EVALUATING THE PREVALENCE AND TRANSMISSION OF LYMPHATIC FILARIASIS IN TWO RURAL COMMUNITIES IN THE WESTERN REGION AFTER TEN ROUNDS OF MASS DRUG ADMINISTRATION

BY

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DECLARATION

This is to certify that this thesis is the result of research undertaken by John Nartey Kanamitie towards the award of MPhil Zoology, from the University of Ghana.

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DEDICATION

This work is dedicated to my mum, Miss Elizabeth Akweley Lawer and my supervisors who challenged and inspired me to start and complete this valuable project.

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LIST OF ABBREVIATIONS

ABR	Annual biting rate
ADL	Acute adeno-lymphangitis
ADLA	Acute dermato-lymphagioadenitis
AFL	Acute filarial lymphangitis
ATP	Annual transmission potential
bp	base pair
BR	Biting rate
CC	Counting chamber
CDC	Centers for Disease Control and Prevention
CFA	Circulating filarial antigen
DEC	Diethylcarbamazine citrate
dNTP	deoxynucleotide triphosphate
DNA	deoxyribonucleic acid
EDTA	Disodium ethylene diamine tetraacetate
ELISA	Enzyme linked immunosorbent assay
GBD	Global Burden of Disease
GFEP	Ghana Filariasis Elimination Programme
GPELF	Global Programme for Elimination of Lymphatic Filariasis

IBR	Infective biting rate
ICT	Immunochromatographic test
LAMP	Loop-mediated isothermal amplification
LF	Lymphatic filariasis
M	Molar
MBR	Monthly biting rate
MDA	Mass drug administration
mf	microfilaria
mg	milligram
ml	millilitre
mM	millimolar
NTD	Neglected Tropical Disease
NTDCP	Neglected Tropical Diseases Control Programme
PCR	Polymerase chain reaction
PSC	pyrethrum spray catches
rDNA	ribosomal DNA
rpm	revolution per minute
s.l	sensu lato
s.s	sensu stricto

TAE	Tris-Acetate EDTA
TAS	Transmission assessment surveys
TP	Transmission potential
TPE	Tropical pulmonary eosinophilia
TS	Thick smear
µM	micromolar
UV	Ultra violet
WHA	World Health Assembly
WHO	World Health Organization

ABSTRACT

Control of lymphatic filariasis (LF) in Ghana is based on annual mass drug administration (MDA) with a combination of ivermectin and albendazole, in order to halt transmission. It is expected that 5-6 rounds of MDA should result in the interruption of transmission. This research present findings from a study in two endemic rural communities in Western Ghana, after ten rounds of MDA. The study was conducted in Agona Princess and Akonu in the Ahanta West District and Nzema East Municipality respectively. Both Districts commenced MDA in 2000 and 2001 respectively.

The effect of treatment on human infection and mosquito transmission was monitored in the communities from December 2014 to June 2015. The screening tool used in this study was CFA-testing with ICT cards followed by examination of only CFA positive individuals for mf using the counting chamber (CC) and thick blood smear (TS) techniques. Mosquitoes were also sampled using the pyrethrum spray collections (PSC) and investigated for *Wuchereria bancrofti* infection using dissection and molecular analysis.

During the December parasitological survey, 370 subjects were examined. 13% were positive for circulating filarial antigens (CFA). The CC and TS detected 4% of the population with microfilariae (mf) in the blood, with an intensity range of 1-226 mf/ml and 1-50 mf/ml respectively. Three months (March, 2015) after treatment of individuals with *W. bancrofti* infections, the parasitological indices decreased considerably to 5% (for CFA), 1% (CC) and 0.3% (TS) with respective mf intensity range of 1-11 mf/ml (CC) and 1 mf/ml (TS). The June 2015 parasitological survey revealed an increase in antigenaemia prevalence (7.6%) while the mf prevalence remained considerably the same. In assessing transmission indices, out of the 132

mosquitoes collected, 125 (94.7%) were *Anopheles gambiae* s.l. Molecular studies identified all anophelines as *An. melas.* One *An. melas* was found infected with three L_2s of *W. bancrofti*, which was confirmed by PCR analysis. There was no recovery of infective stage larva of *W. bancrofti* in the mosquito samples.

In this study, a decline in mf prevalence and intensity was observed after treatment. However, the remaining positive individuals represent a reservoir for the continued transmission of the disease. This calls for the need for bi-annual treatments instead of the current once-a-year treatment. Further, questions have been raised about the possible development of drug resistance following several years of treatment. A continued monitoring for parasite repopulation rates and drug resistance markers would help establish the basis for resistance to ivermectin and albendozole in the study areas.

CHAPTER ONE

INTRODUCTION

1.1.Background

Lymphatic filariasis (LF) is a neglected tropical disease caused by lymphatic lodging filarial parasites; *Wuchereria bancrofti*, *Brugia malayi* or *Brugia timori*. It is painful and profoundly disfiguring disease representing an important economic burden to many developing countries (WHO, 2014). The disease is the leading cause of physical disability and the second leading cause of chronic disability in the world (Zeldenryk *et al.*, 2011; CDC, 2013). The disease is also considered as the second most common vector-borne parasitic disease after malaria (Wynd *et al.*, 2007).

Lymphatic filariasis is prevalent in the tropical and subtropical areas of Asia, Africa, the Western Pacific and some parts of the Americas (Michael *et al*., 1996). The disease causes both acute and chronic morbidity and affects persons of all ages and both sexes (Ottesen *et al.*, 1997). Reports have estimated that at least 120 million people are infected with these filarial parasites in 73 countries and that over 40 million people have apparent lymphatic pathology (Michael *et al.*, 1996; WHO, 2014). The parasitic worms are transmitted through the bites of infective female mosquito vectors of various genera including *Anopheles*, *Culex*, *Mansonia* and *Aedes*, depending on the geographic region. *Coquilletidia* and *Ochlerotatus* have also been reported to be vectors of the LF parasites (de Souza *et al.*, 2012). In West Africa, *Anopheles* mosquitoes are the major vectors of LF (Appawu *et al.*, 2001) with the *An. gambiae* and *An. funestus* complexes being the principal vectors in Ghana (Dunyo *et al.*, 1996; Dzodzomenyo *et al.*, 1999). Studies have concluded that *Culex* spp. are refractory to *W. bancrofti* in Ghana and other parts of West Africa (Zielke & Kuhlow, 1977; Aboagye-Antwi, 2003) but not in East Africa (Magayuka and White 1972; Mwandawiro *et al*., 1997).

Millions of individuals with the infection currently suffer from one or more of the apparent clinical manifestations of the disease. These clinical manifestations include lymphoedema or elephantiasis of limbs, hydrocoele, chyluria, tropical pulmonary eosinophilia (TPE), adenopathy, haematuria and frequent infections associated with damaged lymphatic system (Ottesen *et al.*, 1997).

Several decades of research have identified LF as a disease that can be eliminated due to its biological nature. Firstly, it is a disease that exclusively affects humans. Secondly, the microfilaria (mf) is unable to multiply its numbers in the vector and finally, the mechanism of transmission is relatively inefficient (Ottesen, 2000).

As such, there is a major global effort to eliminate LF as a public health problem. This effort is based on several research works which hypothesized that reduction of circulating microfilariae in the human host would halt transmission through the mosquito vectors of *W. bancrofti*. The International Task Force for Disease Eradication also identified LF as one of the potentially eradicable, infectious diseases (CDC, 1993). Realising the possibilities of elimination of the disease, the World Health Assembly passed a resolution (WHA50.29) in 1997 which requested member states to initiate activities to eliminate LF. This led to the formation of the Global Programme to Eliminate Lymphatic Filariasis (GPELF), launched by World Health Organization (WHO) in 2000 with the goal to eliminate LF as a public health problem by 2020 (Ottesen, 2000; WHO 2014). The programme has two principal objectives in achieving the goal. The first is interruption of the transmission of LF through chemotherapy to all at risk population. Secondly, the programme is geared up to manage morbidity and prevent disability among individuals affected by the disease (WHO, 2013).

To interrupt transmission, the strategy is based on Mass Drug Administration (MDA), where LF endemic districts are mapped and treated through annual administration of single doses of two drug-regimens; 6 mg/kg of body weight diethylcarbamazine citrate (DEC) +

400 mg albendazole or 150 µg/kg of body weight ivermectin + 400 mg albendazole. (Ottesen, 2000; WHO, 2014).

To manage morbidity and decrease the disability caused by LF, the principal strategy focuses on decreasing secondary bacterial and fungal infection of limbs and genitals whose lymphatic function has already been compromised by filarial infection, since secondary infection is the primary pathogenic determinant of worsening lymphoedema and elephantiasis (Ottesen, 2000).

