Assessing Transmission of Lymphatic Filariasis Using Mosquitoes in Communities with At Least Five Rounds of Mass Drug Administration in Ghana

BY

Joseph Harold Nyarko Osei (BSc. Hons.)

A Thesis submitted to the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology in partial fulfillment of the requirements for the degree of

MASTER OF PHILOSOPHY

College of Science

Department of Theoretical and Applied Biology

August, 2013

DECLARATION

I hereby declare that this submission is my own work towards the MPhil and that, to the best of my knowledge, it contains no materials previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

Joseph Harold Nyarko Osei (20253165)

……..... …….. ……

Student Name & ID Signature Date

Certified by:

Prof. Daniel Adjei Boakye

……..... …… ……..

Supervisor Name Signature Date

Prof. Michael David Wilson

……..... ……. ……..

Supervisor Name Signature Date

Prof. Kwasi Obiri-Danso

……..... …….. …….

Supervisor Name Signature Date

Certified by:

Rev. Stephen Akyeampong

……..... …….. …….

Head of Dept. Name Signature Date

DEDICATION

This work is dedicated to my family especially my late mum, Mrs. Evelyn Amankwah Osei, who wanted to see me get at least my doctorate degree. This is also for my dad who is willing to support all his children through to any level on the educational ladder they wish to pursue. To my siblings who would always wish me well in all endeavours. You have all been a strong pillar for me. Thank you all for your immense contributions to all my successes.

ACKNOWLEDGEMENT

I am sincerely gratitude first to my supervisors Profs. Daniel A. Boakye, Michael D. Wilson and Kwasi Obiri-Danso for their support and guidance offered me through the entire study. I thank Profs. Daniel A. Boakye, Michael D. Wilson Dr. Maxwell A. Appawu and Dr. Samuel K. Dadzie who gave me the opportunity to participate in training programmes which were very essential for the completion of my study.

I would also like to acknowledge the wonderful support obtained from the members and staff of the Parasitology Department at the Noguchi Memorial Institute for Medical Research (NMIMR) especially from Mr. Sampson Otoo, Mr. Andy Asafu-Adjaye, Mr. Mba Tihssoumah Mosore, Mr. Osei Kwaku Akuoko, and Ms. Sellase Pi-Bansa who helped in the field collections of my samples, Ms. Millicent Opoku and Ms. Dora Okyere for their support with some of the entomological and molecular laboratory procedures. I will not forget the hard working National Service Personnel and attachment interns who helped in diverse ways. I cannot forget Dr. Kingsley Badu for giving some mosquito samples for the cibarial armature preparation. I appreciate the cooperation of the study communities, field collectors, the transport officer for NMIMR, the various drivers from NMIMR involved in my sampling. To the Director of NMIMR, I say a very big thank you for allowing me work at the Institute.

I cannot forget the wonderful advice offered by my "Senseis" Prof. Takashi Suzuki and Dr. Mitsuko Ohashi-Suzuki. I thank them for allowing me access to some of the equipment in their laboratories.

Whiles thanking God for the strength for my daily schedules, I would also like to thank my family for their tremendous support all these years. I really appreciate your contributions and diverse support.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

µl	Micro Litre
µM	Micro Molar
ABR	Annual Biting Rate
ADL	Acute Adeno-Lymphangitis
ADLA	Acute Dermato-Lymphangio-Adenitis
AFL	Acute Filarial-Lymphangitis
AIBR	Annual Infective Biting Rate
ATP	Annual Transmission Potential
BR	Biting Rate
BSA	Bovine Serum Albumin
Ct	Cycle threshold
ddH_2O	Double distilled water
DEPC	diethylpyrocarbonate
dH_2O	Distilled water
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
dNTP	Deoxynucleotide triphosphate
ELISA	Enzyme Linked Immunosorbent Assay
gDNA	Genomic DNA
GPELF	Global Programme for the Elimination of Lymphatic Filariasis
IBR	Infective Biting Rate
ICT	Immuno Chromatographic Test
ITFDE	International Task Force for Disease Eradication
L_1	First Larval Stage of *Wuchereria bancrofti* in the mosquito vector

L_2	Second Larval Stage of *Wuchereria bancrofti* in the mosquito vector
L_3	Infective Larval Stage of *Wuchereria bancrofti* in the mosquito vector
LDR	Long DNA Repeat
LF	Lymphatic Filariasis
MBR	Monthly Biting Rate
MDA(s)	Mass Drug Administration(s)
MDG	Millennium Development Goals
Mf(s)	Microfilaria(e)
Ml	Milli Litre
mM	Milli Molar
NTDs	Neglected Tropical Diseases
PCR	Polymerase Chain Reaction
PSC	Pyrethrum Spray Catches
RNase	Ribonuclease
RT-PCR	Real Time Polymerase Chain Reaction
STH	Soil Transmitted Helminthes
TAS	Transmission Assessment Surveys
TDR	Tropical Disease Research
TP	Transmission Potential
USAID	United State Agency for International Development
UV	Ultra Violet
WHA	World Health Assembly
WHO	World Health Organization

ABSTRACT

There has been a critical question of when to stop Mass Drug Administration (MDA) in lymphatic filariasis (LF) endemic communities. After at least five (5) rounds of MDA with ivermectin and albendazole, it is expected that microfilaraemia should reach a minimum threshold in the human population such that the vectors (predominantly *Anopheles gambiae*) may not be able to pick up microfilaria during a blood meal.

Using Pyrethrum Spray Catches (PSC), mosquitoes were sampled from Ayensuako, Gyaahadze, and Mankrong communities located in Awutu/Senya, Efutu Municipal and Agona East Municipal districts respectively, all in the Central Region of Ghana where more than 5 rounds of MDA had been done. All mosquitoes caught were morphologically identified and dissected for infection with *W. bancrofti* and the cibarial armature examined for the various species. A total of 550 mosquitoes were collected. Distribution of mosquitoes in these endemic communities was predominantly *An. gambiae* s.l. – 462/550 (84.0%). The proportion of other mosquitoes species were *An. funestus* 9/550 (1.6%), *An. pharoensis* 1/550 (0.2%), *Culex sp.* 57/550 (10.4%), and *Mansonia sp.*, 21/550 (3.8%). For all samples, microscopy was negative for LF parasites. In *Anopheles* mosquitoes the cibarial teeth of the *Anopheles sp.* (average of 15 cibarial teeth (12-19; p = 0.002)) were significantly less than those observed (under the compound microscope) in the *Culex sp.* (average of 26 cibarial teeth (20-34; p = 0.002)).

Although the numbers of mosquitoes collected were low due to the period of collection, the results compared to similar studies in the same region of Ghana indicate that MDA in this area has possibly led to elimination of transmission due to low worm load in the

mosquito vectors. There is, however, residual transmission in some of the endemic communities that had participated in at least five rounds of MDA. The impact of this observation and the analysis of the cibarial armature for the different species as discussed shows that elimination in endemic communities would be realized within the stipulated timelines, 2020, if MDA is combined with vector management.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Introduction

LF commonly referred to as elephantiasisis is a debilitating parasitic disease (Boakye *et al.* 2007). It is known to infect 120 million people worldwide while 1.2 billion people are at risk (Ottesen *et al.* 2008). Apart from being the leading cause of permanent and long-term disability, it happens to be the second largest cause of acute, and /or chronic disability worldwide (Richens 2004, Zeldenryk *et al.* 2011). The disease may manifest as abnormal swelling of the arms, breast (females), male and female genitalia, and legs. Following bacterial infections, a swollen body part may progress to elephantiasis characterized by sores and thickened skin (Palumbo 2008). The disease is transmitted by mosquitoes belonging to the genera *Aedes, Anopheles, Culex,* and *Mansonia* (Koroma *et al.* 2012). These genera alongside *Coquilletidia* and *Ochlerotatus* have been reported to be carriers of the LF parasites (de Souza *et al.* 2012). These vectors transmit the human filarial parasites, *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* in different geographical (tropical and subtropical) areas of Africa, Asia, Western Pacific, the Caribbean and parts of the Americas (Bockarie and Molyneux 2009, Koroma *et al.* 2012, Melrose 2004, Ottesen *et al.* 1997, Molyneux and Taylor 2001, Richens 2004).

LF is a disease of major public health concern due to its burden on affected communities and endemic countries. LF is one of the diseases captured under the Neglected Tropical Diseases (NTDs) and Tropical Disease Research (TDR) for elimination and to ensure its control (Hotez *et al.* 2007). NTDs cause a fourth of the

global burden of diseases (Hotez *et al.* 2004) with LF alone contributing 38% to this. Advances in diagnostics, availability of drugs against LF and the donation of these drugs (ivermectin by Merck and Co and albendazole by GlaxoSmithKline) created the environment for the elimination of the disease. These factors together with a strategy of MDA to whole endemic communities thus made it possible for LF to be targeted as an eliminable disease by the WHO (Ottesen *et al.* 1997, Alleman *et al.* 2006, Molyneux 2003). LF has thus been targeted for elimination by one such programme, the Global Programme for the Elimination of Lymphatic Filariasis (GPELF) in Ghana. In 1993, the International Task Force for Disease Eradication (ITFDE) which was formed and in 1988 declared that LF was one of the six eliminable diseases (dracunculiasis (Guinea Worm), LF, mumps, poliomyelitis, rubella and cysticercosis). This led the World Health Assembly (WHA) in 1997 to pass a resolution – WHA.50.29, purposely for the elimination of LF as a public health disease (WHO 2010, Mohammed *et al.* 2012). This was a call to the WHO member states. Around that same time, WHO had put in place measures to help eliminate LF through MDA (Ottesen *et al.* 2008). Following this resolution (WHA.50.29), the GPELF was established in early 2000 (Molyneux 2003). LF had already been marked for elimination by the year 2020 and the GPELF had two main strategies to meeting this goal. The first was interruption of the transmission of *W. bancrofti* that causes LF through annual MDA to all at risk population in endemic areas. Secondly, the programme was going to manage morbidity and prevent disability among people already affected by the disease. Out of the 83 endemic countries worldwide, 39 of them are from Africa. Ghana is one of the LF endemic countries found in West Africa. The GPELF is based on the hypothesis that Anophelines are inefficient vectors

at low level microfilariaemia (< 1%) and hence 'transmission interruption' could be achieved after five rounds of MDA. In Ghana, *Anopheles* mosquitoes are the only known vectors. Surveys spanning the entire length of the country (Gyapong *et al.* 1996; Dunyo *et al.* 1996) confirmed *Anopheles gambiae* s. l. and *Anopheles funestus* as the vectors of LF in Ghana. The fact that *Anopheles* mosquitoes exhibit the phenomenon of facilitation makes the GPELF strategy seemingly a laudable one. However, if the transmission cycle involves culicines, it would be difficult to achieve elimination of the disease via MDA alone. This is because culicines are capable of transmitting LF even at low Mf (< 1%). In Ghana, recent studies have indicated that some vectors particularly *An. melas* (Amuzu *et al.* 2010) and *Mansonia spp* (Ughasi *et al.* 2010, 2012) could transmit at low levels of microfilaraemia thus keeping a residual transmission of the disease even after more than seven rounds of LF MDA.

This means that when MDA is stopped in an endemic community having culicines as vectors, after observing < 1% Mf prevalence, there could be resurgence of the disease shortly afterwards.

1.2 Rationale of Study

Prior to 1990, scanty LF surveys had been conducted and reported in Ghana. The disease was recorded as being common in the northern regions of Ghana in 1936 by the Gold Coast Medical Department Reports. For some strange reason this information was only found in London but not Ghana (Gyapong 2000). After 1990, however, a nationwide LF studies begun. These studies were well documented this time and had treatment as a component in the various study sites. LF is endemic in Ghana (Dunyo *et al.* 1996, Gyapong *et al.* 1994). The LF surveys in Ghana restarted around 1992. A

preliminary LF study was conducted in the Kasena-Nankana District, Upper East Region of Ghana probably as a pilot project (Gyapong *et al.* 1993). Gyapong and his colleagues selected three communities in the same district, for the first LF survey in August-September, 1992 based on a vitamin A trial database. The study revealed an overall prevalence of 32.4% and it was shown that there was no significant difference between the three study communities in clinical and parasitological findings (Gyapong *et al.* 1994). Following this observation, a nationwide LF survey was conducted in 1996 to determine prevalence and distribution of *W. bancrofti* Mf and its associated clinical diseases. A prevalence map was therefore obtained for Ghana. LF was found to be more prevalent in the northern guinea savannah and the southern coastal savannah, though with considerable regional variations, while the middle forest belt was appreciably free. Realising LF to be of major public health significance, especially in the northern guinea and southern coastal savannahs, the study advocated for a design and implementation of a control programme (Gyapong *et al.* 1996a). Since then, several LF studies had been conducted in Ghana before the year 2000 (Gyapong *et al.* 1996d, Dunyo *et al.* 1996, Dzodzomenyo *et al.* 1999, Gyapong *et al.* 1996b, 1996c, 1998a, 1998b). These studies may have influenced the decision to implement a programme such as the GPELF in Ghana as these studies evidently showed LF endemicity in the country. The GPELF commenced their activities in Ghana in the year 2000. After 2000, several LF surveys had been conducted in the country (Biney *et al.* 2010, Boakye *et al.* 2004, 2007, de Souza *et al.* 2012, Aboagye-Antwi 2003, Amuzu *et al.* 2010, Appawu *et al.* 2001, 2006, de Souza *et al.* 2010, Gbakima *et al.* 2005, Gyapong 2004, Ughasi *et al.* 2010, 2012). These studies implicated *Anopheles* mosquitoes as the vectors for the disease in Ghana.

Prior to these studies, in 1993, the Carter Center's International Task Force for Disease Eradication had found that with the tools and technologies at that time, LF could be eradicated. Realising the possibilities of elimination of the disease, the WHA made a landmark resolution (WHA.50.29) leading to the formation of the GPELF in the year 2000. That same year, the United Nations during the Millennium Summit established the Millennium Development Goals (MDG). These goals were adopted into the working plans of all member states and some international organizations. A programme such as the GPELF incorporated these MDG into their work plans in a way that would help it achieve its goals as described by Molyneux (2003). The GPELF at its inception in the year 2000 thus targeted some endemic districts/communities in Ghana. Mass chemotherapy was the strategy for elimination of LF. MDA therefore started in five endemic districts (Awutu-Efutu Senya, Ahanta-West, Sissala, Kassena-Nankana, Builsa) in Ghana. This gradually expanded to eventually gain nationwide coverage in all the 61 endemic districts in 2006. At the beginning of the programme, there were 49 districts in Ghana. After a re-demarcation exercise, the number of endemic districts became 61 and it is currently 82. Evaluation of the MDA after 2005 revealed high Mf levels in the five districts. Another detailed evaluation in 2007 still revealed high Mf levels in these selected sentinel sites. The MDA had to therefore continue in those districts. The expansion began in the five districts mentioned above in 15 sub districts. These 15 sub districts included Kunchugu, Jeffisi, Gwollu of Sissala; Chuchuluga, Wiaga, Fumbisi of Builsa; Navrongo, Paga, Kasena-Nankana District South of Kasena-Nankana; Apowa, Dixcove, Agona/Princess of Ahanta West; and Winneba – Awutu, Winneba – Efutu and Bontrase – Senya of the Awutu Efutu Senya districts (Ghana

Filariasis Elimination Programme 2008). According to this report, few sub districts had Mf prevalence < 1% with the rest having prevalence between 1.1% - 10.2%. Work done by Kwansa-Bentum in 2005 (unpublished) in nine endemic communities and Amuzu *et al.* (2010), in two endemic communities had different annual transmission potentials (ATP). *An. melas*, one of the *An. gambiae* complex sibling species transmitted even at low level microfilaraemia (Amuzu *et al.* 2010). This poses a big challenge to the GPELF exercise in the study areas and wherever *An. melas* happens to be a vector worldwide. Transmission of LF in endemic communities of sentinel sites in Ghana was expected to have halted after 2006. This has not been the case because there is still evidence of residual prevalence of the disease in these communities (Gbakima *et al.* 2005). Ughasi *et al.* (2010, 2012) and Coulibaly, M., 2010 (unpublished) revealed infective stages of *Wuchereria bancrofti* in samples of *Mansonia* mosquitoes both stored and collected since 2000 and 2010 respectively. *Mansonia* mosquitoes sample collected between 2001 and 2008 after dissection in 2010 revealed infective stages of the filarial worm (Coulibaly, M., 2010 – unpublished). The likelihood of *Mansonia* mosquitoes being a competent vector or developing into one in Ghana and thus West Africa could not be overemphasized. Laboratory experiments had been successfully conducted to determine the role of *Mansonia* mosquitoes in the transmission of LF by Onapa *et al.* (2007) but not in the wild. Ughasi *et al.* (2012) recently gave the first report of two *Mansonia species* possibly contributing to the observed residual LF transmission in some endemic communities in Ghana. This happens to be the only report having *Mansonia* mosquitoes as part of the LF transmission cycle in Africa confirming an earlier report in 1958 (Toumanoff 1958). *Culex* mosquitoes, however,

have been reported to be refractory against *W. bancrofti* in Ghana, West Africa (Aboagye-Antwi 2003) but not in East Africa (Magayuka and White 1972, Mwandawiro *et al.* 1997). In a neighbouring West African country, Nigeria, though not conclusive, there is some evidence of *Culex* mosquitoes transmitting LF in some of their endemic communities (Anosike *et al.* 2005, Agi and Ebenezer 2009, Nwoke *et al.* 2010). The involvement of culicines in the transmission of LF in Ghana would seriously challenge efforts to eliminate the disease using MDA alone. There would obviously be the need for entomological components in LF elimination programmes in Ghana. Currently, there had been over 5 rounds of MDA across the country. It is therefore advisable to re-evaluate the LF status in all endemic foci in Ghana. For global evaluations in LF surveys, Transmission Assessment Surveys (TAS) is used to standardize evaluations from different endemic foci. The TAS is designed to help programme managers determine whether or not endemic areas have reached the critical threshold of infections for stoppage of MDA. This is done with the aid of the Survey Sample Builder Software. Several flexible factors are considered to best fit each of the local situations. These factors include the net primary school enrolment ratio; the population size; the number of schools or enumeration areas and the feasibility of different survey methods (WHO/HTM/NTD/PCT/2011.4 2011, 2012). As flexible as this design may be, there is no entomological component among the listed factors. This may not offer programme managers a comprehensive information to take that critical decision of stopping MDA or otherwise in endemic areas. This work is therefore to assess all possible vector species. It will also determine by inference, indices that could enable predict the transmission phenomenon exhibited by the vectors of LF in Ghana.

