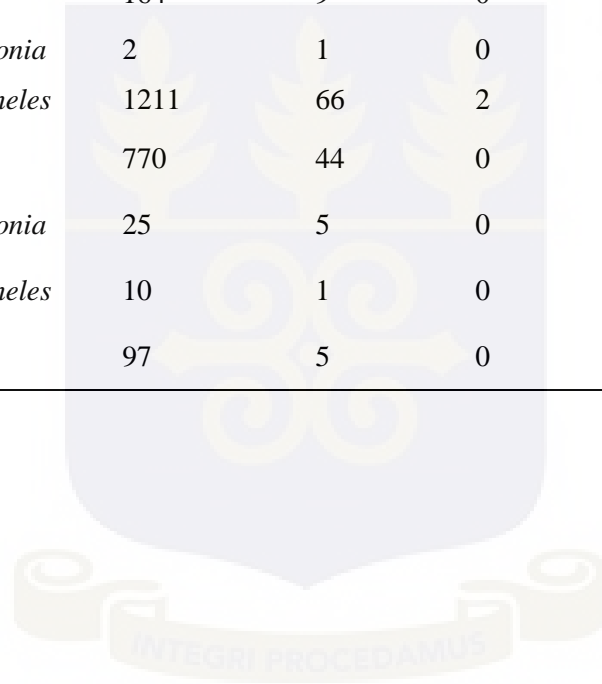


Table 33: Calculated Maximum Likelihood of Infection per district (2014)

Year		2014			
EA	Species of Mosquito	Total No. of Mosquitoes	No. of Pools	No. of Pools Positive for <i>W. bancrofti</i>	Max. Likelihood of Infection (%) (CI)
Agona East	<i>Anopheles</i>	753	44	0	0.0 (0.00 – 0.003)
	<i>Culex</i>	88	10	0	0.0 (0.00-0.02)
	<i>Mansonia</i>	7	5	0	0.00 (0.00-0.24)
Agona West	<i>Anopheles</i>	259	16	1	0.004 (1x10 ⁻⁴ -2x10 ⁻³)
	<i>Culex</i>	164	9	0	0.00 (0.00-0.012)
	<i>Mansonia</i>	2	1	0	0.00 (0.00-0.62)
Awutu Senya	<i>Anopheles</i>	1211	66	2	0.001 (2x10 ⁻⁴ - 6x10 ⁻³)
	<i>Culex</i>	770	44	0	0.00 (0.00-0.002)
	<i>Mansonia</i>	25	5	0	0.00 (0.00-0.07)
Effutu	<i>Anopheles</i>	10	1	0	0.00 (0.00-0.17)
	<i>Culex</i>	97	5	0	0.00 (0.00-0.02)



.4 DISCUSSION

Monitoring infection in the human host for the presence microfilaria is important but there is the need also to assess *W. bancrofti* infection in the mosquito vector population. This is to evaluate the success of interventions for eliminating lymphatic filariasis (LF) transmission from endemic communities (Pedersen et al., 2009). The decision to stop treatment does not require the complete absence of filarial parasites but rather the reduction of parasite numbers to such low levels that transmission will cease (Duerr et al., 2005; Michael et al., 2007).

The presence of infection in mosquito vectors serve as an alternate means to detect LF infection in an affected population, indicate the presence of a reservoir of microfilariae in humans and also detect early recrudescence or the transmission potential if the infective third stage larva is detected (Laney et al., 2010). This aspect of the study sought to find out if the mosquito vectors still carried the *W. bancrofti* parasites which may lead to recrudescence of LF in the population of interest.

The number of communities sprayed per year did not influence the number of mosquitoes collected and *Anopheles gambiae s.l.* the species responsible for LF transmission In Ghana were the highest number of mosquitoes collected. Comparison of the number of *An. gambiae s.l.* collected showed that there was a statistically significant difference in the number of *An. gambiae s.l.* collected per district. Poolscreen analysis showed that six pools of mosquitoes were positive for *W. bancrofti* using the LAMP assay while four of the six pools tested positive for *W. bancrofti* using conventional PCR.

Infection rates were relatively high as compared to the threshold of 0.65% for overall larval infection as suggested by Pedersen et al., (2009) for *An. gambiae s.l.* transmission areas and Farid et al., (2007) for *Culex* transmission areas. The calculated maximum likelihood of infection was however low. Night biting rates were low and similar for both years as compared to the threshold of 0.66 bites/man/hr above which transmission may be sustained by anopheline mosquitoes (Webber & Southgate, 1981). Since no cut-off points have been determined for biting rates and infection rates, it was difficult to determine whether the rates obtained in this study were high enough to sustain transmission or low enough to say that transmission cannot be sustained.

To be able to test large numbers of mosquitoes, the availability of efficient collection methods for local species is very important (Schmaedick et al., 2014). It is necessary to have knowledge of the species responsible for transmission and their feeding and resting habits to be able to employ the right method of collection. In this study, the pyrethrum spray catch (PSC) technique was used in the collection of mosquitoes. This is because the PSC technique is useful in the collection of indoor resting mosquitoes.

The pyrethrum spray catch (PSC) method has the ability to capture large numbers of mosquitoes, both resting blood-fed and gravid female mosquitoes and unfed mosquitoes searching for a blood meal as compared to human landing catches, exit traps and the light traps (Mwandawiro et al., 1997). *Anopheles* species, which are known to be the major vectors of LF in Africa (Subramanian et al., 1998; WHO, 2002b; WHO, 2010) were the most abundant species collected in both years of xenomonitoring activities. *Anopheles*

gambiae s.l and *Anopheles funestus* constituted 65.7% of catches. Very few (0.07%) *Anopheles pharoensis* were collected.

Large numbers of mosquitoes of the *Anopheles gambiae* complex were collected due to their endophilic nature (indoor resting habits) (Aniedu, 1993; Mnzava et al., 1995; Pates & Curtis, 2005; Paaijmans & Thomas, 2011). After taking a blood meal *Anopheles* mosquitoes are most likely to rest indoors making it easy to capture a lot of them using the PSC method. Collection of blood-fed mosquitoes by indoor resting catch or pyrethrum spray catch and analysing them to detect the presence of microfilaria larvae is the optimal technique used for measuring the uptake of microfilaria from the humans (Pedersen et al., 2009).

Culex quinquefasciatus, the species known to facilitate transmission in some parts of East Africa was the second most dominant species (33.4%) collected. *Culex* mosquitoes exhibit exophilic characteristics (rest outdoors at the end of blood feeding) while in search of an oviposition site (Pates & Curtis, 2005). They only tend to exhibit endophilic characteristics when they cannot find a way out of a human dwelling after a blood meal hence the ability to catch them in rooms with no opening in the eaves.

The third most abundant species collected were *Mansonia* species. These constituted 0.81% of collections done in both years (Table 27). *Mansonia* species are known vectors of filariasis in some LF endemic areas, but until recently they had not been considered as potential vectors in Africa. A study by Onapa et al., (2007) however showed that infective *Mansonia uniformis* was possible in laboratory experiments but not in the wild.

Some work also done by Ughasi et al., (2012) in Ghana showed that all stages of *W. bancrofti* were found in *Mansonia* mosquitoes caught from six LF endemic villages.

The study areas were mainly rural, reflecting in the numbers of *Anopheles* mosquitoes captured. *Anopheles* mosquitoes are mainly rural species thus accounting for the significant difference in the numbers of *Anopheles* collected per district (Dunyo et al., 1996; Appawu et al., 2001). Urbanization, industrialization and overcrowding have however brought about abundance in breeding sites.

Culex mosquitoes thrive in these urban environments which have pockets of dirty stagnant water and therefore a large number of *Culex* mosquitoes were collected from mainly the peri-urban areas located in the study sites; mainly Kasoa and its surrounding areas and Bawjiase which are in the Awutu Senya district. The availability and proximity of human settlements to breeding sites have made it easier for more *Culex* to be found in homes and *Anopheles* adapting to the changing conditions (Ahmed et al., 2016; Manyi et al., 2014).