The GPELF strategies to interrupt transmission rely on the hypothesis that Anophelines are incompetent vectors at low density microfilaraemia. A competent vector is capable of ingesting mf from an infected person and supports the development of the parasite to the infective stage and afterwards transmitting them to other uninfected persons (Cran, 1973; McGreevy *et al.*, 1982). Low-density microfilaraemia is the situation where the density of circulating mf is less than 200 mf per ml of capillary blood or 30 mf per ml of venous blood in an LF endemic area (Southgate, 1992). The idea supporting this approach is based on results of research on vector-parasite relationships that determine whether mosquito vectors will be effective in ingesting and transmitting infection at low parasitaemia levels (Gyapong *et al.*, 2005). These vector-parasite relationships described are "Facilitation" and "Limitation".

The phenomenon of "facilitation" refers to a process where ingested circulating mf below a certain threshold, called the Webbers Critical Point, would not sustain the transmission of LF by *Anopheles* vectors (Southgate and Bryan 1992, Pichon *et al.*, 1974, Pichon, 2002, Webber, 1991). "Limitation" describes a process where there is a stable transmission of LF even where there is low microfilaraemia. This is exhibited by culicines (Subramanian *et al.*, 1998; Duerr *et al.*, 2005). The fact that Anopheline vectors demonstrate the phenomenon of facilitation makes the GPELF commendable. This is so because in West Africa, *Anopheles*

mosquitoes are the important vectors of LF (Appawu *et al.* 2001). However, if culicines are involved in the transmission cycle, it would be difficult to eliminate the disease through chemotherapy alone. This is because culicines are capable at ingesting and sustaining the development of low-density mf.

In Ghana, some studies have indicated a probable 'limitation' in some *Anopheles* vectors particularly *An. melas* (Amuzu *et al*., 2010). This proposes that most likely not all Anopheline vectors demonstrate facilitation in their transmission of LF thus keeping a detectable transmission after several rounds of MDA.

1.2. Rationale of study

In Ghana, the current control programme adopts MDA using albendazole and ivermectin. There has been an important question of when to discontinue MDA in LF endemic areas. After at least five (5) rounds of MDA, it is expected that mf should reach a minimum threshold in the human population such that the vectors (predominantly *An. gambiae* in Ghana) may not be able to sustain the transmission of LF (WHO, 2010).

However, a study conducted by Boakye *et al*. (2004) in the Bongo district of the Upper East Region of Ghana indicates a possible "limitation" in *An. gambiae* s.l. and/or *An. funestus* in the transmission of the *W. bancrofti* contrary to other studies (Southgate, 1992). Further, results from a study conducted by Amuzu *et al.* (2010) in two endemic communities at the Gomoa district in the Central Region of Ghana implicated *An. melas*, as a competent vector even at low-parasitaemia levels. Thus, *An. melas* was exhibiting 'limitation' in the transmission of LF. Ughasi *et al*. (2012) also detected the L_3 stage of *W. bancrofti* in samples of *Mansonia* mosquitoes collected in the Gomoa and Komenda-Edina-Eguafo-Abirem (KEEA) districts, both in the Central Region of Ghana. This suggests that *Mansonia* mosquitoes becoming competent vectors in Ghana cannot be underestimated. The work

done by Ughasi *et al.* (2012) reinforces an initial research conducted in Guinea which associated *Mansonia* mosquitoes in the transmission cycle of LF (Toumanoff, 1958). This information is a threat to the success of the GPELF exercise in areas where *An. melas* and *Mansonia* species are the principal vectors. The information regarding the varied vector competence in Anopheline vectors is also fundamental to the success of the GPELF programme. This implies that most likely not all Anophelines exhibit facilitation in their transmission of LF.

Evaluation of MDA carried out in 2006, sixth year of the programme, demonstrated reduction of mf prevalence to less than 1% in some endemic sites while others have prevalence not compatible with elimination of transmission due to a number of systematic non-compliers. Another detailed evaluation of the LF programme carried out in 2007 in 15 sites of the five pilot districts (Awutu-Efutu Senya, Ahanta-West, Sissala, Kassena-Nankana and Builsa) in Ghana showed high mf prevalence and density and therefore the need to continue with MDA until the marked improvements in the microfilarial prevalence are attained (Ghana Filariasis Elimination Programme, 2008).

Presently, there had been between 8 to 14 annual rounds of MDA across the country. It is therefore prudent to undertake a parasitological monitoring for human infection as well as entomological surveillance in order to unravel the transmission potential and re-evaluate the LF status in endemic areas in Ghana.

1.3. General objective

The principal objective of this study is to evaluate the effect of 10 annual rounds of MDA on the prevalence of human infection and transmission of LF in two rural endemic communities.

1.4. Specific objectives

The specific objectives are to:

1. Undertake parasitological surveys to assess the effect of 10 annual rounds of chemotherapy on the prevalence of LF in the study communities.
2. Evaluate the effect of MDAs on transmission indices (biting rates and transmission potentials) of vector mosquitoes in the two endemic communities.
3. Investigate the abundance and diversity of LF vectors in the selected communities.
4. Identify *W. bancrofti* parasites in mosquito samples.

CHAPTER TWO

LITERATURE REVIEW

2.1. Lymphatic filariasis: The Disease

In 1995, the World Health Organization (WHO) identified LF as the second leading cause of long-term permanent and chronic disability after mental illness (Addiss, 2010; Ottesen, 1997). Three species of lymphatic lodging filarial parasites, *W. bancrofti*, *B. malayi* and *B. timori*, cause lymphatic filariasis in humans (CDC, 2013; WHO, 2014). *Wuchereria bancrofti* is responsible for about 90% of all LF cases. The remainder of the cases are caused by *B. malayi* while *B. timori* is also known to cause the disease (WHO, 2014). Bancroftian filariasis is sometimes used to refer to infection by *W. bancrofti*, while brugian filariasis refers to infection by the *Brugia* spp. (Simonsen, 2008). The adults of these parasitic nematodes lodge within the lumen of the lymphatic vessels (Nelson, 1979) for an average of 6-8 years, producing millions of immature microfilariae (mf) that circulate in the blood (WHO, 2014).The vectors of the disease are species of mosquitoes belonging to the genera *Anopheles, Culex, Mansonia* and *Aedes*. These genera alongside *Coquilletidia* and *Ochlerotatus* have been associated to be vectors of the LF parasites (de Souza *et al.*, 2012).

2.1.1. Geographical distribution

Lymphatic filariasis, ranked as the second most common vector-borne parasitic disease after malaria, is found in over 80 tropical and subtropical countries (Wynd *et al.*, 2007).

According to WHO, *W. bancrofti* is predominant in areas with hot and humid climate. It is therefore widespread throughout the tropical regions (Figure 1) of Asia, Africa, the Americas and the Pacific. *Brugia malayi* is mainly found in Southeast Asia and in areas of south-west India (Figure 1), but *B. timori* is only restricted to some islands in Indonesia

(Figure 1) (WHO, 1992; Simonsen, 2008). Currently, it is globally known that about 120 million people are infected, with about 40 million disfigured and incapacitated and over 1.4 billion people at risk of infection (WHO, 2014). Globally, an estimated 15 million people are affected by lymphoedema, which includes swelling of the limbs, breasts or genitals, and approximately 25 million men are afflicted with genital swelling, mainly scrotal hydrocoele (Michael *et al.,* 1996; WHO, 2014). Though these clinical features are not often lethal, they lead to the ranking of LF as the second leading cause of permanent and long-term disability (WHO, 1995).

Below are figures 1a and 1b showing the geographical distribution of bancroftian and brugian filariasis case prevalences based on the crude Global Burden of Disease (GBD) estimates.