Surveillance studies in Ghana so far, involve entomological studies that concentrate solely on the *Anopheles species* aside the Parasitological surveys and MDAs. This study will reiterate the importance of examining all known vectors of LF. It will ensure that entomological surveillance teams will incorporate all LF vectors into their working plans especially when there is suggestive evidence of *Culex* mosquitoes transmitting in West Africa.

1.2.1 Aim of study

- To determine and characterize the mosquitoes involved in the maintenance of LF transmission in areas with about five years of MDA and detect the filarial nematode (*W. bancrofti*) infection.

1.2.2 Specific objectives

- To identify mosquitoes caught in the study area using morphology as described by Gillies and De Meillon (1968); Danilov (1982); Gillies and Coetzee (1987); Huang (2004); and molecular methods (Fanello *et al.* 2002, Scott *et al.* 1993).
- To morphologically compare the cibarial armature of the mosquitoes species in the study area.
- To detect *W. bancrofti* parasites in mosquito samples caught using morphology (microscopy) and molecular method (Boakye *et al.* 2007, Zhong *et al.* 1996).
- To detect *W. bancrofti* parasites in pooled mosquito samples using Real Time Polymerase Chain Reaction (RT-PCR).

CHAPTER TWO

LITERATURE REVIEW

LF is a painful, disfiguring and a parasitic disease that causes long-term disabilities to people in endemic areas (Boakye *et al.* 2007, Kumari *et al.* 2010, Laney *et al.* 2010, Simonsen *et al.* 2010, Zeldenryk *et al.* 2011).Towards the late twentieth century (1997), a lot of plans to eliminate LF were discussed by the WHA after passing the resolution at their 50th session. The World Health Organization's (WHO) 50th WHA session launched the GPELF to help curb the burden of LF and also eliminate the disease by 2020 (Filariasis 2012). The efforts of the Merck & Co., GlaxoSmithKline (donors of Diethylcarbamazine citrate, Ivermectin and Albendazole), GPELF and other local stakeholders (e.g. the Health Services of various endemic countries) in pursuing the successful elimination of LF are gradually materializing. LF was known to affect 128 million people with over 1.2 billion people at risk (Boakye *et al.* 2007) decreasing to 120 million affected individuals with over 1.3 billion people at risk in about 83 endemic countries (Chandra 2008). Currently, over 120 million people are infected with about 40 million disfigured and incapacitated by the disease with 1.4 billion people at risk in about 72 countries (Filariasis 2012, de Souza *et al.* 2012). More endemic countries and communities may have LF successfully eliminated as the years go by.

2.1 Neglected Tropical Diseases (NTD) Intervention Programmes

Currently, as stated in the first WHO report on Neglected Tropical Diseases (NTDs), there are eighteen (18) diseases captured under the NTDs. These include Buruli ulcer, Chagas disease, Cystiscercosis, Dengue fever, Dracunculiasis (Guinea worm disease), Echinococcosis, Fascioliasis, Human African Trypanosomiasis (African sleeping

sickness), Leishmaniasis, Leprosy (Hansen's disease), Lymphatic filariasis, Onchocerciasis, Rabies, Schistosomiasis, Soil-transmitted helminthes (Ascaris, Hookworm and Whipworm), Trachoma and Yaws. Of these, six are controllable by MDA with effective interventions (Savioli and Daumerie 2010). To combat these diseases, a number of Global NTD Programmes under titles such as Global Control Efforts, Global Eradication Efforts and United States Government Initiatives to Address NTDs have been put in place. Programmes classified under the Global Control Efforts include Global Programme to Eliminate Lymphatic Filariasis, African Programme for Onchocerciasis, Onchocerciasis Elimination Programme for the Americas, Global Efforts to Control Schistosomiasis, Global Efforts to Control Soil-transmitted Helminths (STH), and Global Efforts to Control Trachoma. The Guinea Worm Eradication Programme happens to be the only programme under the Global Eradication Efforts. The United States Government Initiatives to Address NTDs which aims at reducing the prevalence of seven NTDs has the Global Health Initiative and the United State Agency for International Development (USAID) NTD Programme to ensure the success of set targets. The bottom line of the set targets for the lymphatic filariasis programmes is achieving elimination of the disease by 2020.

2.2 The Lymphatic Filariasis Disease

This debilitating and disfiguring parasitic disease is caused by the nematode *Brugia malayi*, - Palumbo (2008), *B. timori*, - Koroma *et al.* (2012), and *Wuchereria bancrofti* (Laney *et al.* 2010). Bancroftian filariasis is known to cause about 90% of the world's human filariasis infections and disease manifestations and the remaining 10% caused by

Brugian filariasis (Palumbo 2008). LF also presents some acute and chronic disease conditions which may include Acute Adeno-Lymphangitis (ADL), Acute Filarial-Lymphangitis (AFL), lymphoedema, elephantiasis, genito-urinary complications or lesions, hydrocoele, chylocele, chyluria, chylous ascites, microhaematuria and macrohaematuria just to mention a few.

2.2.1 Acute Disease Conditions

The most common of these conditions is the ADL. The other type is the AFL.

2.2.1.1 Acute Adeno-Lymphangitis (ADL)

This condition is usually the first manifestation of LF and may occur in both early and late stages of the infection. This comes with episodes of fever and transient local swelling. Patients experience frequent inflammation of the lymph nodes in the groin and axilla and the affected area is usually warm, tender, reddened, swollen and painful. Patients may have recurring acute ADL attacks in a year increasing with the degree of lymphoedema. ADL attacks causes persistence and progression of lymphoedema that leads to elephantiasis of the breasts and external genitalia (in females) as well as the limbs (Palumbo 2008, Wijesinghe and Wickremasinghe 2012).

2.2.1.2 Acute Dermato-Lymphangio-Adenitis (ADLA)

ADLA usually presents fever and chills as acute clinical manifestation. It is observed frequently in higher grades of lymphoedema. It however, occurs in both early and late stages of the disease (DREYER *et al.* n.d.). Secondary infections from bacteria such as Streptococci may cause acute episodes. Fungal infections are also known to aggravate the disease conditions which may progress to elephantiasis. This usually occurs in the

raining season or in settings that constantly gets feet of infected individuals soaked in water (Shenoy *et al.* 1999). Frequent ADLA attacks may give an indication of lymphoedema progression (Addiss and Brady 2007).

2.2.1.3 Acute Epididymo-orchitis And Funiculitis

There is inflammation of the testes and epididymis (spermatic cord) which may become swollen and very tender. This is medically referred to as epididymo-orchitis or funiculitis. This is also characterized by severe pains, shivering and fever. Bacterial infections are also known to aggravate this condition (Wilensky and Samuels 1924).

2.2.1.4 Acute Filarial-Lymphangitis (AFL)

This is a rare condition caused by the adult filarial worm and usually subsides without treatment. It occurs following death of the adult filarial worm. The lymph nodes harbouring dead adult worm in the scrotum and along the lymphatics form small tender nodules and these dead adult worm also cause lymphatic dysfunction as a results of the obstruction of the affected lymph channel (Palumbo 2008, Richens 2004, Mohan *et al.* 2012).

2.2.2 Chronic Disease Conditions

The following conditions may be classified as chronic disease manifestations of LF. Lymphoedema, elephantiasis, genito-urinary lesions, hydrocoele, chylocele, chyluria, adenopathy, chylous ascites, microhaematuria and macrohaematuria are some chronic forms of the disease. The most common of these conditions is lymphoedema (Palumbo 2008, Wijesinghe and Wickremasinghe 2012). Explained below are disease conditions that arise mainly due to lymphatic drainage complications caused by *W. bancrofti*. Most

microfilariaemic individuals may have both micro~ and macrohaematuria as well as proteinuria which is an indication of renal damage. These conditions are also known to be caused by the microfilariae rather than the adult filarial worm(s). There is a complete reversal of renal abnormalities associated with haematuria and proteinuria upon clearing microfilariae from the blood (Nwoke *et al.* 2010).

2.2.2.1 Lymphoedema

This is a swelling due to accumulation of lymph in the tissues at the infected area(s). Lymphoedema has the potential of progressing to Elephantiasis (Palumbo 2008, Cheng *et al.* 2006).

2.2.2.2 Elephantiasis

This may be described as a chronic form of filariasis (Mohan *et al.* 2012) progressing from lymphoedema of various body parts due to obstruction of the lymphatic drainage (Palumbo 2008, Cheng *et al.* 2006). The affected body part may have the skin looking warty, thickened and sometimes friable.

2.2.2.3 Genito-urinary complications/lesions

A group of conditions may be characterized under the genito-urinary complications. These include chylocele, chyluria, haematuria, hydrocoele and others.

2.2.2.3.1 Chylocele

Chylocele is known to be strongly associated with *W. bancrofti* infections in LF endemic areas. It occurs when a dilated lymphatic ruptures to force the flow of lymph out of the lymphatic channels and accumulates in surrounding tissue of the affected part of the body (Dreyer *et al.* 2000).

2.2.2.3.2 Chyluria

This is the excretion of lymphatic fluid called chyle from urinary tracts. Chyle contains absorbed fat in the form of chylomicrons in the intestinal lacteals and responsible for the milky appearance of the urine passed by patients suffering this condition. This is a blockage of lymphatic drainage close to intestinal lacteals, that cause(s) abnormal swelling or dilation and an eventual rupture of the lymphatic vessel(s) into the urinary tract, forming a lymphaticourinary fistula - an abnormal duct formed to connect lymphatic drainage to the urinary tract for the discharge of chyle from the former into the latter (Cheng *et al.* 2006).

2.2.2.3.3 Hydrocoele

This is a common chronic manifestation of lymphatic filariasis caused by *W. bancrofti*. This occurs in only males. This condition manifests following accumulation of fluid in the tunica vaginalis (sac surrounding testes). The scrotal sac may gradually swell to bigger sizes over a long period of time. Microfilariae may be found in scrotum of some individuals with this condition (Amaral *et al.* 1994).

2.2.2.3.4 Haematuria

This may be described as microhaematuria or gross haematuria (macrohaematuria). Haematuria is the detection of blood in urine. Microhaematuria may be detected during routine urine examination (Nwoke *et al.* 2010). In the case of gross haematuria, blood in the urine turns urine colouration from straw to red. Haematuria may be an indication of renal damage or abnormality. This condition is also strongly associated with the presence of microfilariae in blood of affected persons (Nwoke *et al.* 2010, Alli *et al.* 2003).

2.3 Biology and Life Cycle of the Vectors

The vectors and carriers of *W. bancrofti* parasites include *Aedes, Anopheles, Culex, Coquilletidia, Mansonia* and the *Ochlerotatus* genera (de Souza *et al.* 2012). Mosquitoes may be diurnal, crepuscular or nocturnal and this to a great extent influence their blood feeding times in the wild. Bionomics of these LF vectors would be described to the genus level. Mosquitoes in general have a holometabolous development – having four distinct stages in their life cycle (Rueda 2007).

2.3.1 Life Cycle of *Aedes species*

Aedes species exhibit holometabolism (complete metamorphosis). This involves both aquatic and terrestrial phases. Adult females may lay as many as several hundred single eggs per batch. Eggs may be laid on the surface of shallow temporary/permanent pools, natural containers of plant/animal/other origins and artificial containers. Examples of these include treeholes, nuts, pitchers, pods, shell of snail, clams, arboreal and ant nest, crab holes, tires, cans, flower vases, bottles, tanks, troughs, drums, and gutters among others (Laird 1988). The eggs of *Aedes* species are smooth. They are long and ovoid measuring about 1mm long. Adult females may also lay eggs on dump surfaces most likely to get temporarily flooded. In some species eggs are laid on moist substrates, in and around depressions of breeding sites with tidal wetlands. A batch of eggs may be laid over a few hours or days depending on availability of suitable substrate. Eggs may also be laid at varying distance above water line. This is to ensure that this batch of eggs is dispersed over a number of breeding sites. The eggs would have to dry for about 24 hours before they become viable. These desiccant resistant eggs may remain viable for up to about 5 years. The eggs may usually sink to the bottom of the water. *Aedes*

species prefer clean water for larval development. Development to emergence may take 4-5 days or more depending on water temperature, availability of food and predators. In very cold temperatures, the larvae may not develop for months (diapause). Hatching occurs once there is adequate amount of water at the breeding site. The various larval stages hangs head down just below the water surface breathing through their siphons. They feed on microbes and phytoplanktons in the water. They dive to the bottom of the water when disturbed and wiggles back to the surface. Fourth instars may molt into pupae. Adult development occurs in the pupal case within 2 days. The pupa does not feed and rest mainly at the surface of the water. They dive to the bottom when disturbed and floats back to the surface of the water. Developed adult in the pupal case ingest air to expand their abdomen. This split opens the pupal case dorsally for the adult mosquito to emerge head first. Males usually emerge before females. They wait till their exoskeletons and wings dry up before flying away.

Adult *Aedes* species feed on nectar for survival. Only the adult female blood feed to mature its eggs. It takes about 2-7 days for the blood meal to be digested. They bite around the twilight periods of dusk and dawn (crepuscular). They may also bite during the day (diurnal). These mosquitoes are known to exhibit both zoophily and anthropophily in their feeding behaviour (Thompson *et al.* 1963, Crans 1964, Crans *et al.* 1996). They may also be described as being predominantly endophagic and endophilic in nature.

2.3.2 Life Cycle of *Anopheles species*

This vector undergoes complete metamorphosis (egg, larva, pupa and adult). Its life cycle involves both aquatic and terrestrial phases. The first three stages are aquatic and the last stage is terrestrial. Adult female after mating lay between 50-200 single brown/black boat shaped eggs having lateral floats. The floats aid dispersal of the eggs on the surface of the water. The eggs may measure 1mm long and 2-5mm wide. After oviposition, viable eggs hatch within 2-3 days but 4-7 days in the temperates (Service 1980). It takes roughly 12-14 days to complete a life cycle. *Anopheles* species may breed in cool clean rivers with shades, streams, pools having water lettuce (*Pistia stratiotes*), rivers with grass at the edges, rice fields, open exposed ground pools, brick-pits, foot-prints, tire ruts, wheelbarrows, mortar-pans, and rarely in domestic ant-traps as recounted by (Gillet 1972) and according to Sattler *et al.* (2005), they have adapted to breeding in urban settlements with stagnant drains, railway lines, shallow wells, irrigation channels, organically polluted water, large drains, swamps, and puddles. Female mosquitoes bite (blood feed) to get blood for maturation of their eggs. In these various breeding sites, the larvae usually lay flat (parallel) just below the surface of the water with the help of its palmates (abdominal setae) and forage for microbes and phytoplanktons. Larvae are filter feeders. They may pupate in about 6-9 days after four molts. This may, however, depend largely on water temperature, nutriments and predators. They grow bigger after every molt. After 2-3 days the non-feeding pupa may split open its pupal case dorsally for emergence of adult mosquito.

Every generation of mosquitoes has the males from that batch of eggs emerging first and maturing within 24 hours to be ready for mating by the time the females emerge. After mating, most males may die. Adult males and females survive on nectar. The females may take blood meal to develop and mature their ovaries before laying a batch of eggs (Gillies 1955). A combination of cues such as carbon dioxide, temperature, moisture, odour, host movement and colour from a vertebrate may attract a female mosquito to take a blood meal (Zimmerman *et al.* 2009, Rebollar-Tellez 2005). *Anopheles* are nocturnal so their blood feeding, mating, egg laying and even emergence of pupae is usually in the evening to early morning. Some *Anopheles* species are exophagic (bite outdoors) biting after sunset to about 2100 hours and others are endophagic (bite indoors) biting usually after 2100 hours (Gilles 1999). They may be exophillic (rest outside) or endophillic (rest indoors) or both. Most may also be endophilic, exophilic, endophagic and exophagic with this behaviour not exclusively exhibited. A few *Anopheles* feed exclusively on humans and others are mostly zoophilic. Anthropophilism and zoophilism varies according to availability of host and the species of mosquitoes that feed on both humans and animals (Gilles 1999).

2.3.3 Life Cycle of *Culex species*

Culex species undergo complete metamorphosis requiring both aquatic and terrestrial phases. Adult females after mating lay eggs in cluster. This may be several hundred per batch on the water surface. Apart from shallow temporary pools, *Culex* species may oviposit in flowing streams (creeks, irrigation ditches, drainage), pond streams (flooded stream beds, chlorophyta-rich habitats, polluted ponds), lake edges, swamps and marshes (coastal marshes, mangrove swamps, irrigated fields), shallow permanent

ponds (fishponds, duckweed ponds), intermittent ephemeral puddles, natural containers of plant origin (treeholes, nuts, pitchers, pods), natural containers of animal and other origins (shell of snail, clams, arboreal and ant nest, crab holes), and artificial containers such as tires, cans, flower vases, bottles, tanks, troughs, drums, gutters and others (Rueda *et al.* 2005, 2006, Laird 1988, Rueda 2007). Some *Culex* species may breed in the most stinking water (Gillet 1972). Females lay eggs one after the other, sticking the eggs together to form a raft with about 200-300 eggs. This egg raft stays afloat using the air trapped underneath the raft. The approximately 6.25 mm long and 3.13 mm wide eggs may hatch and the larvae escape from the bottom of the egg raft into the water in about 1-2 days. Larvae hang at an angle just below the surface of the water with their siphons (air tubes) to breathe. These filter feeders feed on microbes and phytoplanktons. The development of the wigglers (larvae) involves four stages with each of the stages referred to as instars. After development through the four larval stages the fourth instars metamorphose into pupae. The pupae do not feed. They breathe through their pair of trumpets at the surface of the water. When disturbed, they dive in a jerking tumbling motion and float back to the surface. It takes approximately 1-4 days depending on the species and importantly water temperature for adult mosquitoes to emerge from the pupal case.

Adult male mosquitoes may emerge 24 hours before females to mature in readiness for mating with females after their emergence. *Culex* mosquitoes feed on nectar. Adult females, however, take blood meals in other to mature their ovaries and lay viable eggs. It may take about 2-7 days for the blood meal to digest. Some prefer biting around dusk

outdoors and throughout the night indoors. *Culex* may be zoophagic (both zoophilic and anthropophilic). Some mostly prefer domestic and wild birds (ornithophagic) to man, cattle horses and other mammals (Kent *et al.* 2009). In the temperate, they may hibernate in the late summer until early spring before finding water bodies to oviposit. Their feeding and resting behavior may be described as endophagic and endophilic respectively.