Biting rate and infection rate are transmission indices that are important in post-MDA surveillance activities. They aid in the determination of the transmission status of an endemic area hence very useful for xenomonitoring studies in post-MDA surveillance areas. In this study, calculated vector biting rates were low (Table 31). Cumulative biting rates for all four study areas were much lower than that suggested by Webber & Southgate, (1981) to sustain transmission of LF in anopheline transmission areas.

Within districts, over the two years, biting rates remained low and comparable (Table 31). Transmission can therefore be said to be unsustainable in these areas. Studies conducted by Webber & Southgate, (1981) and de Souza et al., (2015) showed that vector biting rates of anopheline mosquitoes less than 0.66 bites/man/hr are unlikely to sustain transmission of LF. The effect of malaria control activities on LF elimination efforts has been demonstrated in The Gambia and Gabon when completed remapping surveys reported no evidence of LF transmission in both countries where altogether 2.5 million people were estimated to be at risk of the disease (Rebollo et al., 2015; Bockarie & Rebollo 2016).

The low biting rates may be attributed to the successful implementation of the malaria control programme on-going in these districts which make use of insecticide treated nets (ITNs), long lasting insecticide nets (LLNs) and indoor residual spraying (IRS). Mosquitoes were collected in the rainy season which is the peak breeding season for mosquitoes. During this time, it is expected that biting rates will be high because but climate change may have led to changes in the rainfall pattern and mosquitoes were not in abundance as expected; therefore, the low biting rates seen in this study. Biting rate however, is a surrogate marker of mosquito density and in the presence of vector control measures biting rate decreases as mosquito density decreases (Stone et al., 2014)

Another reason is that in areas where LF and malaria are co-endemic such as Ghana, vector control continues to play a major role in ameliorating the burden of vector-borne diseases. This brings about a reduction in biting rate and subsequently infection rate of mosquitoes when implemented successfully.

After the London declaration in 2012, integrated vector management (IVM) has become a key part of elimination activities. IVM is seen as a way to make informed decisions about the choice of vector control tools, improve cost effectiveness and sustainability of control and limit the use of insecticides based on an understanding of local ecological conditions pertaining in the area of interest (WHO, 2004; van den Berg et al., 2013).

One of the approaches of IVM is the use of one or more interventions against more than one vector-borne disease. In Ghana, malaria and LF are transmitted by the same vector and even though there is no well documented evidence that the malaria control programme has an impact on the LF control programme, the use of LLINs in the study areas may have had an impact on the LF elimination programme. The potential for integrating control across both diseases stems from their broad geographic overlap, shared vectors across much of this range, and susceptibility to the same interventions (Manguin et al., 2010; van den Berg et al., 2013).

Mosquito infection rates provide immediate evidence for community LF transmission (Bockarie, et al., 2002; Pedersen et al., 2009). The decision to stop MDA does not require that filarial parasites are completely absent but rather that parasite numbers are reduced to levels that transmission no longer occurs (Pedersen et al., 2009). Vector infection rates calculated in this study were higher as compared to thresholds suggested by Pedersen et al., (2009).

In areas where anopheline mosquitoes are the vectors responsible for transmission of LF, the vector infection threshold for overall larval infection has been suggested to be about 0.65% (Pedersen et al., 2009). In this study, the anopheline vector infection threshold for 2013 was 0.97 (CI: 0.17-3.17) and this increased to 1.3 (CI: 0.36-3.65) in 2014. This showed that even though mf and antigen prevalence in humans was below the 1% required to stop MDA, vectors still carried the *W. bancrofti* parasite.

Even though MDA has had a profound effect on *W. bancrofti* in humans, the effect cannot be said to be the same in the vectors due to the different modes of parasite uptake at low microfilaria prevalence affect the rate of transmission in post MDA areas. Contrary to the results obtained in this study, studies conducted in Papua New Guinea and Haiti recorded significant declines in *W. bancrofti* infection rates in mosquitoes by dissection after MDA (Bockarie et al., 2002; Goodman et al., 2003) while work done by (Farid et al., 2007) demonstrated a dramatic decrease in the clearance of filarial DNA in mosquitoes after MDA using molecular xenomonitoring.

Baseline prevalence of microfilariae in mosquitoes was not measured. Therefore, a conclusion cannot be drawn on whether there has been a decrease or not. Some mathematical models have suggested that residual L₃ may circulate between humans and mosquitoes even after the transmission breakpoint has been reached. An example is when *W. bancrofti* population is in irreversible decline but not enough to bring about active transmission and infection (Michael et al., 2004., Duerr et al., 2005).

The LAMP and PCR assays used in this study were not specific for the parasite infective stages (L₃ stage); therefore, the infections found may not be the infective stages of the parasites. Work done in Zanzibar showed that MDA resulted in a decrease in the proportion of mosquitoes with L₃ stage leading to an overall 99.7% decrease in the number of infective bites per year in the area (Rebollo et al., 2015). This led to a reduction of microfilaria prevalence from 49.5% to 10.3% with no sign of resurgence of LF a year after treatment (Maxwell et al., 1990). PCR cannot differentiate infected mosquitoes from infective mosquitoes. Therefore, in order to determine transmission risk, it is necessary to have an assay that specifically detects infective L₃ larvae (Laney et al., 2010)

Culex mosquitoes are not known vectors of LF in West Africa (Kouassi et al., 2015), more so Ghana. In spite of this, the vector infection rate in *Culex* mosquitoes collected was 0.86 (CI: 0.05-4.15) in 2013. There was however no infection seen in 2014. This threshold was also much higher than the suggested threshold of 0.25% for *Culex* transmission areas which are the WHO's South East Asian region, the Americas, the Eastern Mediterranean region and the Western Pacific region (Rao et al., 2014; de Souza et al., 2015). The DNA of parasites can be found in both vector and non-vector mosquitoes long after ingestion of microfilaria, even in situations where the microfilaria do not survive in the mosquito (Fischer et al., 2002; Fischer et al., 2007; Erickson et al., 2009) thus accounting for the presence of *W. bancrofti* microfilaria in *Culex* mosquitoes.

Culex do not transmit LF in Ghana (Dunyo et al., 1996), but they are able to pick up mf at low levels of prevalence because they exhibit the phenomenon of limitation. However, there is no evidence to support the fact that mf picked up develops to the infective L₃ stage to facilitate transmission in Anopheline transmission areas. Thus, this study demonstrates that there is the potential of using non-vector species as a proxy for determining the presence of *W. bancrofti* in a population but not the presence of infection. Through laboratory experiments as well (Fischer et al., 2007) have also shown that in both vector and non-vector mosquitoes for LF, parasite DNA can be detected for two weeks or even longer after feeding on mf positive blood.

Vector infection rate is not only used to assess the elimination of LF by determining whether *W. bancrofti* infection thresholds exist in the vector population which could signify interruption of parasite transmission but it is also used to quantify the numerical values of these thresholds (Pedersen et al., 2009). There are two reasons for this interest in vector infection rates. The first is reason is that larval infection thresholds rather than vector biting densities clearly represent the parasite elimination target for drug intervention programmes in vector-borne infections such as filariasis (Michael et al., 2007). This implies that, having thresholds below which infection is no longer said to occur, is important for diseases whose elimination is based on preventive chemotherapy.

The second reason is that information regarding the numerical value of such vector infection thresholds is required if xenomonitoring tools, such as the all-stage parasite PCR-based poolscreen methods and LAMP (Fischer et al., 2002; Williams et al., 2002; Takagi et al., 2011) can be utilized on the basis of mosquito sampling sizes in filariasis

monitoring programmes, compared with methods based on measurement of infection in humans.