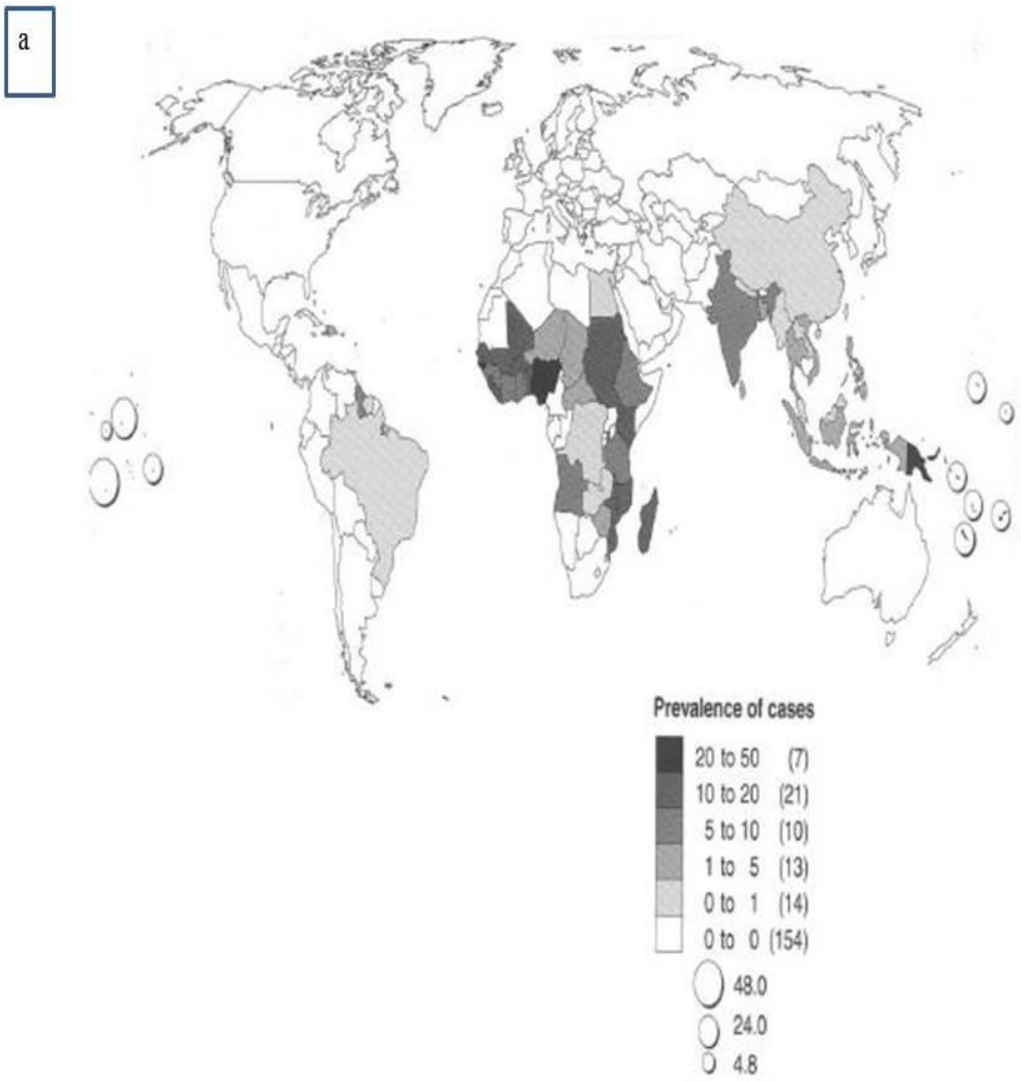

Figure 1a: Geographical distribution of bancroftian filariasis. Circles denote the corresponding prevalences (%) estimated for various Pacific Islands and vary in size proportionately with the prevalence of each island. The figures in brackets indicate the number of countries. Source: Michael and Bundy, 1997.

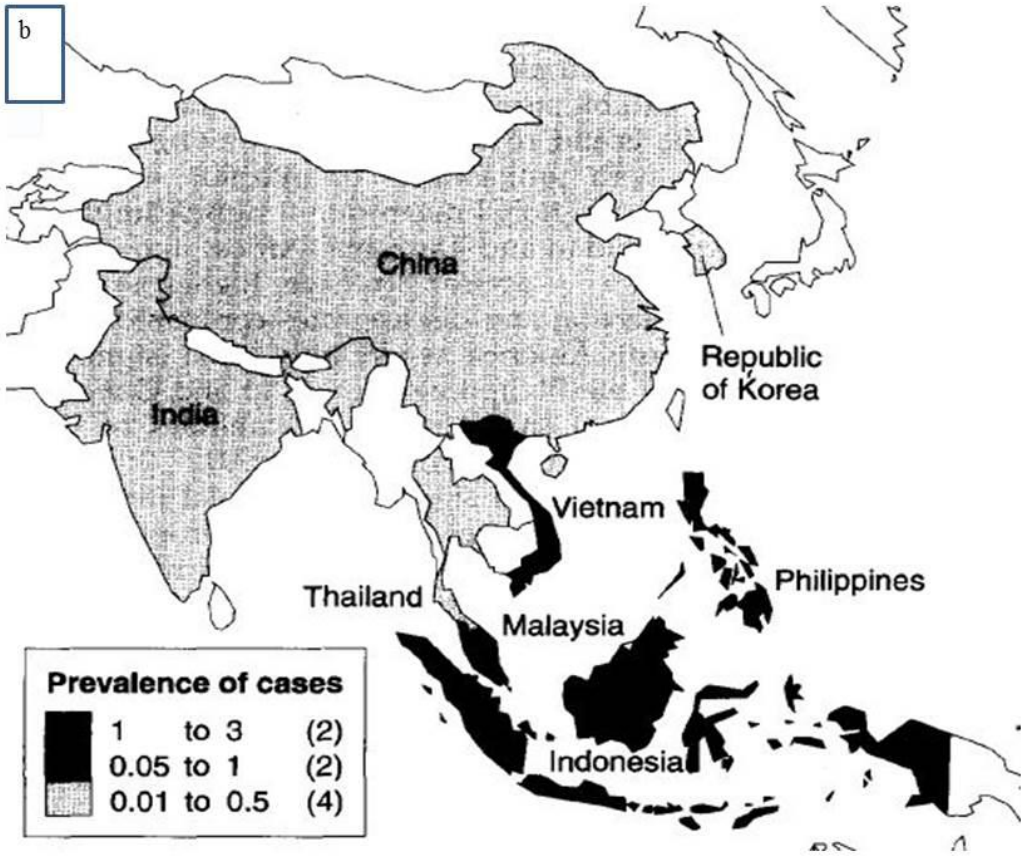

Figure 1b: Geographical distribution of brugian filariasis. The figures in brackets indicate the number of countries. Source: Michael and Bundy, 1997.

2.1.2. Causative agent of lymphatic filariasis

Filariasis is caused by vector-borne nematodes (roundworms) that are parasitic in nature. Depending on the species, adult filariae may inhabit the lymphatics, blood vessels, subcutaneous tissues, connective tissues or serous membranes of body cavities. The adult females produce mf which normally lodge in the bloodstream or the skin and they are transmitted from person to person by dipteran vectors (Simonsen, 2008). Eight species of filarial parasites (nematodes) infect humans: *Onchocerca volvulus, Loa loa, Mansonella streptocerca, M. perstans, M. ozzardi, Wuchereria bancrofti, Brugia malayi* and *Brugia timori*. However, the first three parasites affect the subcutaneous tissues. *Mansonella perstans* and *M. ozzardi* affect the serous cavity of the abdomen while the last three parasites affect the lymphatics and are responsible for the morbidity associated with LF. *Wuchereria bancrofti* (Plate 1 and 3) is the most widespread and responsible for about 90% of all LF cases in the world (Michael *et al*, 1996). *Brugia malayi* (Plate 2 and 3) is mainly limited to Southeast Asia and south-west India, but *B. timori* (Plate 4) is restricted to some islands in Indonesia.

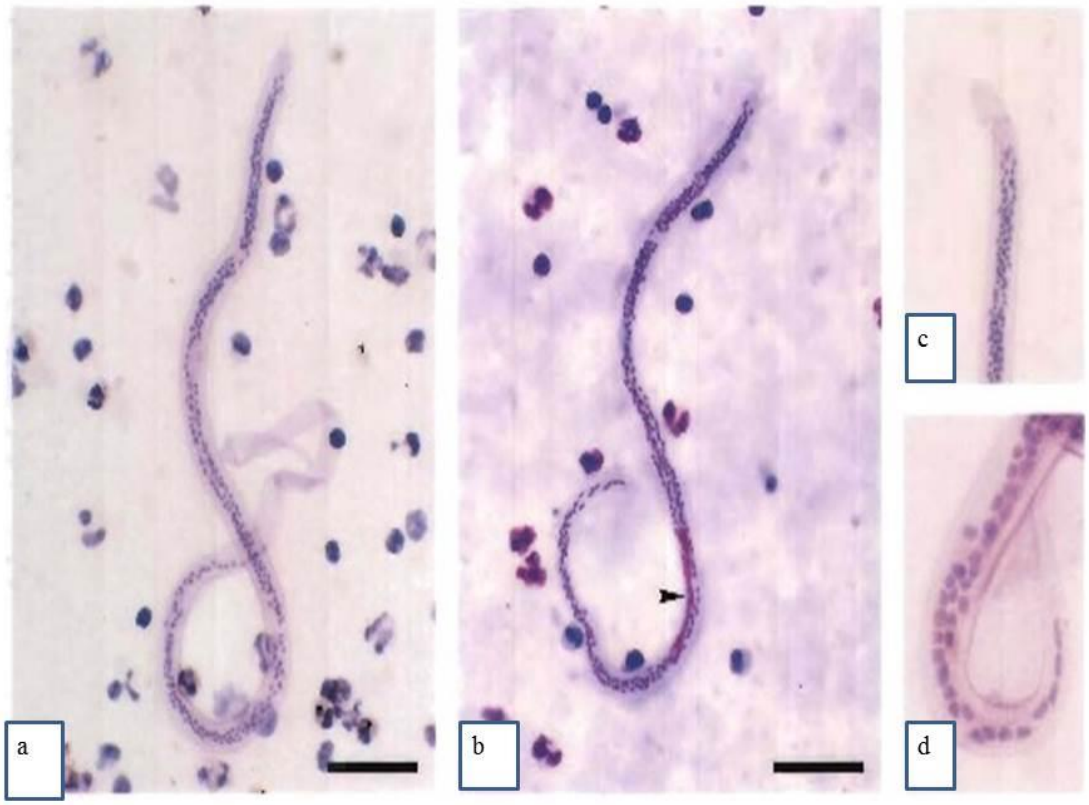

Plate 1: *Wuchereria bancrofti* microfilaria in heamatoxylin (a, c, d) and Giemsa (b) stains. Characteristically, the sheath stains lightly with heamatoxylin (a,c) but not with Giemsa stain (b). Key morphological features include a short head space (a, b, c) and a discrete nuclei in the body. The column of nuclei does not extend to the end of the tail (d). The innerbody stains with Giemsa stain (b, arrowhead) but not with heamatoxylin stain. Image reprinted from Bench Aids for the diagnosis of filarial infections. Copyright World Health Organization 1997. Source:http://www.dpd.cdc.gov/DPDx/HTML/PDF Files/Wbancrofti Lloa benchaid who.pdf