2.3.4 Life Cycle of *Coquilletidia species*

The life cycle of this genus could be described as holometabolous. Adult female mosquitoes search for water bodies with emergent vegetation. They then lay eggs in the part of the water with a high population of emergent vegetation (Carpenter and LaCasse 1955). Some temperate species may even enter diapause (over-winter) at the egg stage when conditions are not favourable (Urquhart *et al.* 1987). Eggs are laid side by side to form a raft. About a hundred eggs or more may be laid per batch (Urquhart *et al.* 1987, Pratt and Moore 1993). Each egg in the raft is vertically arranged with the anterior end pointing downwards towards the water surface (Soulsby 1982). Eggs may hatch after a few days when the temperature of the water is quite suitable (Urquhart *et al.* 1987). There are four larval stages (1^{st} – 4^{th} instar). The larvae reach the 4^{th} larval stage after three molts. Development of larvae is dependent on availability of food (Pratt and Moore 1993). The larvae stay attached to the roots and stem of emergent plants in the water with specialized/modified siphons (Carpenter and LaCasse 1955) with which they breathe whiles foraging for microbes and phytoplankton. The fourth instars molt to become pupae. Pupae do not feed. Unlike other mosquito pupae, *Coquillettidia* species do not need to come to the surface of the water to breathe (Pratt and Moore 1993). The

pupae attach themselves to roots or stems of emergent vegetation with specialized trumpets and remains at that part of the plant until the adults emerge (Carpenter and LaCasse 1955). Adult development occurs within the pupal case before emergence. It takes a few hours for the pupae of certain species in dry climates to emerge. In the tropical and temperate species, however, it may take about 2 days and several weeks respectively (Urquhart *et al.* 1987, Pratt and Moore 1993, Bowman 1999). The male mosquitoes usually emerge first before the females as already discussed in the genera above.

Adult *Coquillettidia* feed on nectar. The females, however, require blood meal to mature their eggs. They use about two or more days to digest the blood meal after which they start laying. In general mosquitoes have a short lifespan. Some gravid *Coquillettidia* species may hibernate/aestivate following unfavourable conditions until the next season before laying eggs (Soulsby 1982). They are nocturnal mostly active in the early hours of the night. They may occasionally bite during the day in dense shady places when disturbed whiles resting. They are mostly described as opportunistic feeders. They feed on a range of animals depending on their availability.

2.3.5 Life Cycle of *Mansonia species*

The genus *Mansonia* is closely related to the *Coquillettidia* (Pratt and Moore 1993). The eggs are laid side by side (oriented vertically with the anterior end pointing downwards

towards the water surface) to form a raft. The aquatic life is as described in the *Coquillettidia* genus.

The adults feed on nectar. Females bite to acquire blood to mature their eggs. Host preference for most species may vary seasonally depending on their availability. The various hosts may include amphibians, birds and mammals. They are known to be savage biters (Pratt and Moore 1993). The adult life is basically as discussed in the genus *Coquillettia*.

2.3.6 Life Cycle of *Ochlerotatus species*

They have a holometabolous life cycle. *Ochlerotatus* species lay single eggs on damp soil. Several hundred eggs may be laid per batch. The eggs would hatch only when flooded with water. The water may either be fresh or brackish. The temperature of the water is also important for hatching and especially larval development. Breeding sites of these mosquitoes include irrigated pastures, tree holes, vinyl tarpaulins covering swimming pools, rain pools, rock pools, wood piles, catch basins, bottom of flooded streams, and depressions flooded by salt water during high tides (Andreadis *et al.* 2001). The larvae hang just below the surface of the water breathing atmospheric air through their siphons. They are filter feeders foraging on microbes and phytoplanktons. There are four larval stages referred to as the instars. The first instars molt three times to get to the fourth instars. They get bigger after each molt. The fourth instars then molt becoming pupae. The pupa may be found resting at the surface of the water breathing through a pair of trumpets. When disturbed the pupa may dive to the bottom of the

water in a jerking motion. The pupa may then float back to the surface of the water (Pratt and Moore 1993). Adult development commences in the pupal case. Depending on the water temperature the pupa may take about 1-3 days to split open dorsally for the emergence of the adult mosquitoes.

The adult *Ochlerotatus* species feed on nectar. Only the females take blood meals to mature their eggs. They are known to give very painful bites and are a nuisance because of their persistent biting behaviour. They are diurnal, mostly anthropophilic and exophilic. They are known to be very strong fliers and could be found miles away from their breeding sites.

2.4 Biology and Life Cycle of *Wuchereria bancrofti*

Wuchereria bancrofti belongs to the family of filarial worms, Filariidae. Other members of this family include *Onchocerca volvulus, O. gutturosa, Brugia malayi, B. timori, Loa loa, Litomosoides carinii, Dirofilaria immitis, Dipetalonema perstans* and *D. viteae (Acanthocheilonema)* (Mehlhorn 1988). In general, the length of filarial worms measures between 20mm and 700mm. The females of each species longer in lengths than their corresponding males (40mm – 700mm in females and 20mm – 180mm respectively). Females produce a lot of larvae that may be sheathed or unsheathed. Larvae of filarial worms are found in circulation and inhabit subcutaneous tissues, the eye, pleural cavity, pulmonary artery, lymph vessels and lymph nodes. These parasites have both intermediate hosts (*Simulium spp., Odagmia spp., Aedes spp., Anopheles spp., Culex spp., Mansonia spp.,* Mites (*Bdellonyssus*), *Culicoides spp.,* and

Ornithodorus moubata) and definitive hosts (humans, cattle, rats, dogs, cats and *Meriones sp.*). Their shape and structure may be used as identification keys because they are species specific. Their terminal nuclei are species-typical (Mehlhorn 1988).

Wuchereria bancrofti male and female adult worms (4cm and 10cm respectively) live in the lymph vessels and nodes. After copulation the female adult worm produces thousands of microfilariae (Mf) that could be found in peripheral blood during the night. Mosquito vectors (*Aedes, Anopheles, Culex* and *Mansonia*), the intermediate hosts, may ingest these Mf after a blood meal. Mf molt in the gut to form the first larval stage (L_1) that penetrate the intestine, enter abdominal cavity and thoracic muscles. They molt again to form the second larval stage (L_2) that could be described as a stumpy or sausage shape. The third and final molt forms the filariform infective stage (L_3). The L_3s (~1.5mm in length) migrate to the proboscis and escape into pool(s) of blood that surround the wound formed as a result of the piercing of the stylet of the proboscis. The L_3s eventually enter circulation and migrate to the lymph vessels and lymph nodes where they mature into adults after two molts within a year.

2.5 Detection of *Wuchereria bancrofti*

Current existing methods for the identification of *W. bancrofti* parasite (microfilaria) in blood include microscopy (gold standard), detection of antibodies (immunodiagnostic test, circulating filarial antigen) and detection of DNA (polymerase chain reaction).

2.5.1 Microscopy

Microscopy is used for the morphological identification of microfilariae mounted on a slide prepared as either dry or wet smear. Dry smear slides are stained with Geimsa after drying the blood. The wet slide, however, has the blood diluted with either water or 2% saponin so as to lyse the erythrocytes for the mobile microfilariae to be easily identified (Mak 1989).

W. bancrofti parasites could also be identified in LF vector samples using microscopy. These mosquito vectors are dissected and the carcasses are observed on slides for the presence of any of the parasite stages. This is done using the morphological characteristics of the different stages of the parasite (Boakye *et al.* 2004).

2.5.2 Immunodiagnostic Test

There is an immunological assay that detects antigens of *W. bancrofti* parasites from blood collected during the day. This commercially available enzyme linked immunosorbent assay (ELISA) test kit uses a monoclonal antibody, Og4C3, to detect this LF parasite (Itoh *et al.* 1998, Pani *et al.* 2000). The results of this test may take quite some time to be ready.

2.5.3 Circulating Filarial Antigen

The Immuno Chromatographic Test (ICT) card is a rapid way of detecting circulating filarial antigens in blood samples. Weil *et al.* (1997) has confirmed that aside getting an instant result, the test is very sensitive and also specific. With this test, as little as 100µl of blood is required (Pani *et al.* 2000). The ICT card can therefore be used in the field to get rapid results during surveys (Omar *et al.* 2000).

2.5.4 Polymerase Chain Reaction (PCR)

Molecular techniques can also be used to identify LF parasites. Purified genomic DNA from blood or mosquito vector samples could be used in a PCR reaction to detect *W. bancrofti* parasite DNA (Zhong *et al.* 1996, Boakye *et al.* 2007). PCR can now be used to determine infection rates as well as transmission of LF in a study area (Laney *et al.* 2010). This is because Laney *et al.* (2010) successfully established a PCR assay that was able to differentiate the infective stage of the *W. bancrofti* parasite from the other larval stages of the worm.

2.6 Factors Affecting the Transmission of *Wuchereria bancrofti*

The factors affecting transmission of *Wuchereria bancrofti* could be seen from the relationship existing between the parasite, vector and human host.

2.6.1 Distribution of Vectors

The disease is endemic in tropical and subtropical regions such as equatorial Africa (West and Central Africa), Indian subcontinent, East and Southeast Asia, the western Pacific, eastern Mediterranean region, parts of South and Central America, and the Caribbean (Greene 1992, McMahon and Simonsen 1996, Weil *et al.* 2008). This is an indication of how widespread the vectors of LF are distributed around the world. It is worth noting that different geographical regions may have different local vectors (Erickson *et al.* 2009). *Anopheles* and *Culex* mosquitoes are confirmed vectors of LF in East Africa, *An. gambiae* s.l., *An. funestus, An. pharoensis* and *Mansonia* mosquitoes are vectors in Ghana and West Africa, and *Aedes, Culex* and *Mansonia* mosquitoes transmit LF in the Americas, Pacific and Asia (Dunyo *et al.* 1996, Dzodzomenyo *et al.* 1999, Appawu *et al.* 2001, Weil *et al.* 2008, Zhou *et al.* 2009).

2.6.2 Vector Composition

This refers to the number of mosquitoes known to be involved in the transmission of a particular disease in a given geographical area or study sites. The local vector for the transmission of LF in endemic communities informs control programmes the kind of strategies to use in managing or even eliminating the disease in that community. Knowing the vector composition of a study site, one may use the information and the biology of incriminated vectors in endemic areas to control the transmission of LF.

2.6.3 Facilitation, Limitation and Proportionality

The phenomenon of "facilitation" refers to a process where ingested Mf below a certain threshold, called the Webber's Critical Point (Pichon 2002), would not support the transmission of LF by *Anopheles* vectors (Southgate and Bryan 1992, Pichon *et al.* 1974, Webber 1991). "Limitation" is usually exhibited by culicines. This is a process where there is a stable transmission of LF even where there is low parasitaemia (low Mf load in blood of the study population) as reported by (Subramanian *et al.* 1998, Duerr *et al.* 2005). These vectors when found transmitting in endemic areas would have serious implications on the transmission dynamics. Even with several rounds of MDA, such vectors may maintain residual transmission of the disease in endemic communities (Amuzu *et al.* 2010, de Souza *et al.* 2012). Vectors may also exhibit "proportionality". Here, a constant number of Mf develop to the infective (L3) stage after the ingestion of blood meal (Duerr *et al.* 2005). This implies that when such vectors are involved in transmission, they would constantly be contributing a good percentage to the Annual Transmission Potential (ATP) in a given endemic area.

2.6.4 Vector Abundance

The proportion of vector mosquitoes among the species diversity of a given study area may be referred to as vector abundance. The proportions of vectors in a study area would have a direct impact on transmission (Simonsen 2008). The higher the proportions of vectors, the greater the ATP, especially when no interventions have been put in place. In the absence of interventions, there would be high biting rates in endemic communities. This would be translated to high infective bites and consequently high ATP.

2.6.5 Vectorial Capacity

Vectorial capacity refers to how competent a given vector may be when it comes to the transmission of vector borne diseases. This involves vector density in relation to host preference, the frequency of successful ingestion of infected blood meal by a vector, 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).

2.6.6 Behaviour and Cultural Practices

The behaviour of indigenes and their way of life may ensure residual disease transmission even after several rounds of MDA. In most endemic villages without electricity, most people turn to stay under the bright moonlight either to socialize or to enjoy the fresh breeze when the rooms are relatively hot. This may expose them to a lot of mosquito bites. In endemic communities with buildings having gaps in the eaves, endophagic vectors blood feed on occupants of such rooms. Indoor bites from vectors

may be high if indigenes of endemic communities do not sleep under treated bed nets. In geographical regions with diurnal vectors such as the *Aedes* mosquitoes, when the indigenes do not wear clothing long enough to cover most of their body parts, they may stand the risk of getting infected.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Sites

The study was conducted in three communities in three different districts of the Central Region of Ghana. Two of these communities, Ayensuako (05° 42' N, 000° 36' W) and Mankrong (05° 40' N, 000° 36' W) are located in the Agona East and West Districts respectively and Gyahadze (05° 23' N, 000° 35' W) is found in the Awutu Efutu Senya District. All three communities lay west of Accra, the capital city of Ghana. These districts have an estimated population of 85,920, 11,5358, and 6,8597 respectively (Ghana Statistical Service 2012).

Gyahadze is about 2.9 km from the sea. This community has a water body called Gyaha which serve as a major breeding site in the community for most part of the year. Gyahadze lies in the Coastal Savanna Zone of Ghana. Indigenes of Gyahadze are primarily subsistent farmers; but a few are petty traders and fishermen. Houses are built with sandcrete blocks or clay with most of the rooms having no ceilings. The eaves of the houses also have gaps. Ayensuako and Mankrong are 40.0 km and 35.6 km from the sea, respectively. Ayensuako and Mankrong are approximately 4.4 km apart. These two communities do not have fisher folks but do subsistence farming. Their housing structures are similar to those found in Gyahadze.

The main vectors of LF in Ghana found in these communities are *An. gambiae* s.l., *An. funestus* s.l. and *Mansonia* mosquitoes (Dunyo *et al.* 1996, Appawu *et al.* 2001, Ughasi *et al.* 2012). Farming runs through all three communities as the main occupation.

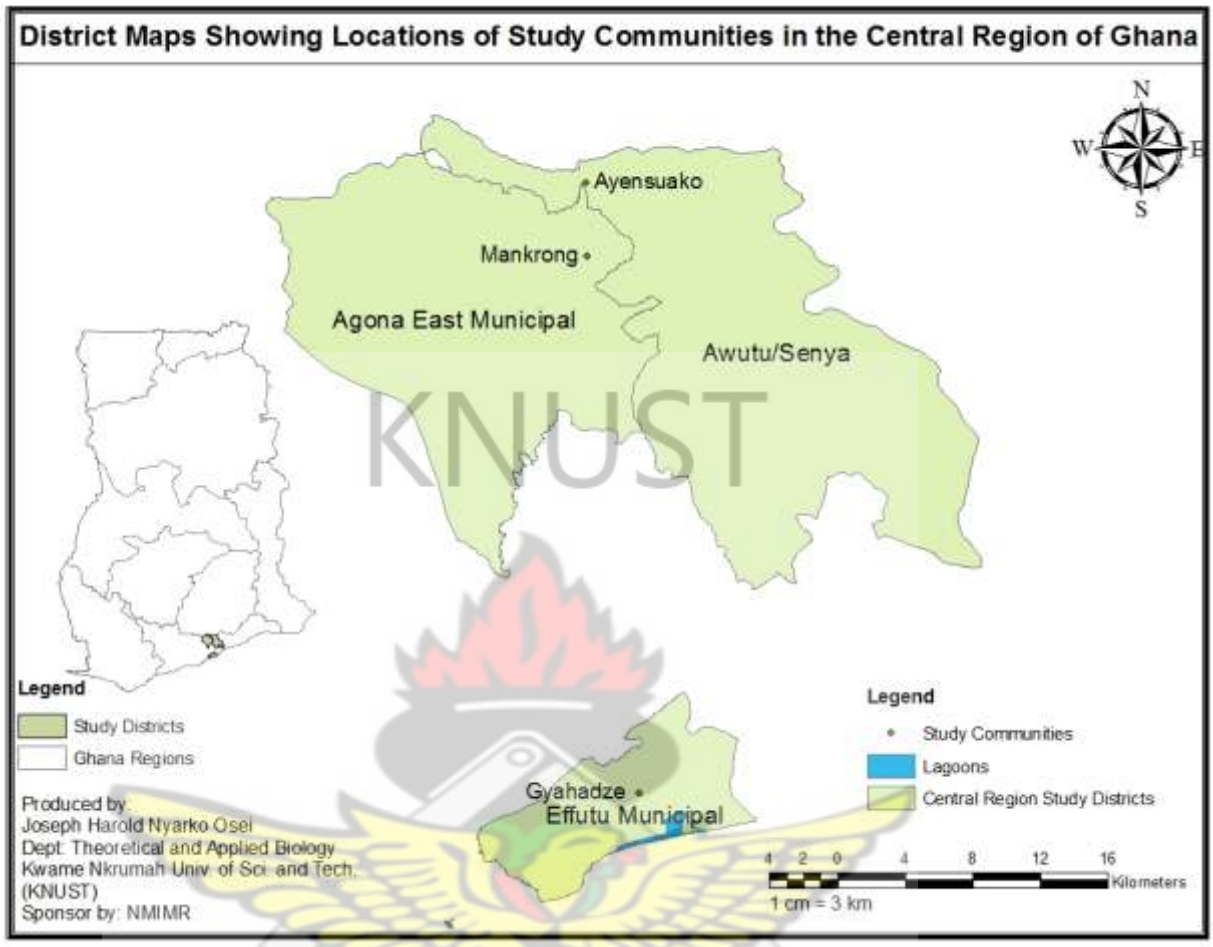


Plate 1: District maps showing the three study communities in the Central Region of Ghana.

3.2 Mosquito Sampling

Each of the three communities was divided into four sections. Ten houses were then selected randomly in each section. A total of 40 houses were therefore sampled in each community. Pyrethrum Spray Catch (PSC) was employed in sampling mosquitoes between 0500 hours and 0800 hours each sampling morning. Five months of sampling (December 2011, September 2012, October 2012, November 2012 and January 2013) were conducted from 2011 to 2013. Gray baft sheets were laid on the entire floor of the room and over furniture too heavy to move. The spray guns were filled with Raid® insecticide (Pynamin Forte - 0.05%, Neopynamin - 0.05%, Deltamethrin - 0.015%,

Solvent, Fragrance - 99.885%). The eaves of the buildings or houses to be sampled were sprayed first from the outside - windows, doors and eaves - before spraying inside the room. After spraying, the gray baft sheets were carefully removed from the room folding them from the edges so that the knockdown insects would collect in the middle of the gray baft sheets. The knockdown mosquitoes were sorted with forceps outside the room in the open after ten 10 - 15 minutes. This was done to separate all genera of mosquitoes caught from the other haematophagous insects. These mosquitoes were transported to the laboratory in well labelled Petri dishes lined with wet filter paper to prevent desiccation of the samples for processing (identification, molecular characterization and dissections for infections). The label information included the house number, number of persons that slept in the sprayed room that night, and the number of mosquitoes caught in that room.