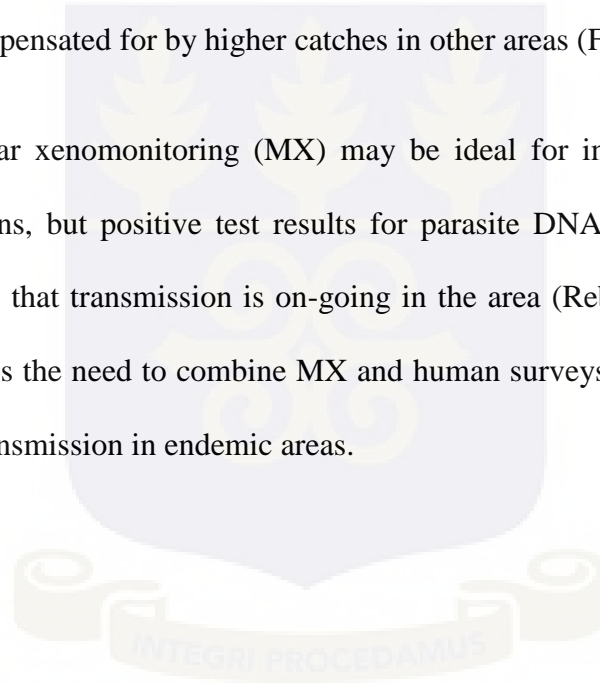
Studies in Papua New Guinea and Mali where anophelines are vectors of LF have shown that even though in some areas there is the possibility of transmission due to the presence of residual microfilaraemia, this has not resulted in the resurgence of the disease (Bockarie, 1994, Coulibaly et al., 2015). Also, before China was certified to be free of LF in 2007, studies showed the presence of residual microfilaraemia in the population (Shi, 1994). This study also points to residual transmission. However, this has not resulted in a resurgence of infection in the human population.

These studies however noted that there were two characteristics of filariasis that make it prone to interruption and ultimately elimination. The first is that transmission efficiency is low in situations where prevalence levels are low. Therefore, residual low density of microfilaraemia cases may not have a significant impact (Bockarie, 1994; Coulibaly et al., 2015; Shi, 1994). The second is that “if the basic elimination of filariasis has been achieved, the residual infection sources will gradually disappear without treatment. This takes about 4 to 5 years for brugian filariasis and 5 to 7 years for bancroftian filariasis (De-jian et al., 2013). Therefore, the “Transmission Threshold” for active transmission of LF in areas where facilitation is exhibited by *Anopheles* mosquitoes is higher than in those areas where *Culex* and other Anopheline species exhibit limitation (Duerr et al., 2005; de Souza et al., 2015). Therefore, with continued surveillance and early detection of recrudescence, vector infection rate is expected to reduce to very low levels as seen in

the humans because all positive cases will be treated on a case by case basis to achieve the elimination goal.

The results obtained from this study show that sampling multiple species which includes non-vectors with different feeding and resting behaviours provides a more complete assessment of *W. bancrofti* infections than sampling only a single important vector species (Horsfall, 1955; Ramalingam et al., 1968). Finally, if multiple species are included in xenomonitoring, the reduced sensitivity resulting from low catches in some areas will be compensated for by higher catches in other areas (Figure 18).

Overall, molecular xenomonitoring (MX) may be ideal for indirectly detecting LF in human populations, but positive test results for parasite DNA in mosquitoes does not necessarily prove that transmission is on-going in the area (Rebollo & Bockarie, 2014). Therefore, there is the need to combine MX and human surveys to come out with a firm conclusion on transmission in endemic areas.



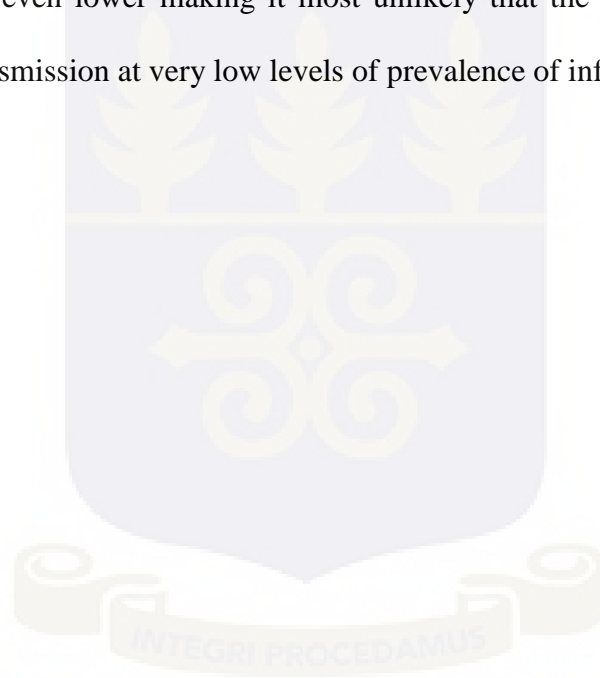
5.5 CONCLUSION

The current strategy of the GPELF of interrupting transmission of LF by treating entire at-risk populations using community wide MDA has been effective in the selected study areas. Data from reviews show that the strategy is effective in stopping transmission in areas where *Anopheles* mosquitoes transmit the disease (Bockarie et al., 2009). The results of this study showed that LF transmission was no longer ongoing in the study areas based on the infection rates and the biting rates.

Testing for the presence of infection in a mosquito using a diagnostic tool that is not stage specific implies that people may be exposed to infective bites (Ramzy et al., 1997) however the annual biting rate that is derived from the infection rates assuming the mosquitoes were harbouring infective larvae were approximately 21 infective bites per person per year in this study. Based on studies by Hairston & de Meillon, (1968) on estimates for infective biting rates of *Culex quinquefasciatus* and a number of other studies involving *Aedes* and *Anopheles*, data have been provided which shows that between 2,700 and 100,000 infective bites per person are needed to bring about infection in an uninfected person (Southgate, 1984). Therefore, an average of 21 bites per person per year is not likely to facilitate transmission of LF in the study areas.

Nonetheless, the high infection rate seen in the mosquitoes demonstrates the presence of microfilaria carriers in the human population but maintaining transmission will depend on the efficiency of the *Anopheles* vector. Since *Anopheles* mosquitoes are known not to transmit efficiently at low levels of prevalence, as infection levels decline, large numbers of mosquitoes need to be analysed to demonstrate a significant decline in infection

prevalence. There is no evidence that *Culex* mosquitoes play a role in transmission of LF in West Africa (de Souza et al., 2014; Kouassi et al., 2015) thereby making it safe to say that the infection seen in the *Culex* mosquitoes in this study is artificial. In conclusion, infection rates determined for both *Anopheles* and *Culex* mosquitoes were high as compared to thresholds suggested by Farid et al., (2007), Michael et al., (2006), Pederson et al., (2009) and Rao et al., (2014) but biting rates were exceptionally low as compared to thresholds suggested by (Webber & Southgate 1981). Maximum likelihood of infection was even lower making it most unlikely that the anopheles vector will be able to cause transmission at very low levels of prevalence of infection in humans.



CHAPTER SIX: SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1. Summary

Lymphatic filariasis is one of the seven neglected tropical diseases that have been described as eradicable and has therefore been targeted for elimination by the WHO. Global efforts to eliminate the disease begun very slowly at the onset but over the last 5 or 6 years have gained momentum making the elimination target an achievable one. Currently, 73 countries are considered to be endemic for the disease, 18 of which have progressed to the surveillance phase while 55 still require MDA. Eleven countries have not started MDA and have also not reported epidemiological evidence indicating that MDA is not required (WHO, 2015).