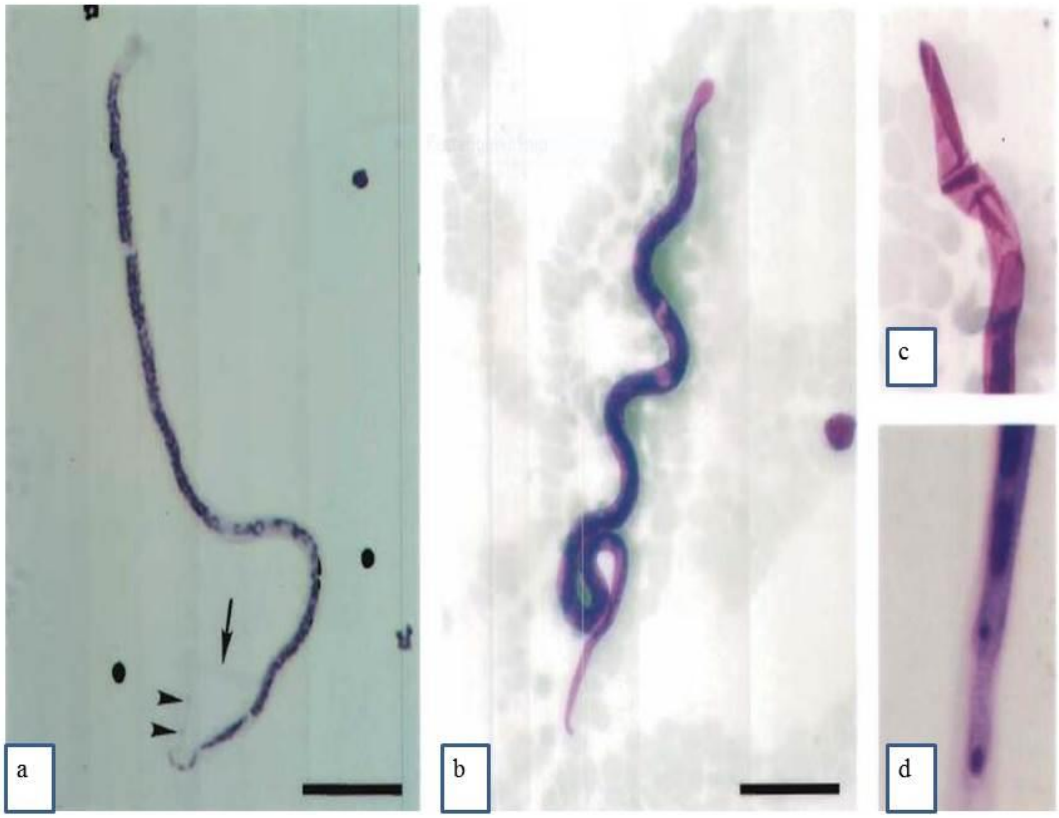

Plate 2: *Brugia malayi* microfilariae in heamatoxylin (a) and Giemsa (b-d) stains. In haematoxylin the sheath does not stain but may be faintly visible (a, arrow).This contrasts with the pink stained sheath in Giemsa preparations (b, c). The column of nuclei is compact and the widely separated sub-terminal and terminal nuclei in the tail are key diagnostic features (a, arrowheads; d). Nuclei are sparse in the region of the innerbody (a). Image reprinted from Bench Aids for the diagnosis of filarial infections. CopyrightWorldHealthOrganization1997.Source:http://www.dpd.cdc.gov/DPDx/HTML/PDF_Files/Brugia benchaid who.pdf

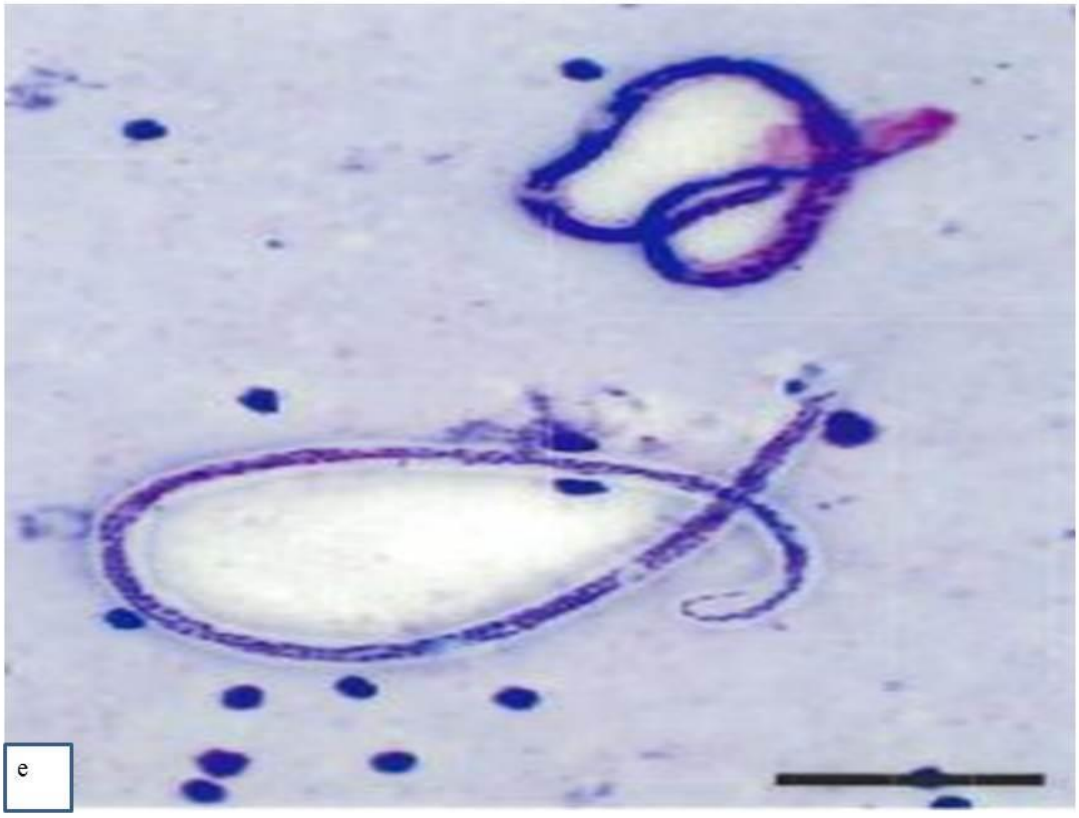

Plate 3: *Brugia malayi* (upper) and *W. bancrofti* (lower) microfilariae in the same field of Giemsa-stained blood film (e). The pink-stained sheath and the darkly stained, compact column of nuclei identify *B. malayi* and distinguish it from *W. bancrofti*. Image reprinted from Bench Aids for the diagnosis of filarial infections. Copyright World Health Organization 1997. Source: http://www.dpd.cdc.gov/DPDx/HTML/PDF_Files/Brugia benchaid who.pdf