Plate 2: Housing structure and adult mosquito sampling

Plate 2A is a clay house with thatch roofing and gaps at the eaves. Plates 2B and 2C are typical sandcrete block houses. Plate 2B is a room without ceiling and having gaps at the eaves. Plate 2C is a room laid with gray baft sheets and being sprayed. Plate 2D is a petri dish containing knockdown mosquitoes sorted.

3.3 Morphological Identification and Dissections

The working bench was cleaned with 70% ethanol. The mosquitoes brought from the study sites were separated using morphological features as described by Gillies and De Meillon (1968); Danilov (1982); Highton (1983); Gillies and Coetzee (1987) to separate

the mosquitoes into anophelines (*Anopheles* mosquitoes) and culicines (*Aedes*, *Culex*,and *Mansonia* mosquitoes). Each mosquito was picked with forceps and into a petri dish and examined under a dissection (stereo) microscope (Olympus SZ60). All *Anopheles* mosquitoes were identified using the pale and dark band patterns on the costal margins of their wings. All culicines are also grouped based on the absence of the pale and dark band patterns on the costal margins of their wings and into their respective genera using morphological identification keys. In all genera of mosquitoes, the males were distinguished from the females by the presence of their plumose (bushy) antennae. The different species that had been morphologically identified were recorded on appropriate entomological data recording forms/sheets.

Dissection: On a well labeded glass slide, each mosquito was divided into head, thorax and abdomen. The wings and legs were detached from the thorax and the legs (of the *An. gambiae* s. l.) used for species identification (using PCR). The three body parts (head, thorax and abdomen) were each teased in a drop of water on the slide.

***Anopheles funestus* s. l.**

This group was identified using the dark and light (pale) band patterns on the costal margins of their wings. The legs are mainly dark, having tibiae and tarsi occassionally with small apical white spot and a few pale scales at the joints respectively. These pale scales are observable only under a microscope.

***Anopheles gambiae* s. l.**

The costa has pale and dark band patterns. The legs have the femora, tibiae and the 1st tarsal segment speckled to a variable degree. Speckling may sometimes fuse to form short lines that may rarely form complete rings.

Anopheles gambiae melas/merus

The palpi were used for distinguishing *An. melas* and *An. merus* from the other members of the *An. gambiae* complex. Here, the ratio of the 4th and 5th segments of the female palp should measure ≥ 0.75 the length of the 3rd segment for these salt-water species, *An. melas* and *An. merus*.

Anopheles pharoensis

The females have shaggy palps with four pale bands situated largely at the apices of segment 2 – 5. The legs have femora and tibiae prominently speckled. The spots usually coalesced to form white patches of varying sizes. Segments 1 – 4 of the hind tarsi are broadly pale apically. The entire 5th segment is pale. The femora, tibiae and 1st tarsal segment are prominently pale internally.

***Aedes* mosquitoes**

Adult *Aedes* species are generally very black mosquitoes with white silvery spots usually concentrated in the thoracic region. The palp usually has three segments and seldom four. When segment four of the palp is present, it is very small. The scutum has pale scales which may be narrow and the scutellum have broad pale scales on all three lobes. The femora, tibiae and tarsi have conspicuous white and black patterns. The hindtarsus has basal white band on at least one tarsomere.

***Culex* mosquitoes**

In the identification of the *Culex* mosquitoes, a few morphological features were observed to categorize them to the genus level. The wings of *Culex* mosquitoes are usually dark-scaled. In the legs, the femora and tibiae are usually generally dark. They may, however, have up to about 10 pale spots (yellow or white).

***Mansonia* mosquitoes**

To identify the *Mansonia* mosquitoes, the costal margins or the wings were observed for the absence of dark and pale band patterns. This was followed by observing the legs

with the unaided eye for the presence of pale markings that may or may not form complete rings on the femora and tibiae. *Mansonia africana* was identified using the tuft of scales on the scutum that formed a rectangular shape. *Mansonia uniformis* was identified by the pale scales that form a pair of parallel lines in the middle of the scutum running from the front of the scutum to the prescutellar area.

3.4 DNA Extraction from mosquitoes collected from study sites

Two different DNA extraction methods were used. These were the boiling method as described by Medici *et al.* (2003) and Queipo-Ortuño *et al.* (2008) excluding the few washing steps which involves vortexing sample in distilled water, centrifuging to pellet samples and discarding the supernatant and reducing incubation temperature from 100 °C to 95 °C; and DNA extraction with the Qaigen kit (according to the manufacturer's protocol). Also DNA samples were extracted from pools of 25 mosquitoes or less where the numbers caught for a given species were not enough.

3.4.1 Boiling method of DNA extraction

One to three mosquito legs were detached from the thorax of each mosquito using dissecting pins. The legs were put into 1.5 ml microcentrifuge tubes using a pair of forceps. The legs were homogenized in 50 µl double distilled water using Konte's pestle. The homogenate was incubated for 10 – 15 min in a water bath (Grant Instrument (Cambridge) Ltd Barrington. Cambridge, CB2 5OZ) set at 95 °C. This was done to denature certain enzymes (e.g. proteases, lipases, etc.) in the homogenate. The resulting homogenate was used as DNA template for PCR. This was used for the molecular identification of *Anopheles gambiae* s.l. to confirm the morphologically identified *Anopheles gambiae* mosquitoes.

3.4.2 DNA extraction using QIAgen kit

The carcasses of dissected mosquitoes were scraped off glass slides in pools of 25 or less (where the numbers in the groups of species were not up to 25) into the 1.5 ml microcentrifuge tubes. 180 µl Buffer ATL was added to each pool of scraped carcasses. A volume of 20 µl proteinase K was then added and mixed thoroughly by vortexing using a vortexer (Standard Mini Vortexer, VWR Scientific Products). This was incubated at 56 °C for 10 min. Each microcentrifuge tube was vortexed briefly for 15 s. Two hundred microlitres of Buffer AL was added to each of the tubes and mixed thoroughly by vortexing then 200 µl absolute ethanol (96 – 100%) mixing thoroughly by vortexing. The mixture was transferred into a DNeasy Mini spin column placed in a 2 ml collection tube. This was placed in a centrifuge (eppendorf Centrifuge 5415D) and span at ≥ 6000 x g (8000 rpm) for 1 min. The flow-through and the collection tubes were discarded. The DNeasy Mini spin columns were each placed in new 2ml collection tubes and 500 µl Buffer AW1 was added and centrifuged at ≥ 6000 x g (8000 rpm) for 1 min. The flow-through and the collection tubes were again discarded. The DNeasy Mini spin columns were again place in new 2 ml collection tubes. 500 µl Buffer AW2 was added to each spin column and centrifuged for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. The flow-through and collection tubes were again discarded. The DNeasy Mini spin columns were each finally placed in 1.5 ml microcentrifuge tubes. 100 µl Buffer AE was added to DNeasy membrane of each spin column and incubated at room temperature for 1 min. These were centrifuged at ≥ 6000 x g (8000 rpm) for 1 min to elute the purified DNA.

3.5 Mounting and Comparing Cibarial Armature of the mosquitoes caught

With the aid of dissecting microscope (Olympus SZ60) and dissecting pins, the heads of morphologically identified mosquitoes were severed on a glass slide. These mosquito heads were placed in well labelled 1.5ml microcentrifuge tubes containing clearing agent (chloral hydrate and phenol) using a pair of forceps. The labels on the tubes include species (morphologically identified), code for study site, date and type of collection. The thoraces and abdomens were teased for the detection of LF parasites via microscopy. The heads were kept for about two to three weeks at room temperature in the clearing agent. Each mosquito head was removed and placed on a glass slide under a dissecting microscope (Olympus SZ60). A drop of mounting medium or Puri's medium (containing distilled water, gum Arabic (acacia), chloral hydrate, glycerin, glacial acetic acid) was then placed on the mosquito head and a cover slip gently placed over it after careful dorso-ventral orientation of the mosquito's head. Each slide was labelled with the species (morphologically identified), code of study site, date and type of collection. The mounted slides were placed on a heat plate (C. S. & E. Slide Warmer No. 26020; Clinical Scientific Equipment Co.; Melrose Park Illinois) to dry. The cibarial armature was viewed under a compound microscope (Leica Galen III) with X100 objective lens. Records of the cibarial teeth of the mounted mosquito heads were taken with the aid of a calibrated eye piece.

3.6 Molecular Identification of *Anopheles gambiae*

Extracted DNA from morphologically identified mosquitoes were used as DNA templates for PCR runs using modifications (troubleshooting to optimize the concentrations of some reagents in the master mix) of Scott *et al.* (1993) and Fanello *et*

al. (2002) protocols. The cocktail PCR master mix included all the standard *Anopheles gambiae* primers except that of *An. bwambae* and *An. quadriannulatus* (see 3.6.1 below for details of master mix).

3.6.1 Species Identification of *Anopheles gambiae sensu lato*

DNA extracts from a single mosquito or a pool of up to 25 mosquitoes were used as templates in a PCR-mix. The PCR-mix contained 1X PCR Buffer, 50 mM $MgCl_2$, 0.25 mM of each dNTP, 0.15 µM primer UN [5'- GTG TGC CCC TTC CTC GAT GT -3'], 0.15 µM primer GA [5'- CTG GTT TGG TCG GCA CGT TT -3'], 0.15 µM primer AR [5'- AAG TGT CCT TCT CCA TCC TA -3'], 0.15 µM primer QD [5'- CAG ACC AAG ATG GTT AGT AT -3'], 0.15 µM primer ME [5'- TGA CCA ACC CAC TCC CTT GA -3'] and 0.5 U *Taq* polymerase. 2 µl DNA extracts (from pooled mosquitoes) were used as templates for the PCR reaction. The PCR runs were done using the Gene Amp PCR System 9700. This had an initial pre-heating step of 3 min at 94 °C to activate the DNA polymerase followed by 33 cycles each consisting 30 s denaturation at 94°C, 30 s annealing at 50 °C and 30 s extension at 72 °C; and the final cycle products are extended for 5 min at 72 °C. After amplification, about 5-10 µl amplicon is mixed with about 2 µl bromophenol blue dye and loaded into wells of a 2% agarose gel for electrophoresis. The electrophoresis is run in a Mupid®-2 plus Submarine electrophoresis system.

Alternatively, for some of the samples, a PCR-mix containing the same concentrations of primers as described above was added to GoTaq and DNA template for amplification. The GoTaq in the PCR-mix comprises 1X Green GoTaq (Flexi Buffer), 25 mM $MgCl_2$, 0.2 mM of each dNTP, and 1.25 Units of GoTaq DNA polymerase. The

PCR reaction conditions remains same as initially described. Loading the wells of the 2% agarose gel did not require any loading buffer because the Flexi Buffer already contains a green loading dye that give the GoTaq its green colouration. The gel is stained in ethidium bromide solution for about 30 min and then observed under a UV illuminator (TOYOBO Transilluminator Model TM-20 connected to a TOYOBO FAS-III monitor for printing pictures of electrophoregrams). The results is read of from the electrophoregram. The expected band sizes for the different sibling species from the PCR products is as shown in Table 1 (Fanello *et al.* 2002).

3.6.2 Identification of Molecular Forms of *Anopheles gambiae sensu stricto*

The PCR products obtained from the procedure described above is used as a template for digestion. A 1X React II Buffer, Bovine Serum Albumin (BSA) and 0.1 U *HhaI* was added to the PCR product and digestion was carried out for a minimum of 3 h following the protocol of Favia *et al.* (1997) and the digested fragments were run through a 2% agarose gel. The gel was then stained in ethidium bromide for about 30 min before observing and photographed under an ultra violet (UV) light illumination (TOYOBO Transilluminator Model TM-20). The expected band sizes are given in Table 1 below (see Appendix)

3.7 Identification of *Wuchereria bancrofti*

For this study morphological identification and molecular techniques (PCR) were the techniques used for the identification of the *W. bancrofti* parasite. See 3.7.1, 3.7.2 and 3.8 below for details.

3.7.1 Identification of *Wuchereria bancrofti* Using Microscopy

In this study, *W. bancrofti* parasites were observed using low power (X10) compound microscope (Leica Galen III) after dissection of mosquito samples. To differentiate between microfilariae (Mf) and the three larval stages, morphological characteristics of the parasites were the criteria for the identification of *W. bancrofti*. In identifying Mf of *W. bancrofti* the features considered included the presence of nuclei running through almost the entire body length; the presence of sheath especially at the cephalic and tail ends; and sometimes morphological dimensions (260 x 8 µm). The three larval stages are also identified by checking for the presence of nuclei along the length of the parasite. Using morphological features and dimensions, the first larval stage (L_1) is about the size of the Mf, the second larval stage (L_2) is thicker and sausage-shaped and the third larval stage (L_3) which happens to be the infective stage is longer and thin with well developed cephalic and tail regions.

3.7.2 Identification of *Wuchereria bancrofti* Using PCR

In the identification of *W. bancrofti*, conventional PCR was used to amplify the *Ssp I* repeats for the detection of the LF parasite given a band size of 188 bp (Derua *et al.* 2012). The PCR-mix contained 0.2 µM each of NV1 [5'- CGT GAT GGC ATC AAA GTA GCG - 3'] – (21-mer) and NV2 [5' – CCC TCA CTT ACC ATA AGA CAA C - 3'] – (22-mer) primers, GoTaq (a mixture of polymerase, buffer, $MgCl_2$, and dNTPs in proportions as earlier described) ddH_2O and 5 µl DNA template giving a final volume of 20 µl. The cycling conditions used were 95 °C for 10 min followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extention at 72 °C for 1 min then a final extention at 72 °C for 10 min. Electrophoresis using 2% agarose gel

stained in ethidium bromide was run for 30 min after loading 10 µl PCR product. The agarose gel was observed and photographed under an ultra violet illuminator (TOYOBO Transilluminator Model TM-20 - TOYOBO FAS-III monitor system).

3.8 Identification of *Wuchereria bancrofti* in pooled mosquito samples using Real Time Polymerase Chain Reaction (RT-PCR)

The "long DNA repeat" of *W. bancrofti* (LDR: GenBank accession number AF093510) was used as a detection target with extracted mosquito genomic DNA (gDNA) templates (Rao *et al.* 2006). This LDR target was amplified with primers and probes specific for these sequences. These primers, Wb-LDR1 [5' – ATT TTG ATC ATC TGG GAA CGT TAA TA – 3'], Wb-LDR2 [5' – CGA CTG TCT AAT CCA TTC AGA GTG A – 3'] and Wb-TaqMan probe [5' – ATC TGC CCA TAG AAA TAA CTA CGG TGG ATC TCT G – 3'] were designed by Primer Express software (Applied Biosystems, Foster City, CA). The probe was labelled with a reporter dye FAM (6-carboxyfluorescein) at the 5' end and the quencher dye TAMRA (6-carboxytetramethylrhodamine) at the 3' end. The primers were not labelled. The RT-PCR reaction was performed with 12.5 µl TaqMan Universal PCR master mix (Life technologies/Applied Biosystems Part No. 4304437), 450 nmol/L of each primer and 124 nmol/L probe and 1 µl DNA template in a final reaction volume of 25 µl. The master mix was loaded into MicroAmp optical 96-well reaction plate (Life technologies/Applied Biosystems Part No. 8010560) placed on MicroAmp splash free 96-well base (Life technologies/Applied Biosystems Part No. 4312063) serving as a rack. The gDNA templates from the pools of mosquitoes were added to the MicroAmp optical 96-well reaction plate (Life technologies/Applied Biosystems Part No. 8010560)

in duplicates according to the plate map designed for the RT-PCR reaction. This was then covered with a MicroAmp Optical Adhesive Film, PCR Compatible, DNA/RNA/RNase Free (Life technologies/Applied Biosystems Part No. 4311971) strip with the aid of MicroAmp Adhesive Film Applicator (Life technologies/Applied Biosystems Part No. 4333183) to form a screed over the reaction plates. The covered reaction plate was then placed in the ABI Prism 7300 instrument which uses SDS software (Life technologies/Applied Biosystems Listing No. 200114) and manufacturer's calibrations for the RT-PCR run. Water was used as a negative control and a standard *W. bancrofti* gDNA was used as a positive control. The cycle threshold (Ct) values for each sample was determined according to the manufacturer's instructions. To ensure a contamination free reaction, all reagents were aliquoted with sterile filtered pipette tips (1-10 µl, 10-20 µl, 100-200 µl and 100-1000 µl). All the reagents were reconstituted or diluted with diethylpyrocarbonate (DEPC) treated water which is certified to be free of all RNase and DNase and therefore used for very sensitive bench work.

Instrument settings

The 96-well reaction plates were placed into ABI 7300 Prism RT-PCR system a new document was created. The system was then calibrated using the manufacturer's protocol. The RT-PCR reaction was run after calibrating the system.

Cycling parameters

The thermal cycling conditions used for the RT-PCR reaction was the default manufacturer's calibrations. The default cycling parameters were 50 °C for 2 min and

95 °C for 10 min (pre-heating) followed by 40 cycles of denaturation at 95 °C for 15 sec and a simultaneous annealing and extension at 60 °C for 1 min.

3.9 Experimental Design

Mosquitoes collected using the PSC sampling method from the three study communities namely Ayensuako, Gyaahadze and Mankrong was transported in petri dish to the NMIMR entomological laboratory. The mosquito species were identified using morphology (morphological identification keys) and molecular method (PCR) as earlier described using DNA extracted from the legs of the mosquitoes to confirm the morphologically identified mosquitoes. Morphologically identified mosquitoes are then divided into head, thorax and abdomen on a glass slide; teased and screened for filarial parasites using microscopy. The transmission indices were estimated after microscopy. The heads of mosquitoes (up to twenty-five) from each genus was cleared using clearing agent and the cibarial armature mounted on a glass slide. The number of teeth was observed under the compound microscope and recorded for each of these genera. DNA was extracted from pools of mosquitoes (up to 25 mosquitoes in a pool) of the same genus and/or species from the same community where applicable for filarial parasite detection using PCR and later confirmed using RT-PCR.