Countries like Egypt, Togo and Malawi have successfully reached the post-MDA surveillance phase and are in the process of conducting TAS to monitor for recrudescence. TAS is the WHO recommended strategy for monitoring recrudescence of LF and also detecting on-going residual transmission in endemic areas. Four sequential steps are recommended by the WHO to eliminate LF through MDA (Ichimori et al., 2014);

- a. mapping of the geographical distribution of the disease;
- b. administering MDA for at least 5 years to reduce the number of microfilariae circulating in the blood to levels that will likely prevent mosquito vectors from transmitting infection;
- c. implementing surveillance after discontinuation of MDA

- d. confirming interruption of transmission at the national level

It is necessary that all these steps be achieved before an endemic area can be declared to be free of the disease. Xenomonitoring is gradually catching up as a post-MDA surveillance tool in areas that have stopped MDA and are undertaking TAS. This is because after confirmation of the absence to microfilaraemia in humans, mosquitoes may still be able to pick up parasites in areas where residual transmission is present especially in areas where *Culex* mosquitoes are the vectors responsible for transmission.

Mass drug administration (MDA) has a profound effect on variables related to infection; microfilaraemia (mf) and circulating filarial antigenemia (CFA) prevalence rates and, transmission i.e. antifilarial antibodies in young children and mosquito infection rates (Ramzy et al., 2006). Current targets for MDA programmes call for the reduction of microfilaraemia prevalence rates to less than 1% and the reduction of infection rates in children born after the start of MDA to less than 0.1%. This is indicated by microfilaraemia or antigen testing (WHO, 2011b).

The areas chosen for this study have undertaken 9-10 rounds of MDA and coverage rates as indicated by the NTDCP are higher than the recommended 65% for 4-6 years (Table 3). Even though the coverage reported by the study was low, and not consistent with what has been reported by the national LF programme in Ghana, repeated annual MDA for 9 and 10 years has led to the reduction in the prevalence of microfilaraemia and antigenemia.

Repeated rounds of MDA in community-based studies with single dose DEC alone or in combination with albendazole or ivermectin has been shown to reduce the burden of LF in many endemic areas (Bockarie et al., 2002; Simonsen et al., 2004; De Rochars et al., 2005). The current data suggest that after 9 to 10 rounds of MDA, LF prevalence has been reduced to levels in the human population where transmission is no longer occurring and after four years of stopping MDA, recrudescence has not occurred. This conclusion is drawn based on the near absence of microfilaraemia carriers in school children aged 6-10 years who were the core group assessed for the presence of LF infection in this study and the adult population who provided an insight into how effective MDA had been in the study areas.

The results from this study showed the absence of microfilaria in blood smears performed on all but two participants who tested positive for filarial antigenemia. The presence of the two mf positives was not enough to continue transmission of the disease due to the inefficient transmission ability of the mosquito vector (Amuzu et al., 2010). Follow-up of the mf positive individuals which is not reported in this study showed clearance of mf a year after treatment.

Treatment coverage in these study areas was good even though baseline endemicity was high. A reduction of baseline prevalence from 23% to 0.04% has shown that single annual MDA has had a profound effect on the adult population; preventing infection and morbidity caused by the disease. This effect has been translated to the school aged population where the “herd immunity” effect has prevented the children from being

infected at early stages in their lives accounting for the zero percent mf prevalence and 0.08% antigen prevalence using ICT.

For xenomonitoring activities, the quest was to provide evidence of the absence of parasites in the vectors responsible for transmission of filariasis in Ghana; the *Anopheles* mosquito. These vectors inefficiently transmit the parasites at low levels of prevalence thereby making xenomonitoring particularly attractive in post-MDA surveillance activities. Biting rates calculated from the study for both years were exceptionally low as compared to thresholds suggested by Webber & Southgate, (1981). Annual biting rates (approximately 21 bites per person per year) were very much below the data provided by Hairston & de Meillon, (1968) which shows that between 2,700 and 100,000 infective bites per person are needed to cause infection in an uninfected person.

Transmission therefore, cannot be sustained with the biting rates calculated in this study. Even though infection rates were quite high for both *Anopheles* and *Culex* mosquitoes as compared to the thresholds suggested by Farid et al., (2007), Michael et al., (2006), Pederson et al., (2009) and Rao et al., (2014), the maximum likelihood of infection was even lower making it most unlikely that the *Anopheles* vector will be able to cause transmission at very low levels of prevalence of infection in humans.

Overall, microfilaria and antigen prevalence were low in humans while parasitemia in mosquito vectors was not enough to cause recrudescence in the study areas. The decision to stop MDA was a good one but continuous surveillance is needed to be able to detect recrudescence as early as possible. Bed net use was averagely good but its use needs to

be encouraged especially now that MDA has stopped. Integration of malaria control efforts with LF control efforts in these areas will improve the health status of residents. For a district like the Awutu Senya which has big industrial towns and a lot of peri-urban areas, it is necessary to encourage bednet use to prevent infective mosquito bites.

MDA has the potential to interrupt transmission of LF provided it is continuous and the directly observed treatment therapy (DOT) system is applied. Also in testing for the presence of the *Wuchereria bancrofti* parasite, more than one diagnostic test should be used to determine the absence of the parasite. A rapid and field applicable test like ICT is necessary to obtain results as soon as possible. Even though the disease does not kill, the discomfort associated with the disease makes it necessary that a rapid test is available to determine the presence of infection and determine ways of management to prevent further infection.

Although some tools are difficult to use under field conditions, others lack sensitivity when the intensity of infection is low (Gounoue-Kamkumo et al., 2015). Therefore, a combination of field applicable and non-field applicable tools is necessary to give accurate information on intensity of infection in endemic areas. Detection of filarial DNA in mosquitoes is a sensitive marker for assessing the endemicity of LF. It is also a useful tool for evaluating the success and progress of control programmes (Ramzy et al., 1997; Bockarie et al., 2000).

Molecular xenomonitoring therefore provides an indirect assessment of human infection and is therefore useful in evaluating the impact of intervention programmes on the

infectious microfilaria reservoir in the human host. However, to have an effective xenomonitoring system, it is necessary to understand the competence of the various mosquito vectors to pick up microfilaria especially when the microfilarial load is low after several rounds of MDA, support the development of ingested microfilariae into infective larvae and transmit to humans (Boakye et al., 2004).



6.2 Conclusion

My research has looked at the absence of *Wuchereria bancrofti* transmission in both humans and mosquito vectors after stopping administration of albendazole and ivermectin in four endemic districts in Ghana in an attempt to help the NTDCP achieve the GPELFs goal. The findings of this study suggest that a combination of xenomonitoring and transmission assessment surveys (TASs) provides a broader and confirmatory picture of the lymphatic filariasis infection status of an area after MDA has been stopped.

It also shows that continuous surveillance is necessary in detecting residual transmission and recrudescence after stopping MDA. Also, the use of more than one diagnostic tool gives a comprehensive picture of the LF status of an area and either confirms the decision to stop MDA or undertake further rounds. Recrudescence has not occurred in the study areas four years after MDA was stopped and mosquitoes are not able to pick up enough parasites to cause recrudescence possibly due to the very low levels of infection and the inability of the vector mosquitoes to pick up parasites at low infection levels.

However, xenomonitoring has to continue as part of post-MDA surveillance to ensure that infection levels fall below the suggested thresholds at which transmission cannot be sustained. This is necessary because mutational changes in the mosquito may cause it to be efficient at transmitting at low levels of prevalence thus reversing all the effort put in place to achieve elimination.

The elimination of LF is now on top of the agenda of all stakeholders and it is necessary to keep it there to be able to achieve the 2020 goal of elimination. Finally, from the current study, it appears that the earliest time for recrudescence to occur in an endemic area like the areas studied goes beyond four years post-MDA. Constant surveillance is however needed to ensure that early recrudescence is detected and dealt with appropriately.