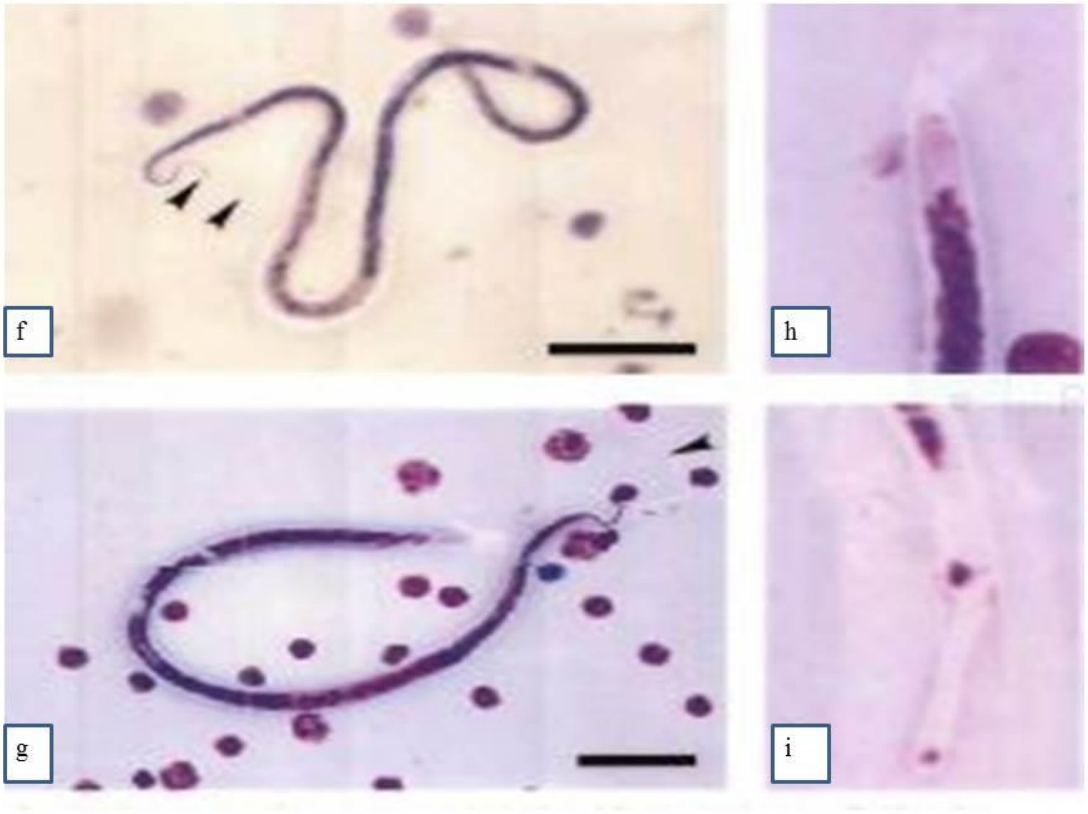

Plate 4: *Brugia timori* microfilariae in heamatoxylin (f) and Giemsa (g-i) stains. *Brugia timori* is larger than *B. malayi* and the sheath does not stain pink (g, arrowhead) with Giemsa stain. The long head space and the subterminal and terminal nuclei are conspicuous features (f-i). Image reprinted from Bench Aids for the diagnosis of filarial infections. CopyrightWorldHealthOrganization1997.Source:http://www.dpd.cdc.gov/DPDx/HTML/PDF_Files/Brugia benchaid who.pdf

2.1.3. Socio economic impact of LF

Lymphatic filariasis is considered as a disease of the poor and 80% of countries which are endemic are classified as low or lower-middle income countries (www.worldbank.org/data/countryclass/classgroups.htm). The chronic complications of the disease mostly affect individuals in their most reproductive stage of life and therefore inflict a major social and economic burden on society. This affects productivity and leads to indirect losses which have a severe drain on the local and national economy (WHO, 2013).

Lymphoedema and hydrocoele cause disfigurement, with serious psychosocial and economic consequences (WHO, 2013). The direct economic costs of managing acute and chronic manifestations are a burden on patients and health systems alike. The cost to patients of treating ADLA episodes ranges from US$ 0.25–1.62, while the cost of hydrocoele surgery, depending on the country and source of care, is US$ 5–60 (Addiss and Brady, 2007). ADLA was estimated to be responsible for losses of US$ 60– 85 million per year in India (Suma *et al.*, 2002; Shenoy *et al.*, 2003) and US$ 38 million per year in the Philippines (WHO, 2004). Haddix and Kestler (2000) report estimated that close to 25 % of surgical operations is for hydrocele repair in endemic communities in Ghana. This on the average costs $30 per operation and represents over a month of income for the average Ghanaian (Gyapong *et al*., 1996b).

According to Addis and Brady (2007), the disease exerts a heavy social burden on affected individuals because the chronic manifestations of the disease are considered shameful and prevent such individuals from playing their role efficiently and leading a fulfilling emotional life in society. Ramaiah *et al.* (2000) disclosed that genital damage is a severe disability for men because it leads to physical limitations and social stigmatization. For women, shame and taboo are associated with lymphoedema and elephantiasis. Such affected women are stigmatized and marriage often becomes impossible for them. These individuals may be

prone to depression and poor mental health (Dreyer *et al.*, 2002). In the coastal areas of Ghana such individuals are subjected to ridicule and considered as unfit marriage partners (Ahorlu *et al.*, 1999).

2.2. Clinical features

Lymphatic filariasis is described by a wide spectrum of clinical manifestations. Some section of individuals in an endemic community are asymptomatic and amicrofilariaemic. These include individuals who have not been sufficiently exposed to the infection, individuals with prepatent infection or adult worm infection without microfilaraemia, and individuals who have cleared the infection. Another section of individuals in the endemic community may be asymptomatic but microfilariaemic. Some of these individuals may remain microfilaraemic and asymptomatic for many years (Simonsen, 2008). Considerable proportion of individuals may also be symptomatic and microfilaraemic. Such group of individuals may show either acute or chronic disease manifestations. One of the pillars of achieving the goal of the GPELF is to provide basic care for persons who suffer from the major forms of filariasis related morbidity, both acute (inflammatory episodes) and chronic (lymphoedema and hydrocele) (Addiss and Brady, 2007).

Lymphatic filariasis presents some acute and chronic clinical manifestations that may include acute adeno-lymphangitis (ADL), acute filarial-lymphangitis (AFL), hydrocoele, lymphoedema, elephantiasis, chyluria, tropical pulmonary eosinophilia.

Chyluria and tropical pulmonary eosinophilia are rarely seen manifestations. Although frequent in males, genital manifestations do not appear to be a substantial problem in females (Bernhard *et al.*, 2000).

2.2.1. Acute disease conditions

Acute adeno-lymphangitis (ADL) is the most common of these conditions. The other type is the acute filarial attacks.

Acute adenolymphangitis may occur in both early and late stage infections and it is the first clinical presentation of LF. Manifestations of acute filarial disease ('filarial fever'), often described as ADL, are characterized by episodic attacks of malaise, fever and chills, and by the appearance of enlarged painful lymph nodes draining the affected part, usually the lower limb, followed by an acute, warm and tender swelling. The episodes usually resolve spontaneously after about a week, and may recur several times within a year (Simonsen, 2008). Regression of the swelling to a normal state after an ADL attack of the leg is commonly followed by unnecessary shedding of the outer layer of the skin (Dunyo *et al.*, 1998).

Dreyer *et al.* (1999) recognized two distinct clinical syndromes of acute filarial attacks: acute filarial lymphangitis (AFL) caused by death of the adult worms, either naturally or after treatment with chemotherapy and acute dermato-lymphangioadenitis (ADLA), associated with secondary bacterial infection. Acute filarial lymphangitis manifests itself as a circumscribed inflammatory nodule or cord centred on degenerating adult worms, with lymphangitis spreading in a descending (centrifugal) fashion. It usually has a mild clinical course and rarely causes residual lymphoedema (Simonsen, 2008).

Acute dermato-lymphangioadenitis, on the other hand develops in a reticular or circumferential pattern, and is clinically similar to erysipelas or cellulitis. Symptoms of local pain and swelling, as well as fever and chills, are present (Addiss and Brady, 2007). In LF endemic communities, ADLA occurs much more commonly than AFL (Dreyer *et al.*, 1999).

2.2.2. Chronic disease conditions

In the year 2000, WHO estimated that more than 40 million people suffer from chronic forms of the disease (lymphoedema, elephantiasis or hydrocoele) caused by the filarial worms and approximately 25 million men globally exhibit urogenital manifestations of LF and over 15 million people are plagued with lymphoedema (WHO, 2014). Chronic disease is incapacitating, leading to limitation in the duration and capacity to work and to changes in activity patterns (Ramaiah *et al.*, 2000; Evans *et al.*, 1993).

Hydrocele is the most common chronic presentation of LF in endemic areas (Mackenzie *et al.*, 2008; Simonsen, 2008). It occurs in only males. Hydrocoele is a source of psychological, social, marital and economic challenge to the patient (WHO, 2002).This condition results following the accumulation of clear, straw-coloured, fluid in the sac surrounding the testicles (tunica vaginalis). The onset may not accompany acute episodes, or it may be preceded by one or more attacks of funiculitis or epididymoorchitis. Following early acute episodes, the swelling around the testes usually disappears completely, but over the years the tunica vaginalis becomes thickened and there is progressive enlargement of the hydrocele. Most cases are unilateral, but bilateral hydrocoele, often with different sizes on the two sides, is not uncommon. Rarely, the fluid may have a milky appearance if lymph from a ruptured lymphatic vessel pours into the hydrocoele to form a chylocoele (Simonsen 2008). *Wuchereria bancrofti* is the only lymphatic filarial parasite that induces genital diseases (De Vries, 2002).