Laboratory Studies

All the laboratory studies were documented using Microsoft Word and Excel 2007. The transmission indices were also recorded and analysed using the Microsoft software mentioned above.

CHAPTER FOUR

RESULTS

4.1 Morphological Identification of mosquitoes caught

A total of 550 mosquitoes were collected in five mornings (dawn) of collection in the three communities. After identification, it was observed that no *Aedes* mosquitoes were caught in the study areas. A total of 472/550 (85.8%) of *Anopheles*, 57/550 (10.4%) of *Culex* and 21/550 (3.8%) of *Mansonia* mosquitoes were identified. Out of the total Anophelines (*An. gambiae*, *An. funestus* and *An. pharoensis*) caught, 231/472 (48.9%) were randomly selected for molecular identification.

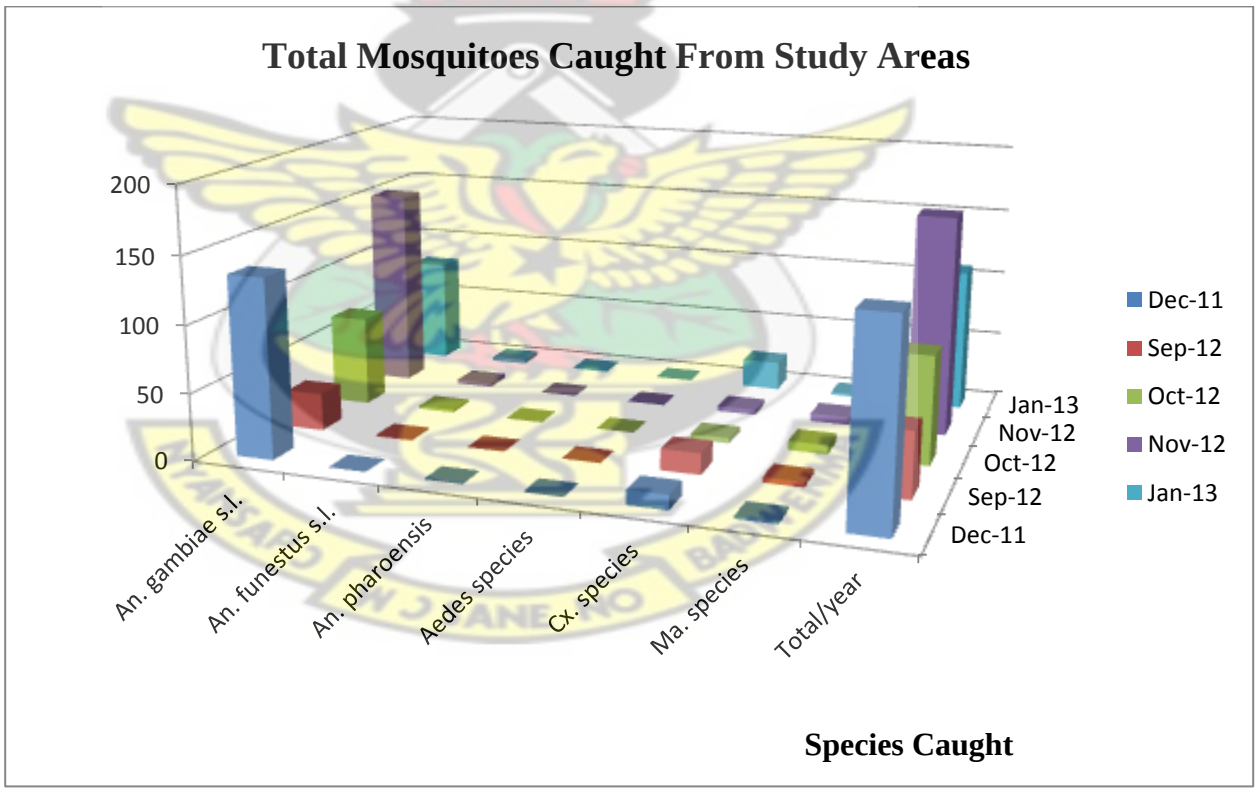


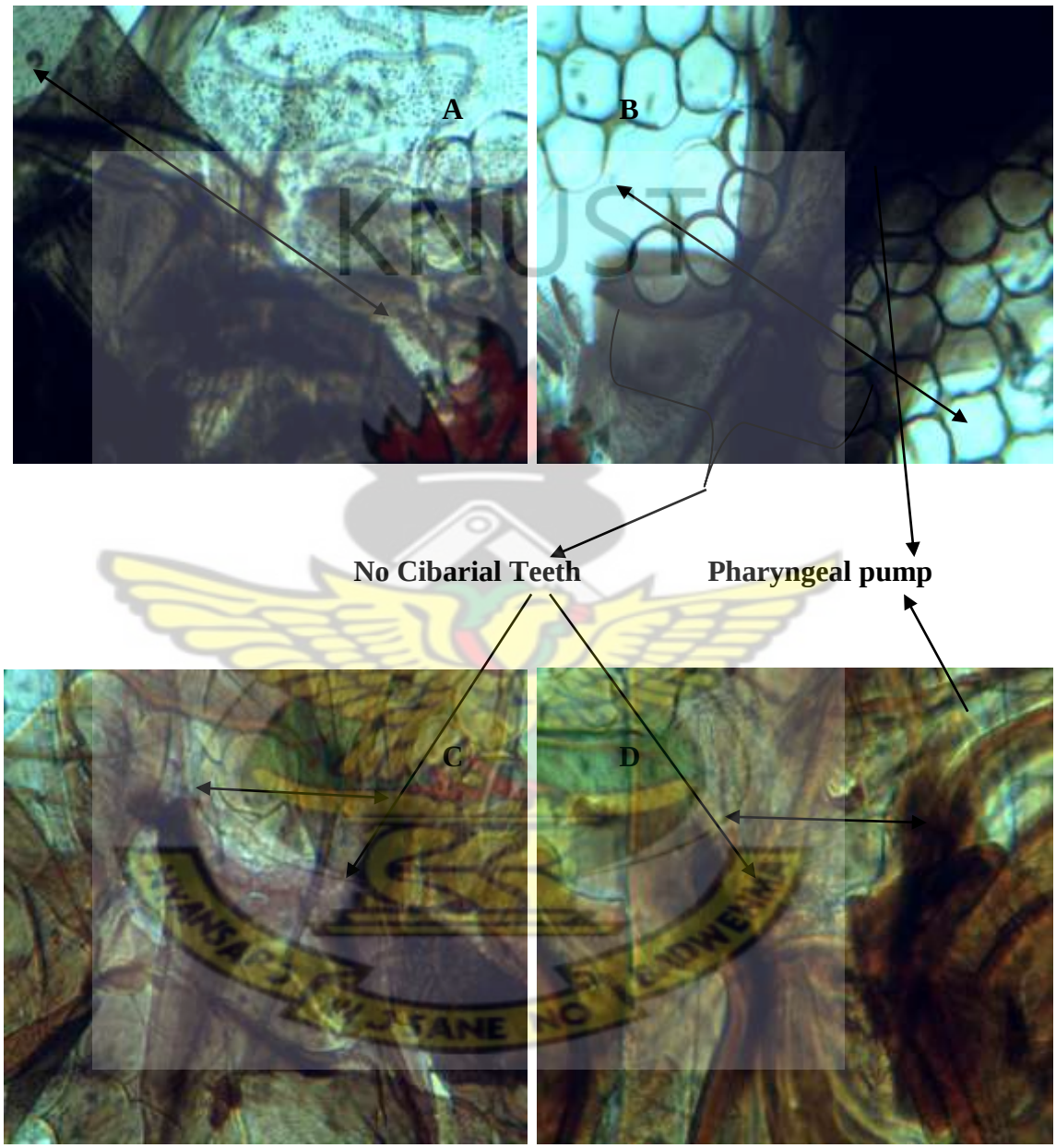
Figure 1: Number of mosquitoes caught from study sites

4.2 Mosquito Dissection

All mosquitoes caught in the study sites were teased and observed for the presence of microfilariae (Mf) or any of the different larval stages (L1-L3) of *W. bancrofti* using microscopy. No Mf or any larval stages of *W. bancrofti* were found via microscopy (Appendix 1, Table 5).

4.3 Mounting and Comparing Cibarial Armature of mosquitoes

No *Aedes* mosquitoes were caught during sampling in the study sites. For the purpose of comparison of the cibarial armature of the *Aedes, Culex* and *Mansonia* genera, *Aedes* mosquitoes (*Ae. aegypti* and *Ae. vittatus*) were sampled from the University of Ghana Campus. Cibarial armature slides mounted using mosquitoes (*Culex* and *Mansonia* genera) from study sites were poor. Mosquito samples belonging to these two genera from Odumase, Dodowa in the Greater Accra region was therefore used in preparing the cibarial armature slides. Observation of the cibarial armature showed no cibarial teeth in both in *Aedes aegypti, Ae. vittatus, Mansonia africana* and *Ma. uniformis* as shown in Plate 3 below. *Culex* mosquitoes, however, had an average of 26 cibarial teeth (range = 20 – 34). See Appendix 2 Table 8 for details.



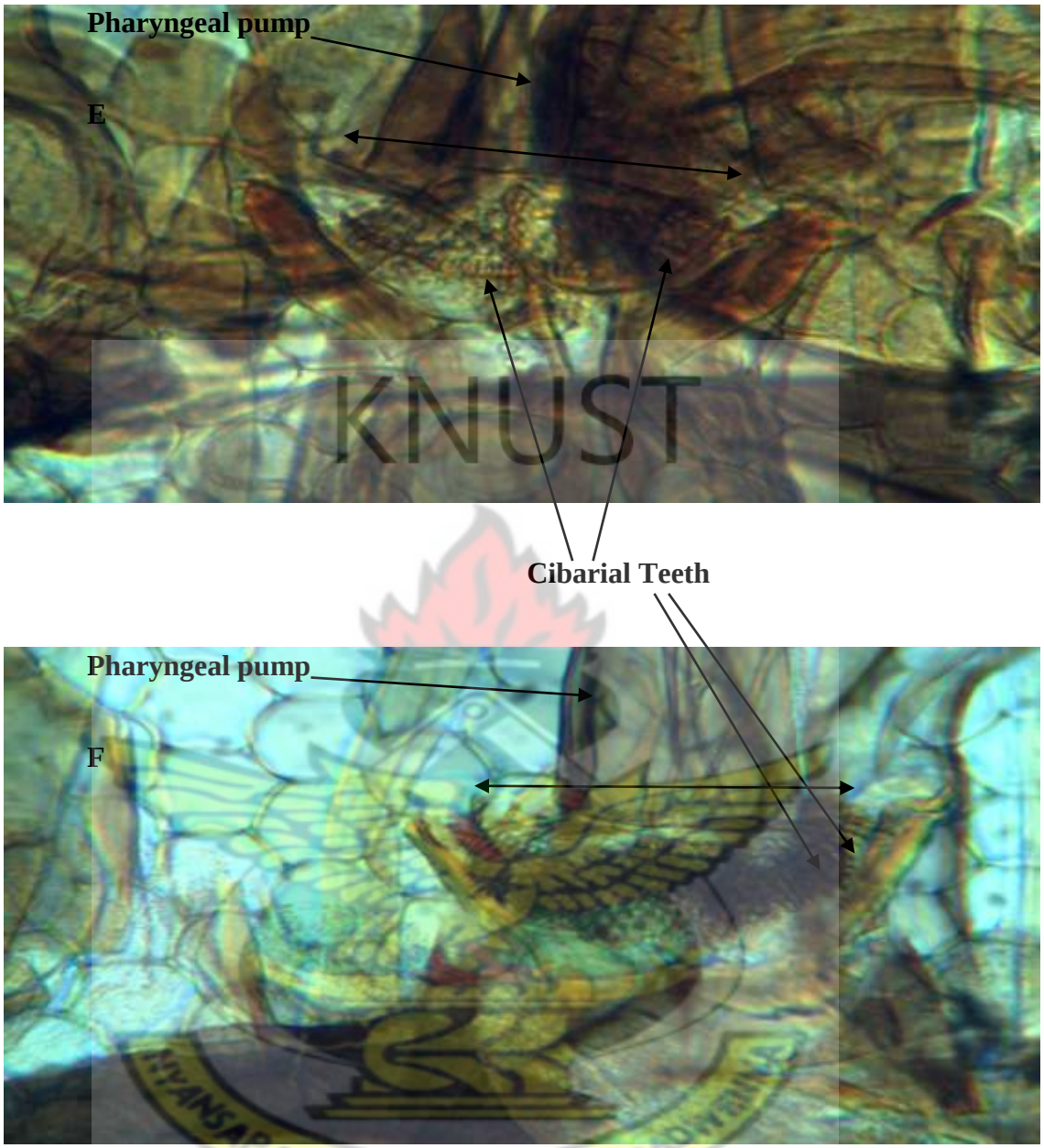


Plate 3: Cibarial armature of Aedes, Culex and Mansonia mosquitoes

Plates 3A and 3B are cibarial armature of *Ae. aegypti* and *Ae. vittatus* with no teeth. Plates 3C and 3D are those of *Ma. africana* and *Ma. uniformis* also without teeth. Plates 3E and 3F are cibarial armature of *Cx.* mosquitoes with teeth. Double arrow shows width of cibarial armature.

4.4 Molecular Identification of *Anopheles gambiae* complexes

A total of 231 morphologically identified *Anopheles gambiae* s. l. were randomly selected for species identification. The results were predominantly *An. gambiae* s. s. recording 222/231 (96.1%). The numbers that came from Ayensuako, Gyahadze and Mankrong were 99/222 (44.6%), 94 (42.3%), and 29 (13.1%), respectively. The only *An. arabiensis* – 1/231 (0.4%) – was from Ayensuako. *An. melas* were found in only Gyahadze, 5/231 (2.2%), and Mankrong, 3/231 (1.3%). A good number, 75/222 (33.8%), of these molecularly confirmed *Anopheles gambiae* s. s. were further characterized into the molecular forms (M and S). Eight out of seventy-five representing about 10.7% were M forms with equal numbers coming from Gyahadze and Mankrong. The rest of the *Anopheles gambiae* s. s., 67/75 (89.3%), were S forms with 58/67 (86.6%), 2/67 (3.0%) and 7/67 (10.4%) coming from Ayensuako, Gyahadze and Mankrong, respectively (see Figure 2 below for details).

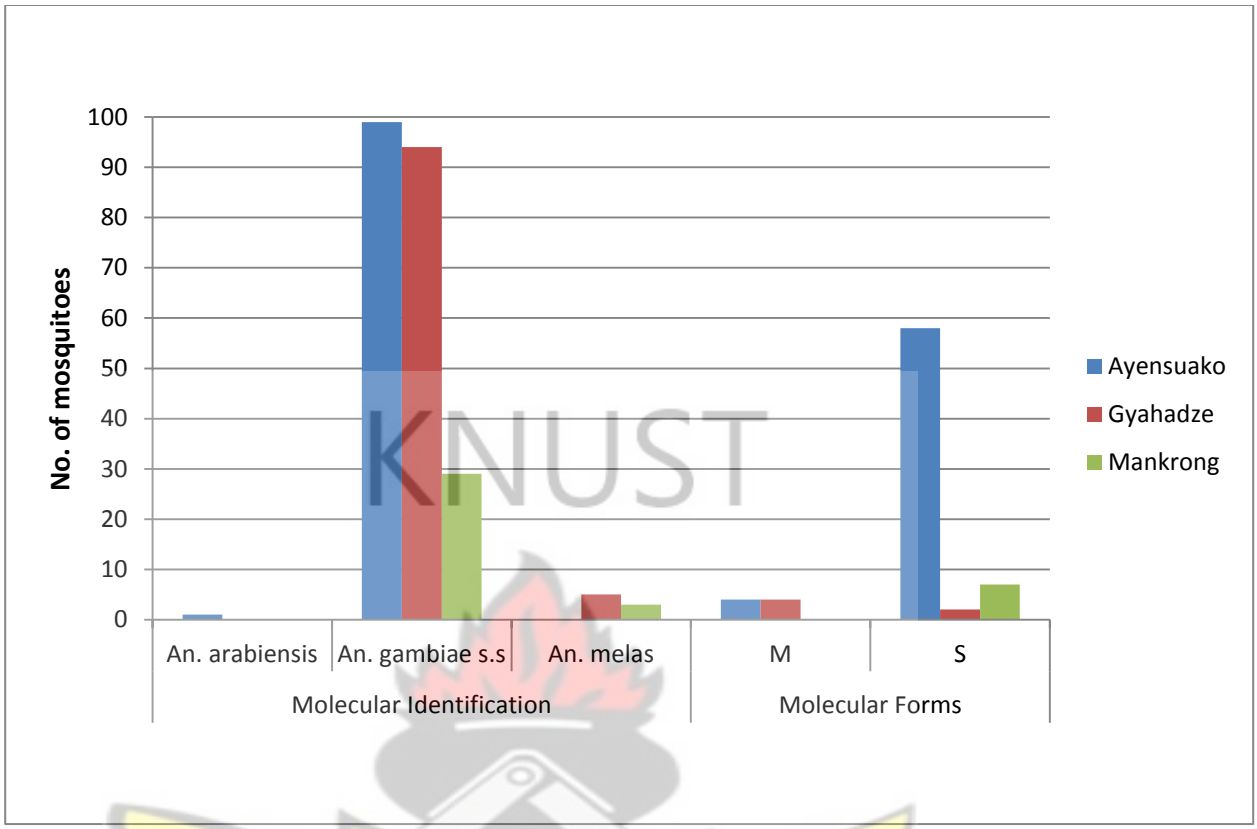


Figure 2: Identification of sibling species of *An. gambiae* complex and molecular forms

4.5 Molecular Identification of *Wuchereria bancrofti*

Microfilaria Load and detection of larval stages of *W. bancrofti* in the mosquitoes caught

Microscopy revealed no microfilariae and larval stages of *W. bancrofti* in all the mosquitoes collected after dissections. The dissected *Anopheles* mosquitoes were pooled 25 per tube per species for DNA extraction. The numbers of the culicines obtained during the study were small and therefore were in pools of less than 25 per tube per species. Extracted DNA was used for the detection of Mf as well as the other LF parasite stages (see Appendix 1, Table 3).

There were no amplifications for the pooled samples screened for *W. bancrofti* using conventional PCR.