6.3 Recommendations

As the elimination goal of lymphatic filariasis is drawing closer, it has become necessary to find innovative ways of achieving the goal and sustaining it. Issues not investigated in this study will help improve ways of achieving the 2020 goal and making the achievement of this goal sustainable. In the next few paragraphs, I make brief suggestions to the WHO, The Neglected Tropical Disease Control Programme and Academia on how future policies and studies could contribute to this area of study.

The World Health Organization

- a. The WHO policies on post-MDA monitoring should be drafted to include xenomonitoring. Pre-TAS, TAS and post-MDA surveillance should all have a xenomonitoring aspect so that not only humans are assessed but the vectors as well. This is to ensure that when the decision is made that an endemic area has eliminated the disease it is the right decision.
- b. A major challenge to the LF elimination programme is the unavailability of a defined threshold of infection rates in mosquitoes. The WHO in consultation with partner organizations need to define a threshold for infection rates in mosquitoes to enable countries in the post-MDA surveillance phase decide when infection has fallen in the vectors and transmission is no longer ongoing.

The Neglected Tropical Disease Control Programme (NTDCP)

- a. The NTDCP should include xenomonitoring in all monitoring activities especially the TAS being conducted in areas that the decision has been made to stop MDA
- b. The NTDCP should pay particular attention to areas which can be described as hotspots where recrudescence is likely to take place after MDA has been stopped.

Academia/Research

- a. It is necessary to define the role of vector control in LF elimination programme.

The issue has arisen about the danger of MDA not being able to maintain adequate treatment coverage to achieve the LF elimination goal. There is the need therefore to explore other ways by which the success of the GPELF can be ensured. Vector control alone or when implemented with chemotherapy has successfully reduced the burden of malaria in many malaria endemic countries. This strategy has also been used in areas like the Solomon Islands and successfully eliminated LF or reduced the burden to levels where active transmission is no longer occurring.

The challenge however is, not knowing the exact levels and duration of mf suppression required for elimination. Even though with adequate sustained treatment coverage MDA has the ability to meet the criteria for elimination it may be difficult to sustain sufficiently high MDA coverage to achieve LF elimination (Burkot et al., 2006). It would be very informative to know if with vector control, between four and six rounds of MDA are still needed to achieve elimination status. This would be very useful information because the adult worm can survive for about six years thus accounting for the recommendation of conducting MDA for between 4 to six years to. Vector control efforts will provide information on the need to integrate LF elimination efforts with malaria and dengue control efforts in areas where *Anopheles* and *Aedes* are responsible for transmission.

- b. Is there a chance for recrudescence of LF in areas that have stopped MDA?

In endemic areas that TAS is being conducted, the focus is on children who were born during MDA. TAS does not take into consideration the older population who may have

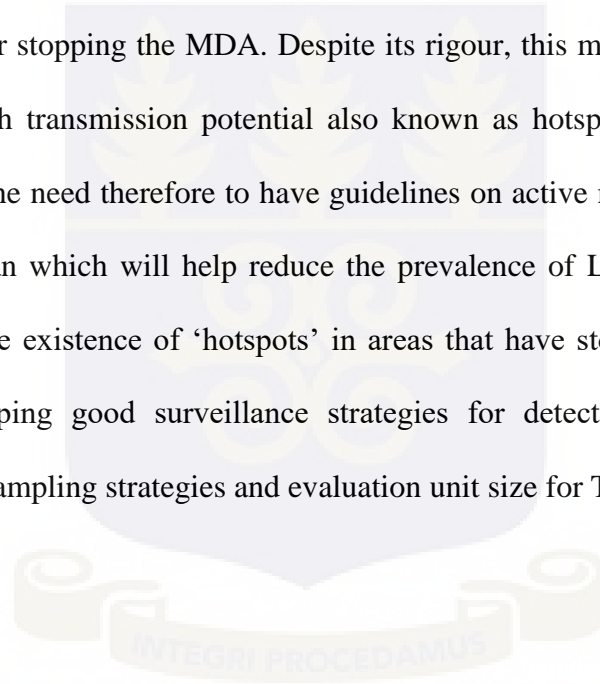
been carrying microfilaria and may not have participated in the annual MDA activities. The assumption is that, an effective minimum MDA coverage of 65% for four to six years should be enough to interrupt transmission. The WHO recommends TAS to be conducted two to three years after MDA is stopped and continued at two-year intervals to ensure that recrudescence does not occur. It may be better to include adults when monitoring interruption of transmission because antigenemia is more prevalent among adults in endemic areas (Simonsen et al., 2002; Weil et al., 1996).

Reproductively viable (mf positive) infections have been found in adults in communities where no antigen-positive children could be identified (Joseph et al., 2011). Using adults in stop MDA assessments and post-MDA surveillance has the disadvantage of potentially failing an area if circulating filarial antigenemia (CFA) is caused by persistent antigen from non-viable worms but it gives a better description of the infection status of the endemic area. Antigen-positive, mf-negative adults in an ongoing MDA setting may or may not be interpreted to mean the presence of reproductively viable worms, because the absence of mf could reflect the impact of successful chemotherapy (Figueredo-Silva et al., 1996).

The LF elimination programs will have no knowledge of whether antigen-positive adults will produce mf until a year or two after MDA has ceased. Therefore, using the all-age antigen criteria gives more confidence in making stop MDA than decisions based on children alone. However, stopping MDA based on antigen levels in either age group must be accompanied by implementation of a surveillance strategy to monitor antigenemia for several years after MDA (King et al., 2012).

- c. Are there hotspots in areas that have stopped MDA providing the opportunity for recrudescence?

Monitoring and evaluation guidelines of the programme to eliminate lymphatic filariasis require impact assessments in at least one sentinel and one spot-check site in each implementation unit (IU). Transmission assessment surveys (TAS) assess antigenemia in children in IUs that have completed at least five rounds of mass drug administration (MDA) each with 65% coverage and with microfilaria levels, 1% in the monitored sites form the basis for stopping the MDA. Despite its rigour, this multi-step process is likely to miss sites with transmission potential also known as hotspots (Swaminathan et al., 2012). There is the need therefore to have guidelines on active monitoring of these areas and an MDA plan which will help reduce the prevalence of LF to interruptible levels. Knowledge of the existence of ‘hotspots’ in areas that have stopped MDA indicate the need for developing good surveillance strategies for detecting ‘hotspots’, adopting evidence-based sampling strategies and evaluation unit size for TAS



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www.filariasis.org



APPENDICES

Appendix I

ETHICAL REVIEW COMMITTEE APPROVAL

GHANA HEALTH SERVICE ETHICAL REVIEW COMMITTEE

*In case of reply the
number and date of this
Letter should be quoted.*



Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra.

Tel: +233-0302681109
233-0302679323

*My Ref.: ERC-
Your Ref. No.*

Fax + 233-0302685424
nanatuesdaykad@yahoo.com

27th September, 2013

IRENE OFFEI OWUSU, Principal Investigator
P.O. Box LG 13
School of Public Health
University of Ghana

**GHS-ERC
Review Summary**

Protocol ID NO: GHS-ERC 14/11/12

Country: Ghana

Protocol Title: “Verification of the Absence of Lymphatic Filariasis Transmission and the Establishment of an Effective POST-MDA Surveillance in Ghana”

Review Date: 25th September, 2013

Dear Irene Offei Owusu,

Please find the review summary of the Protocol titled: “**Verification of the Absence of Lymphatic Filariasis Transmission and the Establishment of an Effective POST-MDA Surveillance in Ghana**” that was submitted to the ERC Secretariat for review.

We wish to inform you that the above-mentioned Protocol underwent full general meeting review and that approval has been granted for its implementation.

Please submit the soft copy of the approved protocol to the secretariat for its records.

The approval letter would be processed and sent to you in due course.

We wish you a successful project implementation.

Accept our congratulations.