The persistent lymphoedema and the subsequent elephantiasis (Plate 5) are the best known clinical manifestations of the disease (Mackenzie *et al.*, 2008). Lymphoedema leading to elephantiasis mostly affect the limbs. Though it may also affect the penis, breast, vulva and scrotum, it is uncommon. Lymphoedema is simply tissue swelling and it results from the accumulation of lymph in the tissues at the infected parts of the body. Elephantiasis

progresses from lymphoedema of the affected body parts due to obstruction of the lymphatic drainage (Cheng *et al.*, 2006). This results in skin/tissue thickening.

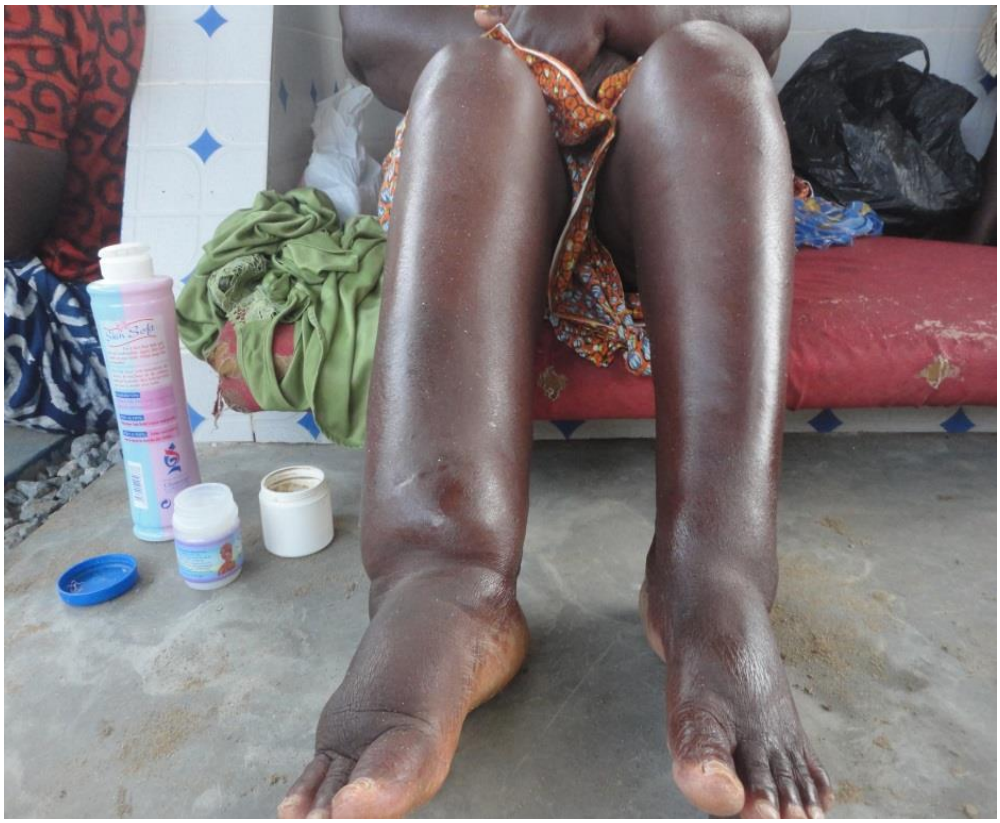

Plate 5: Lymphoedema of the leg. Picture taken during field work in Akonu.

Chyluria results as 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).

2.3. The Life Cycle of *Wuchereria bancrofti*

The parasite, *W. bancrofti* carries out its life cycle in two hosts. Humans serve as definitive host and mosquitoes (vector) as intermediate host. Mosquito species belonging to the *Anopheles*, *Culex*, *Aedes*, *Mansonia, Coquillettidia* and *Ochlerotatus* genera are carriers of the LF parasites (Manguin *et al.,* 2010; de Souza *et al*, 2012; Cano *et al.*,2014). The life cycle is shown in figure 2. The periodicity of most *W. bancrofti* mf bloodstream appearance coincides with the peak feeding activity of the local vectors. The adult worms live in the lymphatic vessels of the human host. The adult female *W. bancrofti* is viviparous and measures 80-100 × 0.25 mm with the male measuring about 40 × 0.1 mm. The adult *Brugia* spp. have only half of this dimension. Microfilariae are produced from ova in the uterus of the female worm. Microfilariae are surrounded by a loose sheath and measure on average 260 × 8 μm (Simonsen, 2008). During a blood meal by the female mosquito, mf are ingested and exsheath in the mosquito abdomen. Some of the parasites pass through the abdominal wall of the mosquito and migrate to the thoracic muscles, where they differentiate into first-stage larvae (L_1). The larvae grow and moult into sausage shaped second-stage larvae (L_2) (Smyth, 1996). They subsequently moult again to produce highly active infective third-stage larvae (L_3).The development of the parasite in the mosquito takes a minimum of 10-12 days.

Mature infective larvae then migrate to the head region within the labium (mouthpart) of the mosquito from where they enter the skin of the human host, probably through the puncture site made by the proboscis of the vector when it takes its blood meal. The L_3 develop to fourth-stage larvae (L_4) as they migrate through the human body to the lymphatic vessels and lymph nodes, where they develop into adult worms in about a year and mate. Female worms produce many sheathed mf which appear in peripheral blood at night between 10PM-2AM reaching a peak about midnight (nocturnal periodicity) [Simonsen, 2008].

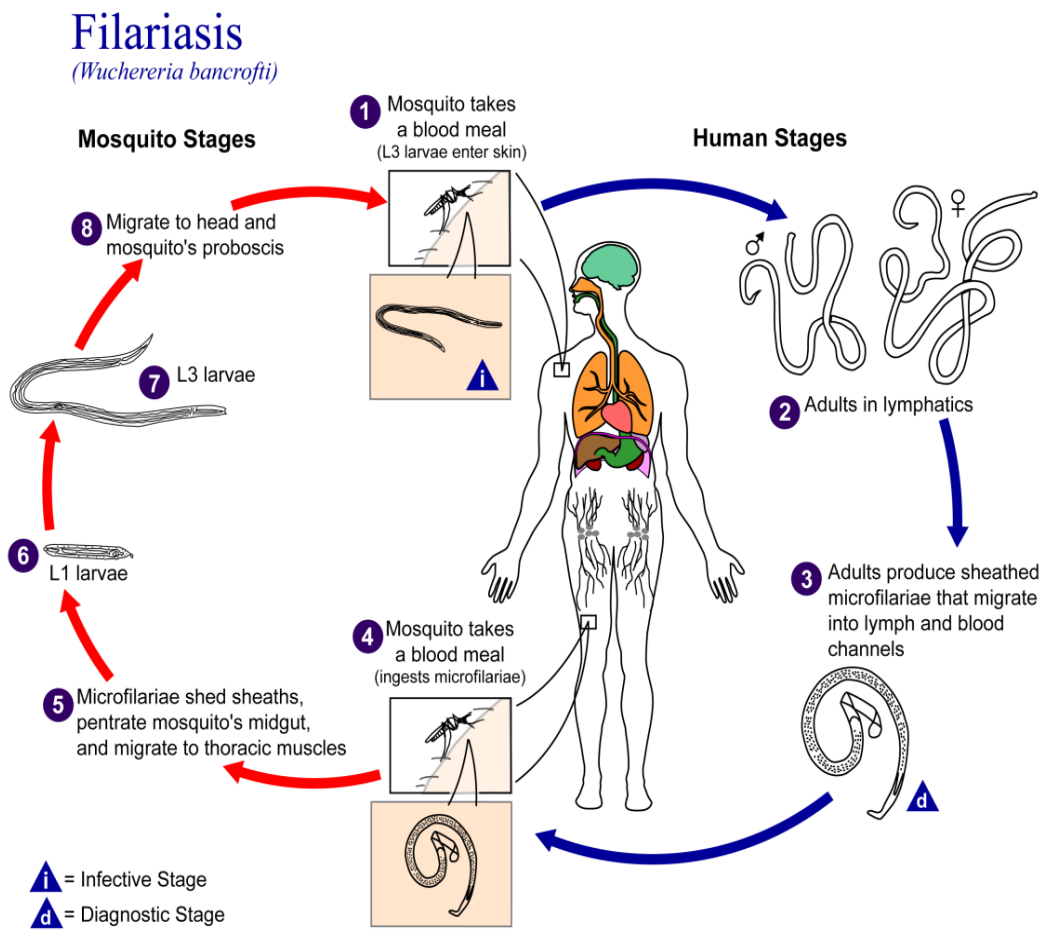

Figure 2: The life cycle of *Wuchereria bancrofti* showing the various developmental stages of the parasite in the vector and the vertebrate host. Source: http://phil.cdc.gov/phil/home.asp

2.4. Vectors of Lymphatic filariasis

The widespread of LF clearly indicates the distribution of the vectors. This also gives an indication that the various geographical regions may have varied local vectors (Erickson *et al*. 2009). In Ghana and other parts of West Africa the vectors responsible for the transmission of *W. bancrofti* are the *An. gambiae* s.l. and *An. funestus* complexes (Appawu *et al.,* 2001; Boakye *et al*., 2004), *Mansonia* (Ughasi *et al.,* 2012) and *An. phorensis* (Dzodzomenyo, 1999). In rural parts of East Africa, *An. gambiae* s.l. and *An. funestus* complexes are responsible for the transmission (McMahon *et al.,* 1981). Nonetheless, *Culex* species, especially *Culex quinquefasciatus* are responsible for the transmission of *W. bancrofti* in urban areas of East Africa and Asia (Kuhlow and Zielke, 1978) and the Americas (CDC, 2013). *Aedes* and *Mansonia*, also transmit the disease in the Pacific Islands and Asia (CDC, 2013).