4.6 Transmission Indices

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

4.6.1 *Biting Rate*

The biting rate refers to the estimated number of vector mosquitoes coming to bite one person exposed during the sampling night. Biting rate (BR) may be expressed as the number of mosquitoes caught or collected divided by the product of the number of persons sleeping in the room during the sampling and the catch nights. To estimate the monthly biting rate (MBR) or annual biting rate (ABR), the man biting rate is either multiplied by 30 or 365 respectively. In Ayesuako, Gyahadze and Mankrong, the respective biting rates were 0.3, 0.2, and 0.1 bites person^{-1} night^{-1}. The ABR for the three communities therefore translated to 122.7, 87.4 and 51.0 bites person^{-1} year^{-1} respectively (Appendix 1, Table 4).

4.6.2 *Infection Rate*

The infection rate is the ratio of the number of mosquitoes with any stage of the *W. bancrofti* parasite and the total number of mosquitoes examined for these parasites. This is usually expressed as a percentage. Microscopy revealed none of the parasite stages. The infection rate for this study was therefore zero.

4.6.3 *Infectivity Rate*

The infectivity rate refers to the ratio of the number of mosquitoes carrying the infective stage (L_3) of the *W. bancrofti* parasite and the total number of mosquitoes examined for

the parasites. This is also expressed as a percentage. No infections were detected using microscopy therefore infectivity rate was also zero (Appendix 1, Table 5).

4.6.4 *Worm Load*

The worm load is defined as the ratio of the number of infective parasites (L_3) and the number of mosquitoes carrying the L_3 parasites. There were no infective mosquitoes. The worm load was therefore zero (Appendix 1, Table 5).

4.6.5 *Infective Biting Rate (IBR) and Annual Infective Biting Rate (AIBR)*

The infective biting rate is the product of the infectivity rate and the biting rate. This was zero (Appendix 1, Table 5). The AIBR is the product of the IBR and 365 days. This was also zero.

4.6.6 *Transmission Potential (TP) and Annual Transmission Potential (ATP)*

Transmission potential is the product of IBR and the worm load. The unit is infective bites person^{-1} night^{-1}The Annual Transmission Potential is, however, the product of the TP and 365 days and the unit(s) is infective bites person^{-1} year^{-1}. These were also zero (Appendix 1, Table 5).

4.7 Detection of *Wuchereria bancrofti* using Real Time Polymerase Chain Reaction (RT-PCR)

Thirty pools of mosquito DNA was extracted for molecular analysis (conventional PCR and RT-PCR). RT-PCR revealed a weak positive from a pool of 25 mosquitoes DNA (*An. gambiae* s. l.) sampled from Ayensuako community. In all, 11/25 (44.0%) were only morphologically identified as *An. gambiae* s. l.; 13/25 (52.0%) were molecularly identified as *An. gambiae* s. s.; and only 1/25 (2%) was identified as *An. gambiae*

arabiensis. Also, 7/12 (58.3%) of the *An gambiae* s. s. were identified as the S molecular forms (see Appendix 1 Table 4). The total number of mosquito pools used for *W. bancrofti* screening was 30. 19/30 of these were *An. gambiae* s. s.; 3/30 were *An. funestus*; 4/30 were *Culex species* and 4/30 were *Mansonia species*. Pools of *An. gambiae* s. l., *An. funestus*, *Culex species* and *Mansonia species* were distributed respectively in the three communities as follows: Ayensuako – 11, 1, 1, 0; Gyahadze – 5, 1, 2, 3; and Mankrong – 3, 1, 1 and 1 (see Appendix 1 Table 8).

The average Cycle threshold (Ct) value was 39.38 (38.8809 – 39.8755) with a standard deviation of 0.703 (see Appendix 2 for print outs of the RT-PCR results).

CHAPTER FIVE

DISCUSSION

A lot of education, morbidity management, mass drug administration (MDA) and transmission studies had been conducted for lymphatic filariasis since the last three decades (Appawu *et al.* 2001, Boakye *et al.* 2007, de Souza *et al.* 2012, Dunyo *et al.* 1996, Dzodzomenyo *et al.* 1999, Gyapong *et al.* 1996a, Ughasi *et al.* 2012, Amuzu *et al.* 2010, Boakye *et al.* 2004, de Souza *et al.* 2010, Gbakima *et al.* 2005, Gyapong *et al.* 1994, 1998b, 1998a, Gyapong 2000, 2004, Gyapong *et al.* 2000, Ughasi *et al.* 2010). These efforts are geared towards the elimination of LF in Ghana within the WHO global stipulated LF elimination schedule, 2020. Having about 83 countries endemic for LF in the recent past, the global intervention programmes have had a positive impact. Currently, LF has been eliminated in some countries such as China, Korea, Zanzibar and recently Togo, leaving about 72 endemic countries worldwide (de Souza *et al.* 2012, Sodahlon *et al.* 2013). Most endemic countries since the inception of MDAs have successfully completed five round of mass chemotherapy. This number of MDAs should be enough to interrupt LF transmission (Grady *et al.* 2007, Pedersen *et al.* 2009, Tisch *et al.* 2011). There is, however, residual transmission in most endemic countries that have had more than five rounds of MDA (Simonsen *et al.* 2010). Reasons such as systemic noncompliance; population coverage < 65%, migration of Mf-positive carriers to/from endemic and non-endemic areas after administration of MDAs; poor sanitation leading to increasing breeding sites for LF vectors; dug-outs created by construction works which adds to existing breeding sites for LF vectors and the rapid rate of urbanization had been cited by several research teams as contributing factors

responsible for the maintenance of residual transmission in such endemic communities (Mohammed *et al.* 2006, Tisch *et al.* 2011, Nwoke *et al.* 2010, Grady *et al.* 2007). Addressing these challenges may have contributed to the successful elimination of LF in some endemic countries thus decreasing the number of endemic countries currently to 72 worldwide (de Souza *et al.* 2012). The observed residual transmission in some endemic countries and communities even after five rounds of MDA called for some critical local assessment of LF transmission. There is an evident decrease in parasitemia and worm load in the vectors after five rounds of MDA (Grady *et al.* 2007, Simonsen *et al.* 2010, Tisch *et al.* 2011). Usually the next step for such endemic countries is an evaluation of the mass chemotherapy programme to know whether or not to stop MDA. In the 34 endemic countries in Africa, Togo is the only country that has successfully eliminated LF (Sodahlon *et al.* 2013). Togo is thus in the surveillance phase of the WHO plans for LF elimination.

It has been a good strategy to put in place formidable nationwide surveillance systems before the decision to stop MDA in any endemic country (Tisch *et al.* 2011, Simonsen *et al.* 2010). In the history of filariases infections and elimination/eradication, there is the likelihood of resurgence of infections in an endemic country certified to have achieved elimination/eradication. The story of Chad is no different. Guinea worm (*Dracunculus medinensis*), though not a true filarial parasite, after its elimination in Chad in 2000 has re-emerged ten years afterwards (MMWR 2011). This makes the decision to stop MDA without the fear of recrudescence very critical. After getting empirical evidence to stop MDA, the right systems would have to be implemented to monitor the disease in order to guard against re-infection of the indigens.

In assessing transmission of LF in endemic communities having low parasitemia due to several rounds of MDA (≥ 5), parasitological as well as entomological surveys are vital field studies performed in order to carry out important laboratory studies with the collected samples. It is difficult using microscopy (the gold standard) to detect filarial parasites at low parasitemia. There are, however, LF vectors such as *Anopheles melas*, *Mansonia africana* and *M. uniformis* that are efficient at low parasitemia (Ughasi *et al.* 2012, Amuzu *et al.* 2010). These vectors when caught during surveillance may present a better picture of the transmission status in these communities. This makes it very important for intervention programmes to know the LF vectors involved and the role they play in transmission in various endemic foci under their jurisdiction. This would inform them on the appropriate control measures to implement in order to confidently decide when to stop MDA.

During the assessment of LF transmission, the importance of xenomonitoring cannot be overemphasized. Its cost effective nature and unbiased random sampling of blood from the populace by the vectors from the whole community ensures effective blood sampling from human reservoirs of *W. bancrofti* who would not willingly participate in parasitological surveys. This study thus used xenomonitoring to assess transmission of LF in endemic communities that had participated in at least five rounds of MDA. This provided the data necessary for calculating the entomological indices that would help determine the transmission status of the study sites. All analyses from entomological indices and conventional PCR suggested there was no transmission of LF in the study communities. The sensitivity of conventional PCR (with the ability to amplify 1 nanogram of DNA material) would convince any LF programme manager to easily

come to a conclusion to stop MDA in endemic foci with at least five rounds of mass chemotherapy. All available options must be exploited before arriving to such a decision. A more sensitive molecular detection of *W. bancrofti*, the RT-PCR (with the ability to amplify 1 femtogram of DNA material) was done to confirm or otherwise results obtained from conventional PCR. One out of the thirty mosquito DNA pools revealed a weak positive result for *W. bancrofti* (see Appendix 2, Table 8). This pool of mosquitoes was identified to be coming from Ayensuako. The mosquitoes that made up this pool were predominantly *Anopheles gambiae* s. s. (13/25 (52.0%)) and only one (1/25 (4.0%)) being *An. gambiae arabiensis*. The molecularly identified ones were all S forms (7/13 (53.8%)) which confirms findings from another study that suggested the S forms to be more involved in the transmission of LF in Ayensuako which is more likely to be a low to medium LF zone (de Souza *et al.* 2010). In this study, the frequency of M forms now known as *An. coluzzii* Coetzee & Wilkerson sp. n. (Coetzee *et al.* 2013) in Ayensuako amongst samples molecularly confirmed was very low (4/62 (6.5 %)). None of these *An. coluzzii* was molecularly identified in the positive pool. Most worrying are the mosquito species identified in the positive pool. These were all *An. gambiae* s. l. known to transmit LF when parasitemia is high. All the *An. coluzzii* as well as the *An. gambiae melas* (known to transmit LF at low microfilaraemia) molecularly identified in the study were not part of the positive pool. This may suggest some form of active transmission in Ayensuako and more so if the molecularly unconfirmed mosquitoes in the positive pool were *An. gambiae melas* as suggested by Amuzu *et al.* (2010). Though not much, *Mansonia* mosquitoes were found in all communities except Ayensuako. The two communities having *Mansonia* mosquitoes (Gyahadze and Mankrong) and no LF

transmission, would have to be monitored closely since these vectors may also transmit LF at low microfilaraemia (Ughasi *et al.* 2010). There is some evidence from this study that MDA have helped decrease parasitemia in the populace. However, the importance of xenomonitoring in these communities should not be trivialized. This is because just as observed at Ayensuako, other endemic communities that had participated in at least five (5) rounds of MDA may still be having residual LF transmission. Xenomonitoring in endemic foci would allow programme managers know the species composition of the vectors in their study area and the phenomenon of transmission (facilitation, limitation and proportionality) employed by these vectors.

With low parasitemia, large numbers of mosquitoes are required for the detection of *W. bancrofti*. Sampling should therefore be done in the rainy season. In this study, sampling was done predominantly in the dry season and not in the peak of the rainy season. This timing was to allow ≥ 6 months interval to elapse after MDA had been administered in these endemic communities. This would ensure clearance of the drug from the blood to a large extent. Nevertheless, though the numbers of mosquitoes caught were very low, there is still evidence of residual transmission in Ayensuako (by RT-PCR). It is possible that with a large number of mosquitoes caught in Ayensuako, all the other four genera of mosquitoes mentioned above may be caught in the community. The evidence from this study shows that the known culicine vectors (*Ma.* mosquitoes) for the transmission of LF in Ghana do not have cibarial teeth. This implies that whiles the anopheline vectors are transmitting by the phenomena of facilitation and limitation (in the case of *An. melas*) as suggested by Amuzu *et al.* (2010), *Mansonia* mosquitoes may also be transmitting by "limitation" (Ughasi *et al.* 2012). Though the

number of cibarial teeth in a given mosquito species may vary as recounted by (Williams and Savage 2009) the absence of cibarial teeth as observed in *Mansonia africana* and *Ma. uniformis* supports the fact that these vectors may be helping maintain residual transmission in endemic communities in Ghana through the phenomenon of "limitation". The transmission cycles in endemic communities may be thus complex than envisaged when *An. gambiae* s. l. was thought to be the only vector in Ghana. The high number of teeth observed in the *Culex* may explain why this genus seems to be refractory to LF in Ghana.

CONCLUSION AND RECOMMENDATION

Conclusion: The annual MDA in various endemic communities in Ghana have reduced parasitemia. There is, however, residual LF transmission in some endemic communities in Ghana. This may be due to *An. melas* and *Mansonia* mosquito vectors in these endemic foci. The possibility of active transmission in some of these endemic communities cannot be ruled out especially when the *An. gambiae* complex is involved in the transmission. Highly sensitive and specific standardized molecular tools or techniques are needed for screening samples from endemic communities before deciding when to stop MDA.

Recommendation: In order to achieve the goal of the GPELF by 2020, there is the need to combine MDA with vector management for a synergistic effect. Vector biology would also be required in planning intervention and surveillance programmes. Larger sample sizes from endemic communities would have to be screened when conducting

evaluations to stop MDA. The criteria for stopping MDA in endemic countries must be standardized for effective evaluation and comparison.

REFERENCES

Aboagye-Antwi F 2003 *Studies on the role of Culex and Anopheles species in the transmission of Wuchereria bancrofti (Spirurida: Filariidae) in the Gomoa District of Ghana* (University of Ghana)

Addiss D G and Brady M A 2007 Morbidity management in the Global Programme to Eliminate Lymphatic Filariasis: a review of the scientific literature *Filaria J.* **6** 2 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1828725&tool=pmcentrez&rendertype=abstract

Agi P I and Ebenezer A 2009 Observations on filarial infection in Amassoma community in the Niger Delta, Nigeria *J. Appl. Sci. Environ. Manag.* **13** 15–9

Alleman M M, Twum-Danso N a Y and Thylefors B I 2006 The Mectizan Donation Program - highlights from 2005. *Filaria J.* **5** 11 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1618829&tool=pmcentrez&rendertype=abstract

Alli R, Bhunia B, Chhotray G P, Reddy M V R and Harinath C B 2003 MICROSCOPIC HAEMATURIA AS AN OCCULT FILARIAL INFECTION IN BHUBANESHWAR AN ENDEMIC AREA FOR BANCROFTIAN FILARIASIS *Journal, Indian Biochem. Clin.* **18** 61–4 Online: http://hinari-gw.who.int/whalecomlink.springer.com/whalecom0/content/pdf/10.1007/BF02867666.pdf

Amaral F, Dreyer G, Figueredo-Silva J, Noroes J, Cavalcanti A, Samico S C, Santos A and Coutinho A 1994 Live Adult Worms Detected by Ultrasonography in Human Bancroftian Filariasis *Am. J. Trop. Med. Hyg.* **50** 753–7 Online: http://www.ajtmh.org/content/50/6/753.short

Amuzu H, Wilson M D and Boakye D A 2010 Studies of Anopheles gambiae s . l (Diptera : Culicidae) exhibiting different vectorial capacities in lymphatic filariasis transmission in the Gomoa district, Ghana *Parasit. Vectors* **3** 1–6 Online: http://www.parasitesandvectors.com/content/3/1/85

Andreadis T G, Anderson J F, Munstermann L E, Wolfe R J and Florin D a 2001 Discovery, distribution, and abundance of the newly introduced mosquito Ochlerotatus japonicus (Diptera: Culicidae) in Connecticut, USA. *J. Med. Entomol.* **38** 774–9 Online: http://www.ncbi.nlm.nih.gov/pubmed/11761373

Anosike J C, Nwoke B E, Ajayi E G, Onwuliri C O, Okoro O U, Oku E E, Asor J E, Amajuoyi O U, Ikpeama C A and Ogbusu F I 2005 Lymphatic filariasis among the Ezza people of Ebonyi State Eastern Nigeria *Ann. Agric. Environ. Med.* **12** 181–6

Appawu M A, Dadzie S K, Baffoe-Wilmot A and Wilson M D 2001 Lymphatic filariasis in Ghana: entomological investigation of transmission dynamics and

intensity in communities served by irrigation systems in the Upper East Region of Ghana. *Trop. Med. Int. Health* **6** 511–6 Online: http://www.ncbi.nlm.nih.gov/pubmed/11469943

Appawu M, Dadzie S, Abdul H, Asmah H, Boakye D and Wilson M 2006 SURVEILLANCE OF VIRAL HAEMORRHAGIC FEVERS IN GHANA : ENTOMOLOGICAL ASSESSMENT OF THE RISK OF *Ghana Med. J.* **40** 137–41

Biney E J, Berkard H, Sakyi K Y, Appawu M, Wilson M D and Boakye D A 2010 The impact of house screening on biting behavior of anopheles species and transmission of lymphatic filariasis and malaria in Gomoa Mampong, a rural community in Ghana *Am. J. Trop. Med. Hyg. Conf. 59th Annu. Meet. Am. Soc. Trop. Med. Hyg. ASTMH* **83**

Boakye D A, Baidoo H A, Glah E, Brown C, Appawu M and Wilson M D 2007 Monitoring lymphatic filariasis interventions: Adult mosquito sampling, and improved PCR – based pool screening method for Wuchereria bancrofti infection in Anopheles mosquitoes *Filaria J.* **6** 13 Online: http://www.scopus.com/inward/record.url?eid=2-s2.0-39049097211&partnerID=40&md5=04ddf76b3bccd92b18e284ba3d7b76d2

Boakye D A, Wilson M D, Appawu M A and Gyapong J 2004 Vector competence , for Wuchereria bancrofti , of the Anopheles populations in the Bongo district of Ghana **98** 501–8

Bockarie M J and Molyneux D H 2009 The end of lymphatic filariasis ? *BMJ* **338** 1470–2

Bowman D D M S . P D 1999 Georgis' Parasitology for Veterinarians - Seventh Edition *W.B. Sanders Company (A Harcourt Health Sciences Company), The Curtis Center, Independence Square West, Philadelphia, PA 19106* (W.B. Sanders Company (A Harcourt Health Sciences Company), The Curtis Center, Independence Square West, Philadelphia, PA 19106)

Carpenter S J and LaCasse W J 1955 *Mosquitoes of North America (North of Mexico)* (London: University of California Press, Berkeley, Los Angeles, London)

Chandra G 2008 Nature limits filarial transmission *Parasites and vectors* **1** 13 Online: http://www.scopus.com/inward/record.url?eid=2-s2.0-64949183367&partnerID=40&md5=3a2d17cd4ba4cc8bcfca81037e2e4cac

Cheng J-T, Mohan S, Nasr S H and D'Agati V D 2006 Chyluria presenting as milky urine and nephrotic-range proteinuria. *Int. Soc. Nephrol.* **70** 1518–22 Online: http://www.ncbi.nlm.nih.gov/pubmed/16871246