Ag. Administrative Secretary, Ghana Health Service Ethical Review Committee

For: Chairman

Name: Abena Kwaa Addai-Donkoh



Appendix II

UNIVERSITY OF GHANA, SCHOOL OF PUBLIC HEALTH
DEPARTMENT OF EPIDEMIOLOGY AND DISEASE CONTROL
INFORMATION SHEET FOR PARENTS/GUARDIANS

We would like to invite your child/ward to participate in a research programme which aims to find out whether elephantiasis (*Lymphatic filariasis*) is no longer found in your district as it was about 10 years ago. This research work will last 4 years after which tangible conclusions can be drawn on whether the disease is no longer found in your district or otherwise. Diagnostic tests designed by the WHO will help determine this so that the annual drug distribution that goes on in your district can be stopped. It is expected that the study will provide the evidence that is needed to stop the annual drug distribution programme and also provide some simple ways of detecting the disease in the population. The research will involve questionnaire administration and collection of blood samples during the day by trained personnel.

Blood samples will be collected once from each participating child unless he/she tests positive for elephantiasis. Upon obtaining a positive result, the home of the child will be visited and night blood samples collected from both the child and all adults within the household. Needle pricks may be a bit painful and there may be the possibility of infection so to prevent this, the site of the prick will be cleaned thoroughly with cotton wool soaked in 70% alcohol before and after blood collection. The subject will be given dry sterile cotton wool to put on the site of prick and asked to hold on to it until bleeding stops. As a benefit, anyone who tests positive will be referred to the National Lymphatic

Filariasis Control Programme to be treated. In doing so the community will be spared of the disease which could lead to disability in members of the community.

Your child/ward's participation is entirely voluntary. You are not under any obligation to allow your child/ward to participate and you also have the right to refuse this invitation.

The research team may terminate the child's participation if he or she is found not able to follow procedures. If a parent or guardian at any time during the study decides that his child/ward should not participate any longer, he or she is free to withdraw his/her ward without further discussion and this will have no consequences.

Active participation of the child will only be required when the researchers visit the selected schools or follow up at home when a child tests positive. These visits will be for the collection of blood samples only. Visits to the schools will be done at times when it is most convenient for the school and home visits will be done when all members of the household are present.

All information related to your child/ward's participation will be kept confidential and will not be revealed to anyone except where required by law and regulations. The child's identity will not be revealed in any reports or publications resulting from the study.

If you have any questions concerning this study or your child's rights as part of this research project, you may contact:

Miss Irene Offei Owusu on 0207576569

Any new important information concerning the results of our study will be made known to you.

UNIVERSITY OF GHANA, SCHOOL OF PUBLIC HEALTH
DEPARTMENT OF EPIDEMIOLOGY AND DISEASE CONTROL
INFORMED CONSENT FORM FOR PARENTS AND GUARDIANS

This form needs to be filled by every parent who agrees that his/her ward participate in the study.

Please return this form after it is filled

Study Number: GHS-ERC 14/11/2012

Title of Project: Verification of the Absence of Lymphatic Filariasis Transmission in Ghana

Name of Researcher: Irene Offei Owusu

- I confirm that I have read and understand the information sheet for the above study.
- I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily
- I understand that my child/ward's participation is voluntary and he/she is free to withdraw at any time, without giving any reason, without any consequences.
- I understand that relevant sections of any of my child/ward's data collected during the study, may be looked at by responsible individuals involved in the study or from regulatory authorities where it is relevant to his/her taking part in this research. I give permission for these individuals to have access to my child's records.
- I agree to allow the enrollment of my child/ward in the above research study

Name of Child

Name of Consenting Parent/Guardian

Date

Signature/ Thumbprint



Appendix III

UNIVERSITY OF GHANA, SCHOOL OF PUBLIC HEALTH
DEPARTMENT OF EPIDEMIOLOGY AND DISEASE CONTROL
INFORMATION SHEET

We would like to invite you to participate in a research programme which aims to find out whether elephantiasis (*Lymphatic filariasis*) is no longer found in your district as it was about 10 years ago. This research work will last 4 years after which tangible conclusions can be drawn on whether the disease is no longer found in your district or otherwise. Diagnostic tests have been designed by the WHO to help determine this so that the annual drug distribution that goes on in your district and other districts can be stopped when the disease is no longer present. It is expected that the study will provide the evidence that is needed to stop the annual drug distribution programme and also provide some simple ways of detecting recurrence of the disease in the population. The research will involve questionnaire administration and collection of blood samples and collection of mosquitoes by trained personnel. Blood samples will be collected once from each consenting participant and tested for the presence of the elephantiasis causing parasite.

A second blood sample will only be collected when a positive result is obtained from a participant after the point of care test is performed. The household of the positive participant will be visited between 9pm and 2am and blood samples collected from both the individual and all other members of their household for further testing. Needle pricks may be a bit painful and there may be the possibility of infection. In order to prevent this, the site of the prick will be cleaned thoroughly with cotton wool soaked in 70% alcohol before and after blood collection. The subject will be given dry sterile cotton wool to put

on the site of prick and asked to hold on to it until bleeding stops. As a benefit, anyone who tests positive will be referred to the National Lymphatic Filariasis Control Programme to be treated. In doing so the community will be spared of the disease which could lead to disability in members of the community which will lead to affected individuals becoming a burden on their household.

Your participation is entirely voluntary and you are not under any obligation to participate. You also have the right to refuse this invitation if you are not comfortable with it in any way. The research team may terminate your participation if you are not able to follow procedures or they do not deem you fit enough to participate in the study. If at any time during the study you decide not to participate any longer you are free to withdraw without further discussion and this will have no consequences.

Active participation will only be required when the researchers visit the selected homes to administer questionnaires and draw about 10 drops of blood and also when follow up is done on a participant who tests positive for the first test conducted. These visits will be for the collection of blood samples only. Visits will be done when all members of the household are present and blood samples collected from all members of the household.

All information related to your participation will be kept confidential and will not be revealed to anyone except where required by law and regulations. Your identity will not be revealed in any reports or publications resulting from the study.

If you have any questions concerning this study, you may contact:

Miss Irene Offei Owusu on 0207576569

Any new important information concerning the results of our study will be made known to you.

UNIVERSITY OF GHANA, SCHOOL OF PUBLIC HEALTH
DEPARTMENT OF EPIDEMIOLOGY AND DISEASE CONTROL
INFORMED CONSENT FORM FOR ADULTS

This form needs to be filled by every person approached to participate in the study.

Please return this form after it is filled

Study Number: GHS-ERC 14/11/2012

Title of Project: Verification of the Absence of Lymphatic Filariasis in Ghana.

Name of Researcher: Irene Offei Owusu

- I confirm that I have read and understand the information sheet for the above study
- I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily
- I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without any consequences.
- I understand that relevant sections of any data collected during the study may be looked at by responsible individuals involved in the study or from regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.
- I agree to take part in the above research study.

Name of Participant

Signature/ Thumbprint

Date



Appendix IV

UNIVERSITY OF GHANA, SCHOOL OF PUBLIC HEALTH
DEPARTMENT OF EPIDEMIOLOGY AND DISEASE CONTROL
INFORMATION SHEET

We would like to invite you to participate in a research programme which aims to find out whether elephantiasis (*Lymphatic filariasis*) is no longer found in your district as it was about 10 years ago. This research will last 4 years after which tangible conclusions can be drawn on whether the disease is no longer found in your district or otherwise. Diagnostic tests have been designed by the WHO to help determine this so that the annual drug distribution that goes on in your district can be stopped. It is expected that the study will provide the evidence that is needed to stop the annual drug distribution programme and also provide some simple ways of detecting recurrence of the disease in the population. The research will involve questionnaire administration and collection of blood samples during the day and sometimes at night by trained personnel. Blood samples will be collected once from each participant unless he/she tests positive for elephantiasis. Upon obtaining a positive result, the participant will be visited again and blood samples collected from both the individual and all other members of their household between 9pm and 2am.