2.4.1. Life cycle and Biology of the vector

The four genera of mosquitoes; *Anopheles*, *Culex*, *Aedes* and *Mansonia*, have several species that play important roles in the transmission of lymphatic filariasis. The biological and ecological features of these genera differ widely. Within a genus, differences in biting and feeding behaviour, resting and breeding preferences, seasonal abundance and affinity to human habitations determine their transmission potential (WHO,GPELF 2013). Mosquitoes may be diurnal, crepuscular or nocturnal. This behaviour to some extent influences their biting and feeding times. Mosquitoes in general exhibit complete metamorphosis-having four distinct stages in their life cycle – (egg, larva, pupa and adult).

The life cycle involves both aquatic and terrestrial phases (Figure 3). The first three stages survive only in water bodies, while the adult fly. All stages except the egg are mobile. Both male and female mosquitoes feed on nectar from flowers, aphid excretions on leaves, and

sap leaking from broken twigs to obtain the carbohydrates that are required for activities like flight. Unfortunately for humans and other vertebrate hosts, the females of most mosquito species require a blood-meal for development of their eggs (Ellis, 2004). Adult females after mating lay their eggs on water or moist surfaces. *Anopheles* lay about 50-200 small, brown or blackish boat shaped eggs with lateral floats scattered on the water surface. The eggs are laid singly and measure 1mm long and 2-5 mm wide. The lateral floats aid dispersal of the eggs on the surface of the water. *Aedes* may also lay as many as several hundred single eggs per batch on moist surfaces or above the waterline. The eggs of *Aedes* species are smooth, long and ovoid measuring about 1mm long (Figure 4). *Culex* mosquitoes lay their eggs one after the other, sticking together in the form of a raft (Figure 4). The eggs are approximately 6.25 mm long and 3.13 mm wide. The eggs of *Mansonia* are laid side by side (oriented vertically with the anterior end pointing downwards towards the water surface) to form a raft. The incubation period, varies widely with species. Viable eggs hatch usually 2-3 days after oviposition, but it may take 4-7 days or longer in temperate zones (Service, 1980).

There are four instars (stages) the larvae go through once the eggs are hatched. The larvae shed their skin at the end of each instar to allow further growth in a process known as 'moulting'. The instars have no legs but have well-developed heads with mouth brushes, and their bodies are covered with hairs (WHO, GPELF 2013). The larvae of the various genera are filter feeders and feed actively on microscopic organisms and plants. They swim by wriggling their bodies and breathe using their siphons. The larvae of *Anopheles* normally lie parallel just below the water surface. For *Aedes*, *Culex* and *Mansonia*, the various larval stages hangs head down just below the water surface. The larvae may pupate after four moults within 6-9 days depending on factors such as the availability of nutrients, temperature conditions and other competing organisms within the habitat (Gimnig *et al.*, 2001).

Unlike those of many other insects, mosquito pupae are very active. They have a comma-shaped body with two well-defined parts, the cephalothorax and the abdomen, with a pair of respiratory trumpets on the upper part. The abdomen consists of movable segments and carries a pair of paddle-like appendages at the tip. Pupae have no functional mouth part and do not feed but come to the water surface frequently to get oxygen. The pupal stage usually lasts 1–3 days, during which time all the larval tissues transform into adult tissues (WHO, GPELF 2013). The non-feeding pupa may split open its pupal case dorsally for the adult mosquito to emerge. It takes about 8-14 days for a mosquito to complete a life cycle.

Every group of mosquitoes has the males from that batch of eggs emerging first as adults and are prepared for mating within 24 hours, such that they may become proficient for mating by the time the females emerge. After a single mating, most males may die. Adult males and females feed on plant nectar, from which they derive energy. But the female requires a blood meal for development of the ovary, followed by the maturation and laying of a batch of eggs (Gillies, 1955). A multiple of factors such as carbon dioxide, temperature, moisture, odour, host movement and colour from a vertebrate may stimulate a female mosquito to take a blood meal (Service, 1980, Rebollar-Tellez, 2005, Zimmerman *et al.*, 2009).

Aedes are usually crepuscular and bite around the twilight periods of dusk and dawn. They may also bite during the day (diurnal). They exhibit both zoophily (biting animals) and anthropophily (biting humans) in their feeding behaviour (Thompson *et al.*, 1963; Crans *et al*., 1996). They may also be described as being predominantly endophagic (feeding indoors) and endophilic (resting indoors) in nature.

Anopheles are nocturnal and as such activities such as blood feeding, mating, egg laying and even emergence of pupae usually occur in the evening to early morning. Some species bite outdoors (exophagic) after sunset to about 2100 hours while others bite indoors

(endophagic) usually after 2100 hours (Gilles, 1999). They may also rest outside (exophillic) or rest indoors (endophillic) or both. Few species of *Anopheles* feed exclusively on humans (anthropophilic) while the rest are principally zoophilic. Most *Anopheles* feed on both human and animals but the extent of anthropophilism and zoophilism varies according to availability of host and the species of mosquitoes (Gilles, 1999).

Some *Culex*, prefer biting outdoors (at dusks) or indoors (throughout the night). *Culex* may be both zoophilic and anthropophilic. Some prefer domestic and wild birds (ornithophagic) to man, cattle, horses and other mammals (Kent *et al*., 2009).

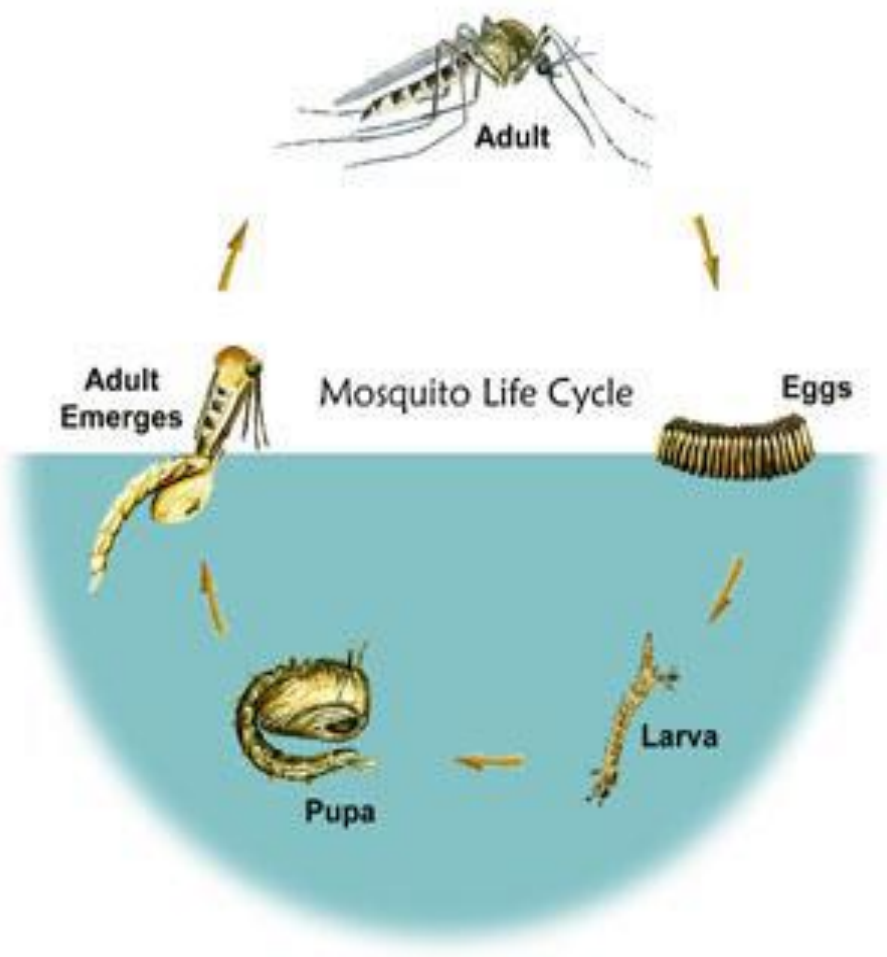

Figure 3: Life cycle of a mosquito, showing the aquatic and terrestrial phases. Source: www.epa.gov/pesticides/health/mosquitoes