Coetzee M, Hunt R H, Wilkerson R, Torre A Della, Coulibaly M B and Besansky N J 2013 Anopheles coluzzii and Anopheles amharicus, new members of the Anopheles gambiae complex *Magnolia Press* **3619** 246–74

Crans W J 1964 Continued host preference studies with New Jersey mosquitoes *Proc. N.J. Mosq. Exterm. Assoc.* **51** 50–8

Crans W J, Sprenger D A and Mahmood F 1996 The blood feeding habits of Aedes sollicitans (Walker) in relation to eastern equine encephalitis virus in coastal areas of New Jersey. II. Results of experiments with caged mosquitoes and the effects of temperature and physiological age on host selection *J. Vec. Ecol.* **21** 1–5

Danilov V N 1982 A KEY FOR THE MOSQUITOES OF THE GENUS MANSONIA *An. Inst. Hig. e Med. Trop. (Lisboa).* **9** 31–6

Derua Y A, Alifrangis M, Hosea K M, Meyrowitsch D W, Magesa S M, Pedersen E M and Simonsen P E 2012 Change in composition of the Anopheles gambiae complex and its possible implications for the transmission of malaria and lymphatic filariasis in north-eastern Tanzania. *Malar. J.* **11** 188 Online: http://www.ncbi.nlm.nih.gov/pubmed/22681999

DREYER G, MEDEIROS Z, NETTO M J, LEAL N C, GONZAGA DE CASTRO L and PIESSENS W F Acute attacks in the extremities of persons living in an area endemic for bancroftian filariasis : differentiation of two syndromes *Trans. R. Soc. Trop. Med. Hyg.* **93** 413–7 Online: http://cat.inist.fr/?aModele=afficheN&cpsidt=1917202

Dreyer G, Noroes J, Figueredo-Silva J and Piessens W 2000 Pathogenesis of Lymphatic Disease in Bancroftian Filariasis:: A Clinical Perspective *Parasitol. Today* **16** 544–8 Online: http://www.sciencedirect.com/science/article/pii/S0169475800017786

Duerr H P, Dietz K and Eichner M 2005 Determinants of the eradicability of filarial infections: a conceptual approach *Trends Parasitol.* **21** 88–96

Dunyo S K, Appawu M, Nkrumah F K, Baffoe-Wilmot A, Pedersen E M and Simonsen P E 1996 Lymphatic filariasis on the coast of Ghana. *Trans. R. Soc. Trop. Med. Hyg.* **90** 634–8

Dzodzomenyo M, Dunyo S K, Ahorlu C K, Coker W Z, Appawu M A, Pedersen E M and Simonsen P E 1999 Bancroftian filariasis in an irrigation project community in southern Ghana. *Trop. Med. Int. Heal. TM IH* **4** 13–8 Online: http://www.scopus.com/inward/record.url?eid=2-s2.0-0033036345&partnerID=40&md5=4fd7dcc2a7dc7c35528456bab2c6c914

Erickson S M, Xi Z, Mayhew G F, Ramirez J L, Aliota M T, Christensen B M and Dimopoulos G 2009 Mosquito Infection Responses to Developing Filarial Worms

ed S Aksoy *PLoS Negl. Trop. Dis.* **3** 11 Online: http://www.pubmedcentral.nih.gov /articlerender.fcgi?artid=2752998&tool=pmcentrez&rendertype=abstract

Fanello C, Santolamazza F and della Torre a 2002 Simultaneous identification of species and molecular forms of the Anopheles gambiae complex by PCR-RFLP. *Med. Vet. Entomol.* **16** 461–4 Online: http://www.ncbi.nlm.nih.gov/pubmed /12510902

Favia G, Torre A, Bagayoko M, Lanfrancotti A, Sagnon N F and Toure Y T 1997 Molecular identification of sympatric chromosomal forms of Anopheles gambiae and further evidence of their reproductive isolation *Insect Mol. Biol.* **6** 377–83

Filariasis W _ L 2012 World Health Organisation: Lymphatic Filariasis, Fact Sheet No. 102, 2012. Updated March 2013.

Gbakima A A, Appawu M A, Dadzie S, Karikari C, Sackey S O, Baffoe-Wilmot A, Gyapong J and Scott A L 2005 Lymphatic filariasis in Ghana: establishing the potential for an urban cycle of transmission *Trop. Med. Int. Heal.* **10** 387–92

Ghana Filariasis Elimination Programme . 2008 *Ghana Filariasis Elimination Programme Summary Report for Centre of Neglected Tropical Diseases , Liverpool , UK*

Ghana Statistical Service 2012 2010 POPULATION AND HOUSING CENSUS FINAL RESULTS GHANA STATISTICAL SERVICE Online: http://www .statsghana.gov.gh/docfiles/2010phc/2010_POPULATION_AND_HOUSING_CE NSUS_FINAL_RESULTS.pdf

Gilles H M 1999 Epidemiology of malaria *Essential Malariology In Gillies, H. M & Warrel, D. A. (Eds.)*

Gillet J D 1972 *Common African Mosquitos and Their Medical Importance* (London: William Heinemann Medical Books Ltd: London)

Gillies M T 1955 The pre-gravid phase of ovarian development in Anopheles funestus *Ann. Trop. Med. Parasitol.* **49** 320–5

Gillies M T and Coetzee M 1987 A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region) *Publication of South African Institute for Medical Research.* vol 55 (The South African Institute for Medical Research, P. O. Box 1038 Johannesburg) pp 99–119

Gillies M T and De Meillon B 1968 The Anophelinae of Africa south of the Sahara (Ethiopian zoogeographical region) *Publ. South African Inst. Med. Res.* **54** 203–24

Gold Coast Medical Department Reports 1936 1936 *Gold Coast Medical Department Reports (1936-37)*

Grady C A, Beau De Rochars M, Direny A N, Orelus J N, Wendt J, Radday J, Mathieu E, Roberts J M, Streit T G, Addiss D G and Lammie P J 2007 Endpoints for Lymphatic Filariasis Programs *Emerg. Infect. Dis.* **13** 608–10 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2725965&tool=pmcentrez&rendertype=abstract

Greene B M 1992 Tissue Nematodes *Infectious Diseases* (W. B. Saunders Company) pp 2008–15

Gyapong J O 2004 2 . 5 MONITORING AND EVALUATION *Am. J. Trop. Med. Hyg.* **71** 20–1

Gyapong J O 2000 Lymphatic of lymphatic filariasis as a public heaith problem in Ghana : from research *Trans. R. Soc. Trop. Med. Hyg.* **94** 599–601

Gyapong J O, Adjei S and Sackey S O 1996a Descriptive epidemiology of lymphatic filariasis in Ghana. *Trans. R. Soc. Trop. Med. Hyg.* **90** 26–30

Gyapong J O, Badu J K, Adjei S and Binka F N 1993 Bancroftian filariasis in the Kasena-Nankana district of the upper east region of Ghana - a preliminary study *J. Trop. Med. Hyg.* **96** 317–22

Gyapong J O, Gyapong M and Adjei S 1996b Epidemiology of acute adenolymphangitis (ADL) due to lymphatic filariasis in northern Ghana *Am. J. Trop. Med. Hyg.* **54** 591–5

Gyapong J O, Gyapong M, Evans D B, Aikins M K and Adjei S 1996c The economic burden of lymphatic filariasis in northern Ghana *Ann. Trop. Med. Parasitol.* **90** 39–48

Gyapong J O, Magnussen P and Binka F N 1994 Parasitological and clinical aspects of Bancroftian filariasis in the Kasena Nankana District, Upper East Region, Ghana *Trans. R. Soc. Trop. Med. Hyg.* **88** 555–7

Gyapong J O, Omane-Badu K and Webber R H 1998a Evaluation circulating of the filter paper blood collection antigen in bancroftian filariasis method for detecting *Trans. R. Soc. Trop. Med. Hyg.* **92** 407–10

Gyapong J O, Webbed R H, Morris J and Bennett S 1998b Prevalence of hydrocele as a rapid diagnostic index for lymphatic filariasis *Trans. R. Soc. Trop. Med. Hyg.* **92** 40–3

Gyapong M, Gyapong J O, Adjei S, Vlassoff C and Wiess M 1996d Filariasis in northern Ghana: some cultural beliefs and practices and their implications for the disease control *Soc. Sci. Med.* **43** 235–42

Gyapong M, Gyapong J, Weiss M and Tanner M 2000 The burden of hydrocele on men in Northern Ghana. *Acta Trop.* **77** 287–94 Online: http://www.ncbi.nlm.nih.gov /pubmed/11114391

Highton R B 1983 Taxanomic keys for the identification of the Afrotropical mosquitoes *Taxanomic keys for the identification of the Afrotropical mosquitoes* pp 1–82

Hotez P J, Molyneux D H, Fenwick A, Kumaresan J, Sachs S E, Sachs J D and Savioli L 2007 Control of Neglected Tropical Diseases *N. Engl. J. Med.* 1018–27

Hotez P J, Remme J H F, Buss P, Alleyne G, Morel C and Breman J G 2004 Combating tropical infectious diseases: report of the Disease Control Priorities in Developing Countries Project. *Clin. Infect. Dis.* **38** 871–8 Online: http://www.ncbi.nlm.nih.gov /pubmed/14999633

Huang Y-M 2004 The subgenus Stegomyia of Aedes in the Afrotropical Region with keys to the species (Diptera: Culicidae) *Zootaxa 700* (Auckland: Mongolia Press) pp 1–120 Online: http://www.mapress.com/zootaxa/

Itoh M, Gunawardena N K, Xu-Guang Q, Weerasoriya M V. and Kimuura E 1998 The use of whole blood absorbed on filter paper to detect Wuchereria bancrofti circulating antigen. *Trans. R. Soc. Trop. Med. Hyg.* **92** 513– 515

Kent R, Juliusson L, Weissmann M, Evans S and Komar N 2009 Seasonal Blood-Feeding Behavior of Culex tarsalis (Diptera : Culicidae) in Weld County , Colorado , 2007 Seasonal Blood-Feeding Behavior of Culex tarsalis (Diptera : Culicidae) in Weld County , Colorado , 2007 *J. Med. Entomol.* **46** 380–90

Koroma J B, Bangura M M, Hodges M H, Bah M S, Zhang Y and Bockarie M J 2012 Lymphatic filariasis mapping by immunochromatographic test cards and baseline microfilaria survey prior to mass drug administration in Sierra Leone. *Parasit. Vectors* **5** 10 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi? artid=3268710&tool=pmcentrez&rendertype=abstract

Kumari K A, Harichandrakumar K T, Krishnamoorthy K and Das L K 2010 The stigmata and discrimination experienced, in southern India, by cases of lymphatic filariasis. *Ann. Trop. Med. Parasitol.* **104** 421–6 Online: http://www.ncbi.nlm .nih.gov/pubmed/20819310

Kwansa-Bentum B 2005 *Vector Competence of the Anopheles (Diptera: Culicidae) Population for Wuchereria bancrofti (Spirurida: Filariidae) After Mass Drug Administration in the Gomoa District of Ghana* (University of Ghana)

Laird M 1988 *The Natural History of Larval Mosquito Habitats* (London: Academic Press)

Laney S J, Ramzy R M R, Helmy H H, Farid H A, Ashour A A, Weil G J and Williams S A 2010 Detection of Wuchereria bancrofti L3 Larvae in Mosquitoes: A Reverse Transcriptase PCR Assay Evaluating Infection and Infectivity ed E Ghedin *PLoS Negl. Trop. Dis.* **4** e602 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2821903&tool=pmcentrez&rendertype=abstract

Magayuka S A and White G B 1972 Hybrid compatibilities and susceptibility of Culex pipiens fatigans Wied. to Wuchereria bancrofti (Cobbold) in East Africa. *Bull. World Health Organ.* **46** 801–5 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2480890&tool=pmcentrez&rendertype=abstract

Mak J W 1989 Recent advances in the laboratory diagnosis of filariasis. *Malays. J. Pathol.* **11** 1–5

McMahon J E and Simonsen P E 1996 Filariases *Manson's Tropical Diseases* pp 1321–65

Medici D De, Croci L, Delibato E, Pasquale S Di, Filetici E and Toti L 2003 Evaluation of DNA Extraction Methods for Use in Combination with SYBR Green I Real-Time PCR To Detect Salmonella enterica Serotype Enteritidis in Poultry *Appl. Environ. Microbiol.* **69** 3456–61

Mehlhorn H 1988 Parasitology in Focus: Facts and Trends © *Springer-Verlag Berlin Heidelberg 1988* (Springer, 1988) pp 95–6, 117

Melrose W D 2004 Lymphatic filiriasis: A review 1862-2002 *Killamey-Australia; Warwick Educational Publishing Inc.*

MMWR M and M W R 2011 *Renewed Transmission of Dracunculiasis --- Chad, 2010* vol 60

Mohammed K a, Deb R M, Stanton M C and Molyneux D H 2012 Soil transmitted helminths and scabies in Zanzibar, Tanzania following mass drug administration for lymphatic filariasis--a rapid assessment methodology to assess impact. *Parasit. Vectors* **5** 299 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3543323&tool=pmcentrez&rendertype=abstract

Mohammed K a, Molyneux D H, Albonico M and Rio F 2006 Progress towards eliminating lymphatic filariasis in Zanzibar: a model programme. *Trends Parasitol.* **22** 340–4 Online: http://www.ncbi.nlm.nih.gov/pubmed/16713740

Mohan H, Bisht B, Goel P and Garg G 2012 Vulval elephantiasis: a case report *Infect. Dis. (Auckl).* 1–3 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi ?artid=3502772&tool=pmcentrez&rendertype=abstract

Molyneux D 2003 Lymphatic Filariasis (Elephantiasis) Elimination: A public health success and development opportunity. *Filaria J.* **2** 13 Online: http://www .pubmedcentral.nih.gov/articlerender.fcgi?artid=200964&tool=pmcentrez&rendert ype=abstract

Molyneux D H and Taylor M J 2001 Current status and future prospects of the Global Lymphatic Filariasis Programme. *Curr. Opin. Infect. Dis.* **14** 155–9 Online: http://www.scopus.com/inward/record.url?eid=2-s2.0-0035086186&partnerID=40 &md5=49d8ad5039d1846df60174e9def76808

Mwandawiro C S, Fujimaki Y, Mitsui Y and Katsivo M 1997 Mosquito vectors of bancroftian filariasis in kwale district, kenya *East Afr. Med. J.* **74** 288–93

Nwoke B E B, Nwoke E A, Ukaga C N and Nwachukwu M I 2010 Epidemiological characteristics of Bancroftian filariasis and the Nigerian environment *J. Public Heal. Epidemiol.* **2** 113–7

Omar M S, Sheikha A K, Al-amari O M, Abdalla S E and Musa R A 2000 RESEARCH NOTE FIELD EVALUATION OF TWO DIAGNOSTIC ANTIGEN TESTS FOR WUCHERERIA BANCROFTI INFECTION AMONG INDIAN *Southeast Asian J. Trop. Med. Public Heal.* **31** 415–8

Onapa A W, Pedersen E M, Reimert C M and Simonsen P E 2007 A role for Mansonia uniformis mosquitoes in the transmission of lymphatic filariasis in Uganda? *Acta Trop.* **101** 159–68 Online: http://www.ncbi.nlm.nih.gov/pubmed/17316535

Ottesen E A, Duke B O L, Karam M and Behbehani K 1997 Strategies and tools for the control / elimination of lymphatic filariasis *Bull. World Health Organ.* **75** 491–503

Ottesen E A, Hooper P J, Bradley M and Biswas G 2008 The global programme to eliminate lymphatic filariasis: health impact after 8 years. ed N De Silva *PLoS Negl. Trop. Dis.* **2** e317 Online: http://www.pubmedcentral.nih.gov/articlerender .fcgi?artid=2556399&tool=pmcentrez&rendertype=abstract

Palumbo E 2008 Filariasis: diagnosis, treatment and prevention. *Acta Biomed.* **79** 106–9 Online: http://www.ncbi.nlm.nih.gov/pubmed/18788504

Pani S P, Hoti S L, Elango A, Yuvaraj J, Lall R and Ramaiah K D 2000 Evaluation of the ICT whole blood antigen card test to detect infection due to nocturnally periodic Wuchereria bancrofti in *Trop. Med. Int. Heal.* **5** 359–63

Pedersen E M, Stolk W A, Laney S J and Michael E 2009 The role of monitoring mosquito infection in the Global Programme to Eliminate Lymphatic Filariasis. *Trends Parasitol.* **25** 319–27 Online: http://www.ncbi.nlm.nih.gov/pubmed/19559649

Pichon G 2002 Limitation and facilitation in the vectors and other aspects of the dynamics of filarial transmission: the need for vector control against Anopheles-transmitted filariasis. *Ann. Trop. Med. Parasitol.* **96 Suppl 2** S143–S152 Online: http://www.ncbi.nlm.nih.gov/pubmed/12625927

Pichon G, Perault G and Laigret J 1974 Redement parasitaire chez les vecteurs de filarioses *Bull. World Health Organ.* **51** 517–24

Pratt H D and Moore C G 1993 *Mosquitoes of Public Health Importance and their Control* (U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, Public Health Practice Program Office, Atlanta, Georgia 30333)

Queipo-Ortuño M I, De Dios Colmenero J, Macias M, Bravo M J and Morata P 2008 Preparation of bacterial DNA template by boiling and effect of immunoglobulin G as an inhibitor in real-time PCR for serum samples from patients with brucellosis. *Clin. Vaccine Immunol.* **15** 293–6 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2238042&tool=pmcentrez&rendertype=abstract

Rao R U, Atkinson L J, Ramzy R M R, Helmy H, Farid H A, Bockarie M J, Susapu M, Laney S J, Williams S A and Weil G J 2006 A REAL-TIME PCR-BASED ASSAY FOR DETECTION OF WUCHERERIA BANCROFTI DNA IN BLOOD AND MOSQUITOES *Am. J. Trop. Med. Hyg.* **74** 826–32

Rebollar-Tellez E A 2005 Human body odor, mosquito bites and the risk of disease transmission *Folia Entomol. Mex.* **44** 247–65

Richens J 2004 Genital manifestations of tropical diseases *Sex. Transm. Infect.* **80** 12–7 Online: http://discovery.ucl.ac.uk/1185/

Rueda L M 2007 Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in freshwater *Hydrobiologia* **595** 477–87 Online: http://link.springer.com/10.1007/s10750-007-9037-x