Needle pricks may be a bit painful and there may be the possibility of infection so to prevent this, the site of the prick will be cleaned thoroughly with cotton wool soaked in 70% alcohol before and after blood collection. The subject will be given dry sterile cotton wool to put on the site of prick and asked to hold on to it until bleeding stops. As a benefit, anyone who tests positive will be referred to the National Lymphatic Filariasis

Control Programme to be treated. In doing so the community will be spared of the disease which could lead to disability in members of the community which will lead to affected individuals becoming a burden on their household.

Your participation is entirely voluntary and you are not under any obligation to participate. You also have the right to refuse this invitation if you are not comfortable with it in any way. The research team may terminate your participation if you are not able to follow procedures. If at any time during the study you decide not to participate any longer you are free to withdraw without further discussion and this will have no consequences.

Active participation will only be required when the researchers visit the selected homes to administer questionnaires and draw about 10 drops of blood and also when follow up is done on a participant who tests positive for the first test conducted. These visits will be for the collection of blood samples only. Visits will be done when all members of the household are present and blood samples collected from all members of the household.

All information related to your participation will be kept confidential and will not be revealed to anyone except where required by law and regulations. Your identity will not be revealed in any reports or publications resulting from the study.

If you have any questions concerning this study, you may contact:

Miss Irene Offei Owusu on 0207576569

Any new important information concerning the results of our study will be made known to you.

UNIVERSITY OF GHANA, SCHOOL OF PUBLIC HEALTH
DEPARTMENT OF EPIDEMIOLOGY AND DISEASE CONTROL
INFORMED ASSENT FORM

This form needs to be filled by every person approached to participate in the study.

Please return this form after it is filled

Study Number: GHS-ERC 14/11/2012

Title of Project: Verification of the Absence of Lymphatic Filariasis Transmission in Ghana.

Name of Researcher: Irene Offei Owusu

- I confirm that I have read and understand the information sheet for the above study.
- I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.
- I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without any consequences.
- I understand that relevant sections of any data collected during the study may be looked at by responsible individuals involved in the study or from regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.
- I agree to take part in the above research study.

Name of Participant

Signature/Thumbprint

Date

Appendix V

QUESTIONNAIRE FOR CHILDREN AND ADULTS

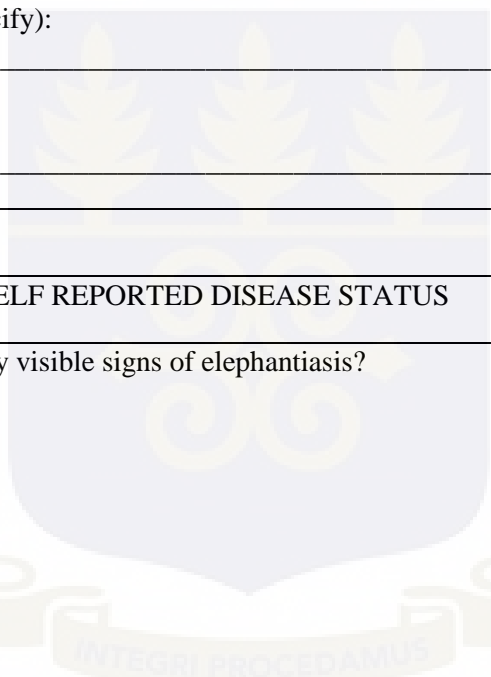
TOPIC: VERIFICATION OF THE ABSENCE OF LYMPHATIC FILARIASIS TRANSMISSION IN GHANA.

NO.	QUESTION	VARIABLE NAME
Q1	Interviewer ID : <input style="width: 30px; height: 20px; display: inline-block; margin-right: 5px;" type="text"/> <input style="width: 30px; height: 20px; display: inline-block; margin-right: 5px;" type="text"/> <input style="width: 30px; height: 20px; display: inline-block;" type="text"/>	Q1INTID
Q2	Name of Respondent : _____	Q2RESNAME
Q3	Participant ID : <input style="width: 30px; height: 20px; display: inline-block; margin-right: 5px;" type="text"/> <input style="width: 30px; height: 20px; display: inline-block; margin-right: 5px;" type="text"/> <input style="width: 30px; height: 20px; display: inline-block; margin-right: 5px;" type="text"/> <input style="width: 30px; height: 20px; display: inline-block; margin-right: 5px;" type="text"/> <input style="width: 30px; height: 20px; display: inline-block;" type="text"/>	Q3PARTID
Q4	Head of Household: _____	Q4HHEAD
Q5	House Number: _____	Q5HNO
Q6	Date D D M M Y Y Y Y <input style="width: 30px; height: 20px; display: inline-block; margin-right: 5px;" type="text"/> <input style="width: 30px; height: 20px; display: inline-block; margin-right: 5px;" type="text"/> <input style="width: 30px; height: 20px; display: inline-block; margin-right: 5px;" type="text"/> <input style="width: 30px; height: 20px; display: inline-block; margin-right: 5px;" type="text"/> <input style="width: 30px; height: 20px; display: inline-block; margin-right: 5px;" type="text"/> <input style="width: 30px; height: 20px; display: inline-block; margin-right: 5px;" type="text"/> <input style="width: 30px; height: 20px; display: inline-block; margin-right: 5px;" type="text"/> <input style="width: 30px; height: 20px; display: inline-block;" type="text"/>	Q6DATE
Q7	District/Municipality: [1] Awutu Senya [2] Effutu [3] Agona East [4] Agona West	Q7DIST <input style="width: 30px; height: 30px; margin-left: 10px;" type="checkbox"/>

Q8	Sub-district: _____ _____	Q8SUBDIST
Q9	School/Community: _____	Q9SCHCOM
SECTION A: BIODATA		
Q10	Age: <input type="text"/> <input type="text"/> <input type="text"/>	Q10AGE
Q10 b	Date of Birth: D D M M Y Y Y Y <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	Q10bDOB
Q11	Sex: [M] Male [F] Female	Q11SEX <input type="checkbox"/>
Q12	Level of education: [1] None [2] Primary [3] Middle/JHS [4] SHS/O/A level [5] Tech/Voc . [6] Tertiary [7] Non formal education [8] Other specify: _____	Q12EDUC <input type="checkbox"/>

Q13	Do you have a bednet in your home? [1] Yes [2] No	Q13HAVNET <input type="checkbox"/>
Q14	Do you sleep under the/a bednet? [1] Yes [2] No	Q14SLEEPNET <input type="checkbox"/>
SECTION B: COMPLIANCE TO MASS DRUG ADMINISTRATION(MDA)		
Q15	Do you know about any drug distribution programme in this community where the heights of people are measured and tablets given? [1] Yes [2] No	Q15KNOW <input type="checkbox"/>
Q16	Have you ever participated in the programme? [1] Yes [2] No	Q16PARTICIPATED <input type="checkbox"/>
Q17	If "No" to Ques 16, why? [1] Less than approved height [2] No parental consent [3] Have been busy during time of administration [4] Dislike for taking medicines [5] Did not find it necessary to take the drugs [6] Religious belief [7] Wasn't around during time of administration [8] Other (specify): _____	Q17NOWHY <input type="checkbox"/>