Rueda L M, Iwakami M, O'Guinn M, Mogi M, Prendergast. B F, Miyagi I, Toma T, Pecor J E and Wilkerson R C 2005 HABITATS AND DISTRffiUTION OF ANOPHELES SINENSIS AND ASSOCIATED ANOPHELES HYRCANUS GROUP IN JAPAN *J. Am. Mosq. Control Assoc.* **21** 458–63

Rueda L M, Kim H, Klein T A, Pecor J E, Li C, Debboun M and Wilkerson R C 2006 Distribution and larval habitat characteristics of Anopheles Hyrcanus Group and related mosquito species (Diptera : Culicidae) in South Korea *J. Vector Ecol.* **31** 199–206

Sattler M A, Mtasiwa D, Kiama M, Premji Z, Tanner M, Killeen G F and Lengeler C 2005 Habitat characterization and spatial distribution of Anopheles sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. *Malar. J.* **4** 4 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=546229&tool=pmcentrez&rendertype=abstract

Savioli L and Daumerie D 2010 *First WHO report on neglected tropical diseases: working to overcome the global impact of neglected tropical diseases* Online: http://whqlibdoc.who.int/?010/9789241564090_eng.pdf

Scott J A, Brogdon W G and Collins F H 1993 Identification of single specimens of the Anopheles gambiae complex by the polymerase chain reaction. *Am. J. Trop. Med. Hyg.* **49** 520–9 Online: http://europepmc.org/abstract/MED/8214283

Service M W 1980 A guide to medical entomology *Macmillan tropical and sub-tropical medical texts* (London: London: Macmillan) pp 204–10

Shenoy R K, Kumaraswami V, Suma T K, Rajan K and Radhakuttyamma G 1999 A double-blind, placebo-controlled study of the efficacy of oral penicillin, diethylcarbamazine or local treatment of the affected limb in preventing acute adenolymphangitis in lymphoedema caused by brugian filariasis *Ann. Trop. Med. Parasitol.* **93** 367–77 Online: http://www.ingentaconnect.com/content/routledg/catm/1999/00000093/00000004/art00005

Simonsen P E 2008 Helminthic Infections pp 1–38

Simonsen P E, Pedersen E M, Rwegoshora R T, Malecela M N, Derua Y A and Magesa S M 2010 Lymphatic filariasis control in Tanzania: effect of repeated mass drug administration with ivermectin and albendazole on infection and transmission. *PLoS Negl. Trop. Dis.* **4** e696 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2879369&tool=pmcentrez&rendertype=abstract

Sodahlon Y K, Dorkenoo A M, Morgah K, Nabiliou K, Agbo K, Miller R, Datagni M, Seim A and Mathieu E 2013 A success story: Togo is moving toward becoming the first sub-Saharan African nation to eliminate lymphatic filariasis through mass drug administration and countrywide morbidity alleviation. *PLoS Negl. Trop. Dis.* **7** e2080 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3623714&tool=pmcentrez&rendertype=abstract

Soulsby E J L 1982 *Helminths, Arthropods and Protozoa of Domesticated Animals Seventh Edition* (Bailliere Tyndall, London)

Southgate B A and Bryan J H 1992 Factors affecting transmission of Wuchereria bancrofti by anopheline mosquitoes. 4. Facilitation, limitation, proportionality and epidemiological significance *Trans. R. Soc. Trop. Med. Hyg.* **86** 523–30

De Souza D, Kelly-Hope L, Lawson B, Wilson M and Boakye D 2010 Environmental factors associated with the distribution of Anopheles gambiae s.s in Ghana; an important vector of lymphatic filariasis and malaria. *PLoS One* **5** e9927 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2847902&tool=pmcentrez&rendertype=abstract

De Souza D, Koudou B, Kelly-Hope L, Wilson M, Bockarie M and Boakye D 2012 Diversity and transmission competence in lymphatic filariasis vectors in West Africa, and the implications for accelerated elimination of Anopheles-transmitted filariasis *Parasit. Vectors* **5** 1–6 Online: http://dx.doi.org/10.1186/1756-3305-5-259

Subramanian S, Krishnamoorthy K, Ramaiah K D, Habbema J D F, Das P K and Plaisier A P 1998 The relationship between microfilarial load in the human host and uptake and development of Wuchereria bancrofti microfilariae by Culex quinquefasciatus: a study under natural conditions *Parasitology* **116** 243–55

Thompson E G, Hayes D E and Ludlam K W 1963 Notes on the feeding habits of Aedes sollicitans in the Chincoteaque-Assateaque Island area of Virginia. *Mosq. News* **23** 297–8

Tisch D J, Alexander N D E, Kiniboro B, Dagoro H, Siba P M, Bockarie M J, Alpers M P and Kazura J W 2011 Reduction in Acute Filariasis Morbidity during a Mass Drug Administration Trial to Eliminate Lymphatic Filariasis in Papua New Guinea ed H Da Costa Santiago *PLoS Negl. Trop. Dis.* **5** 9 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3134431&tool=pmcentrez&rendertype=abstract

Toumanoff C 1958 Filariose humaine et sa transmission dans la Basse-Guinée (Estuaire du Rio Nunez) *Bull. Soc. Pathol. Exo.* **51** 908–12

Ughasi J, Bekhard H E, Coulibaly M, Adabie-Gomez D, Gyapong J, Appawu M, Wilson M D and Boakye D A 2012 Mansonia africana and Mansonia uniformis are Vectors in the transmission of Wuchereria bancrofti lymphatic filariasis in Ghana. *Parasites vectors* **5** 89 Online: http://www.ncbi.nlm.nih.gov/pubmed/22564488

Ughasi J C, Berkard H, Gomez D, Appawu M, Wilson M D and Boakye D A 2010 Mansonia species are potential vectors of lymphatic filariasis in Ghana. *Am. J. Trop. Med. Hyg.* **83** 302

Urquhart G M, Armour J, Duncan J L, Dunn A M and Jennings F W 1987 *Veterinary Parasitology* (Longman Scientific & Technical, Harlow, Essex, England.)

Webber R H 1991 Can anopheline transmitted filariasis be eradicated? *J. Trop. Med. Hyg.* **94** 241–4

Weil G J, Kastens W, Susapu M, Laney S J, Williams S A, King C L, Kazura J W and Bockarie M J 2008 The Impact of Repeated Rounds of Mass Drug Administration with Diethylcarbamazine Plus Albendazole on Bancroftian Filariasis in Papua New Guinea ed P J Lammie *PLoS Negl. Trop. Dis.* **2** 7 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2586652&tool=pmcentrez&rendertype=abstract

Weil G J, Lammie P J and Weiss N 1997 The ICT Filariasis Test: a Rapid-Format Antigen Test for Diagnosis of Bancroftian Filariasis. *Parasitol. Today* **13** 401–4

WHO R O for S-E A 2010 *The Regional Strategic Plan for Elimination of Lymphatic Filariasis 2010-2015*

WHO/HTM/NTD/PCT/2011.4 2011 Monitoring and epidemiological assessment of Mass Drug Administration in the global programme to eliminate lymphatic filariasis: a manual for national elimination programmes *WHO Report2*

WHO/HTM/NTD/PCT/2011.4 2012 *Transmission assessment surveys in the Global Programmed to Eliminate Lymphatic Filariasis*

Wijesinghe, R. S. Wickremasinghe A R 2012 Physical, Psychological, and Social Aspects of Quality of Life in Filarial Lymphedema Patients in Colombo, Sri Lanka *Asia Pac J Public Heal.*

Wilensky A O and Samuels S S 1924 Acute Deferentitis And funiculitis *Ann. Surg.* **78** 785–94

Williams M R and Savage H M 2009 Identification of Culex (Melanoconion) Species of the United States Using Female Cibarial Armature (Diptera : Culicidae) Identification of Culex (Melanoconion) Species of the United States Using Female Cibarial Armature (Diptera : Culicidae) *J. Med. Entomol.* **46** 745–52

Zeldenryk L M, Gray M, Speare R, Gordon S and Melrose W 2011 The emerging story of disability associated with lymphatic filariasis: a critical review. *PLoS Negl. Trop. Dis.* **5** e1366 Online: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3246437&tool=pmcentrez&rendertype=abstract

Zhong M, McCarthy J, Bierwert L, Lizotte-Waniewski M, Nutman T B, Ottesen E A and Williams S A 1996 A POLYMERASE CHAIN REACTION ASSAY FOR DETECTION OF THE PARASITE WUCHERERIA BANCROFTI IN HUMAN BLOOD SAMPLES hasmanydispersed repeat DNA families , each Isolation of DNA from parasites and construction of a *Am. J. Trop. Med. Hyg.* **54** 357–63

Zhou L, Lawrence G G, Vineis J H, Mcallister J C, Wirtz R A, Brogdon W G and Allister J C M C 2009 Detection of Broadly Distributed Sodium Channel Alleles Characteristic of Insect Pyrethroid Resistance in West Nile Virus Vector Culex pipiens Complex Mosquitoes in the United States Detection of Broadly Distributed Sodium Channel Alleles Characteristic o *J. Med. Entomol.* **46** 321–7

Zimmerman R H, Galardo A K R, Lounibos L P, Arruda M & and Writz R 2009 Bloodmeal Hosts of Anopheles Species (Diptera: Culicidae) in a Malaria-Endemic Area of the Brazillian Amazon *J. Med. Entomol.* **43**

APPENDICES

Appendix 1

Table 1: Sizes of the DNA fragment

DNA fragment sizes after PCR-RFLP for the different species of *Anopheles gambiae* complex and for M and S form of the *An. gambiae s.s.* The 23 bp fragment is due to the presence of a restriction site (GCG^C) at position 469 of the rDNA sequenced in Scott *et al.* (1993) in all taxa except *An. merus*. The 6 bp fragments in *An. quadriannulatus*, and *An. melas* and the 29 bp fragments in *An. merus* are due to the presence of a second restriction site at position 475 in these species. The 257 and 110bp fragment in *An. gambiae* S form are due to the presence of a restriction site at position 580.

Species	PCR product sizes (bp)	Fragment Lengths (bp) after digestion with *HhaI*		
Anopheles arabiensis Patton	315	292	23	
Anopheles quadriannulatus Theobald	153	124	6	23
Anopheles melas Theobald	464	435	6	23
Anopheles merus Dönitz	466	437	29	
Anopheles gambiae S form	390	257	110	23
Anopheles gambiae M form	390	367	23	

Source: Fanello *et al.* (2002).

Table 2: Total number of mosquitoes caught in the study areas

	Dec-11	Sep-12	Oct-12	Nov-12	Jan-13	Total/spp.
***An. gambiae* s.l.**	134	28	68	152	80	**462**
***An. funestus* s.l.**	0	1	2	3	3	**9**
An. pharoensis	0	0	0	0	1	**1**
Aedes species	0	0	0	0	0	**0**
Cx. species	11	16	4	4	22	**57**
Ma. species	2	4	7	6	2	**21**
Total/year	**147**	**49**	**81**	**165**	**108**	**550**

Table 3: Species identification of *An. gambiae* s. l. and molecular forms

	Molecular Identification			Molecular Forms	
	An. arabiensis	***An. gambiae s.s***	***An. melas***	**M**	***S***
Ayensuako	1	99	0	4	58
Gyahadze	0	94	5	4	2
Mankrong	0	29	3	0	7
Total	**1**	**222**	**8**	**8**	**67**

Table 4: Biting rate estimation

	Number of Mosquitoes	Number of Sleepers	Number of Catch Nights	Biting Rate (BR)	MBR	ABR
Ayensuako	274	163	5	0.3	10.1	122.7
Gyahadze	176	147	5	0.2	7.2	87.4
Mankrong	100	143	5	0.1	4.2	51.0

Table 5: Estimation of Entomological Indices

	No. of Mosquitoes	Microscopy				Worm Load	Infectivity Rate	BR	IBR	TP	ATP
		Mf	L_1	L_2	L_3						
Ayensuako	274	0	0	0	0	0	0	0.3	0	0	0
Gyahadze	176	0	0	0	0	0	0	0.2	0	0	0
Mankrong	100	0	0	0	0	0	0	0.1	0	0	0

Table 6: Pool screening from *An. gambiae* s. l. *An. funestus, Culex spp.* and *Mansonia spp.*

	An. gambiae* s. l.		***An. funestus***		***Culex spp.***		***Mansonia spp.***	
	No. of Pools	**No. of mosquitoes in Pool(s)**	**No. of Pools**	**No. of mosquitoes in Pool(s)**	**No. of Pools**	**No. of mosquitoes in Pool(s)**	**No. of Pools**	**No. No. of mosquitoes in Pool(s)**
Ayensuako*	11	25	1	3	1	10	0	0
Gyahadze	5	25, 26	1	2	2	17, 18	3	2, 3, 6
Mankrong	3	25, 28	1	5	1	7	1	11

Notice

There was one positive pool revealed in the samples collected from Ayensuako in the *An. gambiae* s. l. population.

Key for Table 6

* = positive filarial parasites revealed

Appendix 2

Table 7: RT-PCR Results

Document Name: Joe-26-07-2013
Plate Type: Standard Curve
User: Administrator

Document Information

Operator: Administrator
Run Date: Friday July 26 2013 10:46:48
Last Modified:
Friday July 26 2013 12:09:35
Instrument Type: Applied Biosystems 7300 Real-Time PCR System

Comments:
SDS v1.4

Thermal Cycler Profile

Stage	Repetitions	Temperature	Time	Ramp Rate	Auto Increment
1	1	50.0 °C	2:00	Auto	
			10:0		
2	1	95.0 °C	0	Auto	
3	40	95.0 °C	0:15	Auto	
		60.0 °C	1:00	Auto	

Standard 7300 Mode
Data Collection : Stage 3 Step 2
PCR Volume: 25 µL

Well	Sample Name	Detector	Task	Ct	StdDev Ct
A1	Mos1	wbldr1	Unknown	Undetermined	
A2	Mos1	wbldr1	Unknown	Undetermined	
A3	Mos2	wbldr1	Unknown	Undetermined	
A4	Mos2	wbldr1	Unknown	Undetermined	
A5	Mos3	wbldr1	Unknown	Undetermined	
A6	Mos3	wbldr1	Unknown	Undetermined	
A7	Mos4	wbldr1	Unknown	Undetermined	
A8	Mos4	wbldr1	Unknown	Undetermined	
A9	Mos5	wbldr1	Unknown	Undetermined	
A10	Mos5	wbldr1	Unknown	Undetermined	
A11	Mos6	wbldr1	Unknown	Undetermined	
A12	Mos6	wbldr1	Unknown	Undetermined	

B1	Mos7	wbldr1	Unknown	Undetermined	
B2	Mos7	wbldr1	Unknown	Undetermined	
B3	Mos8	wbldr1	Unknown	Undetermined	
B4	Mos8	wbldr1	Unknown	38.8196	
B5	Mos9	wbldr1	Unknown	39.8755	0.703
B6	Mos9	wbldr1	Unknown	38.8809	0.703
B7	Mos10	wbldr1	Unknown	Undetermined	
B8	Mos10	wbldr1	Unknown	Undetermined	
B9	Mos11	wbldr1	Unknown	Undetermined	
B10	Mos11	wbldr1	Unknown	Undetermined	
B11	Mos12	wbldr1	Unknown	Undetermined	
B12	Mos12	wbldr1	Unknown	Undetermined	
C1	Mos13	wbldr1	Unknown	Undetermined	
C2	Mos13	wbldr1	Unknown	Undetermined	
C3	Mos14	wbldr1	Unknown	Undetermined	
C4	Mos14	wbldr1	Unknown	Undetermined	
C5	Mos15	wbldr1	Unknown	Undetermined	
C6	Mos15	wbldr1	Unknown	Undetermined	
C7	Mos16	wbldr1	Unknown	Undetermined	
C8	Mos16	wbldr1	Unknown	Undetermined	
C9	Mos17	wbldr1	Unknown	Undetermined	
C10	Mos17	wbldr1	Unknown	Undetermined	
C11	Mos18	wbldr1	Unknown	Undetermined	
C12	Mos18	wbldr1	Unknown	Undetermined	
D1	Mos19	wbldr1	Unknown	Undetermined	
D2	Mos19	wbldr1	Unknown	Undetermined	
D3	Mos20	wbldr1	Unknown	Undetermined	
D4	Mos20	wbldr1	Unknown	Undetermined	
D5	Mos21	wbldr1	Unknown	Undetermined	
D6	Mos21	wbldr1	Unknown	Undetermined	
D7	Mos22	wbldr1	Unknown	Undetermined	
D8	Mos22	wbldr1	Unknown	Undetermined	
D9	Mos23	wbldr1	Unknown	Undetermined	
D10	Mos23	wbldr1	Unknown	Undetermined	
D11	Mos24	wbldr1	Unknown	Undetermined	
D12	Mos24	wbldr1	Unknown	Undetermined	
E1	Mos25	wbldr1	Unknown	Undetermined	
E2	Mos25	wbldr1	Unknown	Undetermined	
E3	Mos26	wbldr1	Unknown	Undetermined	
E4	Mos26	wbldr1	Unknown	Undetermined	
E5	Mos27	wbldr1	Unknown	Undetermined	

E6	Mos27	wbldr1	Unknown	Undetermined	
E7	Mos28	wbldr1	Unknown	Undetermined	
E8	Mos28	wbldr1	Unknown	Undetermined	
E9	Mos29	wbldr1	Unknown	Undetermined	
E10	Mos29	wbldr1	Unknown	Undetermined	
E11	Mos30	wbldr1	Unknown	Undetermined	
E12	Mos30	wbldr1	Unknown	Undetermined	
H7	PTC	wbldr1	Unknown	28.7496	0.0267
H8	PTC	wbldr1	Unknown	28.7119	0.0267
H11		wbldr1	NTC	Undetermined	
H12		wbldr1	NTC	Undetermined	

Table 8: Cibarial teeth observed in *Aedes, Culex* and *Mansonia* mosquitoes

Sp. ID	Cibarial Teeth	Sp. ID	Cibarial Teeth	Sp. ID	Cibarial Teeth
Ma. uni 4	0	*Cx. Sp.*	24	*Ae. aeg 7*	0
Ma. uni 5	0	*Cx. Sp.*	26	*Ae. vit 5*	0
Ma. uni 6	0	*Cx. Sp.*	28		
Ma. uni 7	0	*Cx. sp* 19	26		
Ma. af 4	0	*Cx. sp* 26	20		
Ma. af 5	0	*Cx. sp 32*	34		
Ma. af 6	0				
Ma. af 7	0				