Q18	<p>If “Yes” to Ques.16, how many times have you participated in the programme?</p> <p style="text-align: center;"><input type="text"/> <input type="text"/></p>	Q18NOTIMEPART
Q18 b	<p>Comments:</p> <p>_____</p> <p>_____</p>	Q18bCOMMENT
Q19	<p>What prevented you from receiving the drugs in the years that you did not?</p> <p>[1] Religious beliefs</p> <p>[2] Less than required height</p> <p>[3] Dislike for the drug</p> <p>[4] Travelled</p> <p>[5] Drug reaction</p> <p>[6] No parental consent</p> <p>[7] Pregnant</p> <p>[8] Breastfeeding</p> <p>[9] Other (specify):</p> <p>_____</p> <p>_____</p>	<p>Q19WHATPREV</p> <p style="text-align: center;"><input type="checkbox"/></p>
Q20	<p>Did you accept the drugs when they were given to you?</p> <p>[1] Yes</p> <p>[2] No</p> <p>[3] Not Applicable</p>	<p>Q20ACCEPT</p> <p style="text-align: center;"><input type="checkbox"/></p>
Q21	<p>Did you swallow the drugs that you received?</p>	<p>Q21SWALLOW</p> <p style="text-align: center;"><input type="checkbox"/></p>

	<p>[1] Yes</p> <p>[2] No</p> <p>[3] Not Applicable</p>	
Q22	<p>If “No”, why not?</p> <p>[1] I forgot</p> <p>[2] Had a reaction the last time I took the drugs</p> <p>[3] Do not find it necessary to take the drugs</p> <p>[4] Dislike for drugs</p> <p>[5] Other (specify):</p> <p>_____</p> <p>_____</p>	<p>Q22NOSWALLWH</p> <p>Y <input type="checkbox"/></p>
		
	SECTION C: SELF REPORTED DISEASE STATUS	
Q23	<p>Do you have any visible signs of elephantiasis?</p> <p>[1] Yes</p> <p>[2] No</p> <p>Sign</p> <p>[1] Hydrocoele</p> <p>[2] Lymphedema</p> <p>(Question 23 will be answered mainly by observation of respondent by interviewer)</p>	<p>Q23DXSIGN</p> <p><input type="checkbox"/></p> <p><input type="checkbox"/></p>
THANK YOU		
	SECTION D: PARASITOLOGICAL EXAMINATION	
Q24	<p>Sample/Participant ID</p> <p><input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/></p>	Q24SAMPID

	[3] Indeterminate [4]Not Applicable	
--	--	--



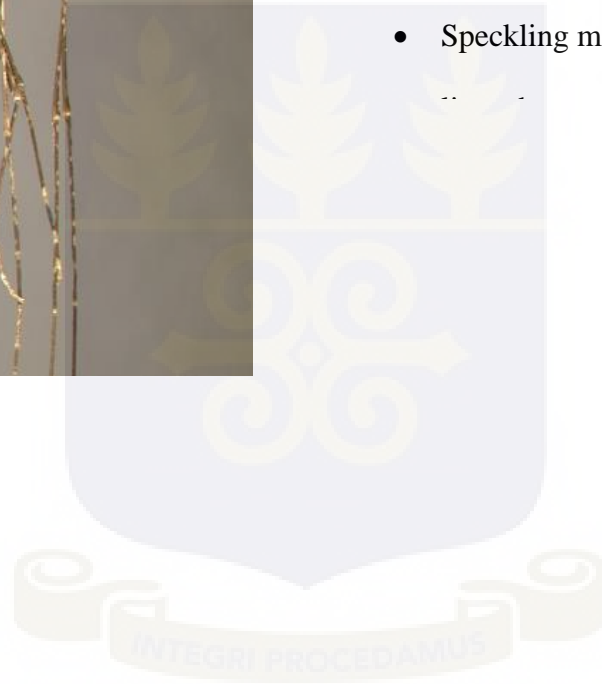
Appendix VI

MOSQUITO IDENTIFICATION KEYS

a) *Anopheles gambiae*



- Pale and dark band patterns on the costa of the wings
- Femora, tibiae and the first tarsal segment of the legs speckled to a variable degree.
- Speckling may sometimes fuse to form short



b) Anopheles funestus



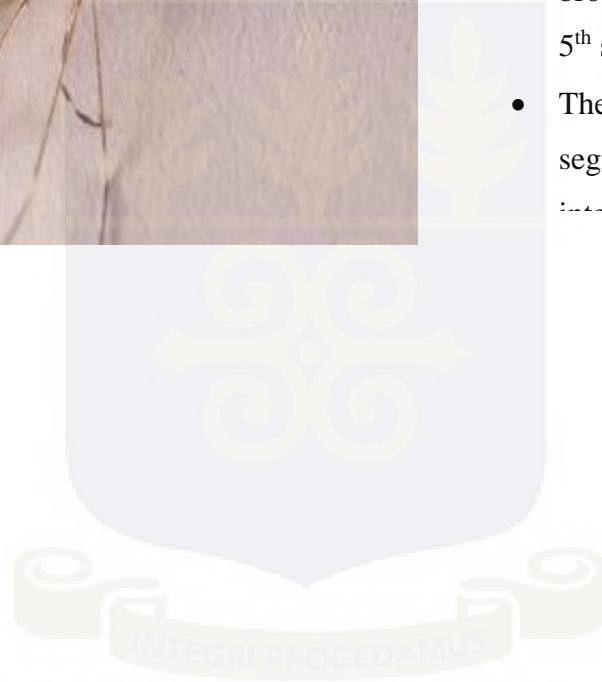
- Dark and pale band patterns on the costal margins of the wings
- Mainly dark legs
- Tibiae and tarsi and occasionally have small apical white spots
- Few pale scales at the joints which can be observed only under a microscope.



c) *Anopheles pharoensis*



- Palps of the females are shaggy with four bands situated largely at the apices of segments 2-5
- The femora and tibiae of the legs are prominently speckled
- Segments 1- 4 of the hind tarsi are broadly pale apically while the entire 5th segment is pale
- The femora, tibiae and 1st tarsal segment are prominently pale



d) *Culex* mosquitoes



- Dark-scaled wings
- The femora and tibiae of the legs are generally dark (may have up to about 10 pale spots which may be yellow or white)



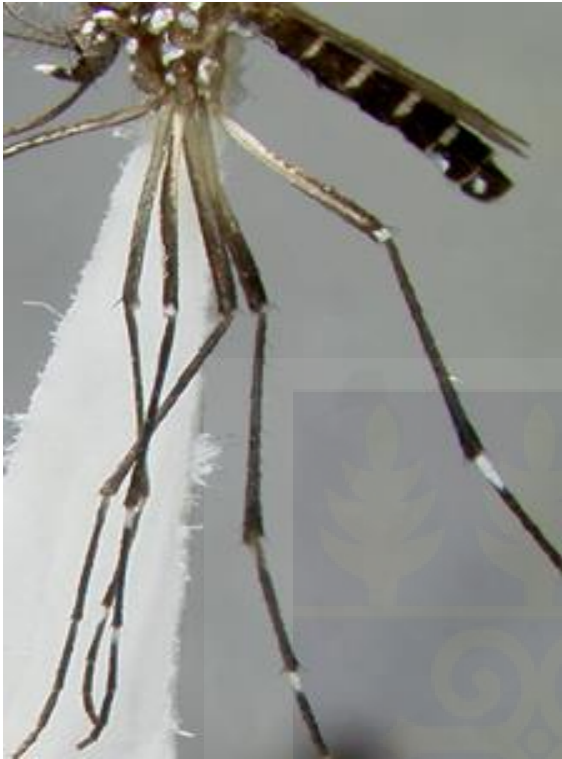
e) *Mansonia* mosquitoes



- Absence of dark and pale patterns on the costal margins or wings
- Presence of pale markings that may or may not form complete rings on the femora and tibiae



f) *Aedes* mosquitoes



- Generally identified as very black mosquitoes with white silvery spots which are usually concentrated in the thoracic region
- Palp has three segments (sometimes four)
- The fourth segment of the palp if present is very small.
- The scutum has pale scales which may be narrow
- The scutellum has broad scales on all three lobes.
- There are conspicuous black and white patterns on the femora, tibiae and tarsi.
- At least one tarsomere of the hindtarsus has a basal white band