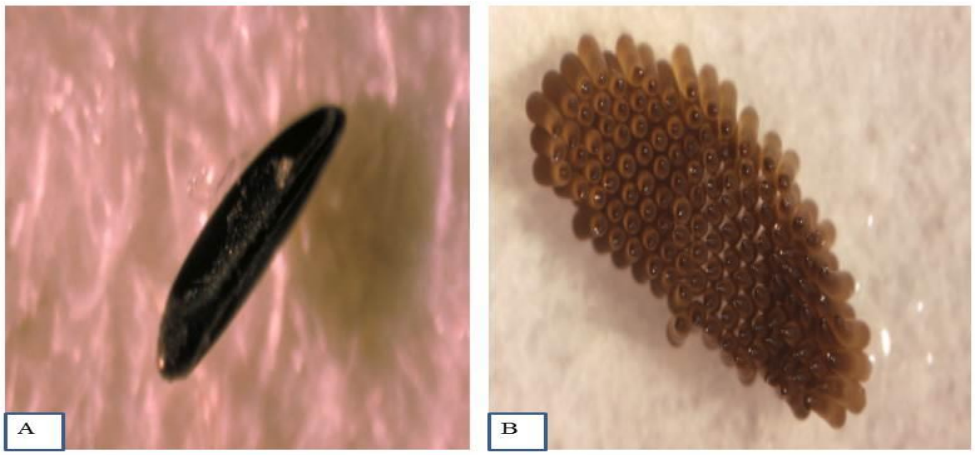

Figure 4: Mosquito eggs. A, single egg of *Aedes aegypti*. B, eggs of *Culex quinquefasciatus* in rafts. Photograph by the Centers for Disease Control and Prevention (CDC) Public Health Image Library; CDC/Harry Weinburgh. Adapted from College of Agriculture and Life Sciences, Cooperative Extension, University of Arizona, Li *et al*. (2001).

2.4.2. Morphological identification of LF vectors

Several techniques have been exploited for the identification of the various genera of mosquitoes as well as distinguishing between closely related species. Morphological identification keys for *Anopheles* mosquitoes have been provided by Gillies and colleagues (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987). *Anopheles* mosquitoes are generally characterized by dark spots on the wings and maxillary palps which are about the same length as their proboscis. *Anopheles gambiae* s.l. can be distinguished by the presence of smooth palps, with three pale bands that are about the same length as the proboscis. The femur, tibiae and first tarsal segment of the legs have short, white lines. The wings have five distinctively pale spots on the coastal margins.

For *Aedes* mosquitoes, their wings are generally uniform and the maxillary palps are shorter than their proboscis. The tip of the female abdomen is usually pointed. At rest, the proboscis

and body of *Aedes* mosquito are at an angle to one another (WHO, 2007). The vertex of the adult head has either a few or numerous forked scales that are restricted or not to the occiput. The posterior margin of the scutellum is trilobed, with a distinct group of setae on each lobe. The thorax has no setae in the prespiracular area and the alula of the wing has narrow fringe scales. The adult abdomen is completely covered with scales (Rueda, 2004).

Culex mosquitoes can be distinguished from *Anopheles* mosquitoes by observing their resting postures. *Culex* mosquitoes rest more or less parallel to the surface whereas *Anopheles* mosquitoes rest at an angle between 45° and 90° to the surface. The wings lack spots and the palps are shorter than the length of the proboscis. The tip of adult female abdomen is usually blunt (WHO, 2007).

Generally, the proboscis and body of *Mansonia* mosquitoes are at angle to one another when at rest. The maxillary palps are shorter than the proboscics and the tip of the female abdomen is blunt (WHO, 2007).

2.4.3. Vector competence

Failloux *et al.* (1995) and Bryan *et al.* (1990) defined vector competence in relation to LF transmission as the ability of mosquito vectors to ingest mf and to support their development after ingestion as well as the rate of mosquito survival until parasite maturation. Vector competence differs between species (Black IV *et al.* 2002; Severson *et al.,* 2001) and therefore has a complete consequence on the epidemiology of vector-borne diseases (Osei-Poku, 2012). The transmission dynamics of LF is related to the relationship between the parasite's behaviour, the circulating mf density, the ingestion of mf and production of infective stage larvae (Brito *et al.*, 1997, Calheiros *et al.,* 1998). Vector competence in relation to *W. bancrofti* transmission can be described based on the phenomenon of 'Facilitation', 'Limitation' and 'Proportionality'.

2.4.3.1. The Phenomena of Facilitation, Limitation and Proportionality

A competent vector is one that has the ability of ingesting mfs from an infected person, sustaining their development to the infective stage larvae (L_3) and successively transmitting them to other persons (Cran, 1973; McGreevy *et. al,* 1982).

In 'facilitation', LF vectors are only able to sustain and support the development of ingested mf when the density of ingested mf has gone above a certain threshold (Bain, 1971; Brengues and Bain, 1972). Thus, *Anopheles* vectors are postulated to be competent vectors of LF when the mf density in the human population is high. Contrarily, 'Limitation' describes a process whereby even at low mf density, LF vectors can support the development of the ingested mf for stable transmission. This phenomenon is exhibited by culicines (Subramanian *et. al.,* 1998, Duerr *et. al.,* 2005). LF vectors may also exhibit 'Proportionality'. In this circumstance, a constant number of ingested mf develops to the infective (L_3) stage in the vector (Duerr *et al.,* 2005).

In Ghana, some studies reveal that members of *Anopheles gambiae* s.l. demonstrate differences with regards to the concepts of facilitation' and 'limitation' in the transmission of LF. Amuzu *et al.* (2010) implicated *An. melas,* as a competent vector even at low parasitaemia levels. Thus, *An. melas* may be exhibiting 'limitation' in the transmission of LF. A study conducted by Boakye *et al.* (2004) in the Bongo district of the Upper East Region of Ghana indicates a possible "limitation" in *An. gambiae* s.l. and/or *An. funestus* in the transmission of the *W. bancrofti* contrary to other reports. The information regarding the varied vector competence in Anopheline vectors is fundamental to the success of the GPELF programme.

2.5. Factors affecting parasite development in the mosquito

In competent vectors, circulating mfs produced by adult female worms in the peripheral blood of humans, are ingested during a blood meal and pass through the midgut epithelium to access the hemocoel (Christensen and Sutherland, 1984). Microfilariae enter the mosquito's hemolymph, penetrate the thoracic musculature and later migrate to the indirect flight muscles. This tissue is the site of parasite development, where mfs undergo two molts and emerge as infective-stage larvae (Erikson *et al*, 2009). Lymphatic filariasis parasites grow nearly seven times in length during the extrinsic developmental period within the mosquito. For instance *B. malayi* grow from approximately 200µm to 1,350µm in length, from mf to L_3 stages respectively (Murthy and Sen, 1981). As LF parasites develop, the mosquito vector must endure a series of injuries due to the activities of the parasites. Migrating mfs normally cause damage to both midgut and muscle cells as they penetrate through them (Perrone and Spielman 1986; Erickson *et al.*, 2009).

Beckett (1971), in his study revealed that nuclear enlargement (a sign of a putative repair response) occurs in both *Brugia*-infected and neighbouring non-infected muscle cells when *Aedes* mosquitoes were infected with *Brugia* parasites and that there was a complete degeneration of infected muscle cells as infective stage larvae exit the flight muscles. Other studies have also confirmed that mosquito flight muscle cells become relatively empty of glycogen granules following infection with *Brugia* parasites (Kan and Ho, 1973; Lehane and Laurence, 1977). This means a successful development of filarial parasites depends on the capability of a competent vector to survive infection (Erikson *et al.*, 2009). This suggests how the immune system of mosquito plays a role in parasite development. However, some mosquito vectors are able to resist or limit filarial worm infections due to the lethal effect of their cibarial armature or the selective barrier imposed by the midgut epithelium.

2.5.1. Mosquito immunity

Mosquitoes are effective vectors to major pathogenic diseases and this biological behaviour put them under continuous threats of infection. Pathogenic agents normally inhabit three primary compartments in the mosquito: the midgut, the hemocoel and the salivary glands. All three of these compartments mount physical and physiological barriers that limit or reduce pathogen development (Hillyer, 2010).

For the perpetuation of their life cycles, females of all mosquito species require a blood meal for the production and development of eggs. This act often exposes mosquitoes to infectious agents that aim to undergo complex developmental and reproductive processes within the mosquito before they can be transmitted during a subsequent blood meal. Apart from getting infection through blood feeding, mosquitoes also acquire pathogens through sugar feeding, through breaks in their cuticle that are created after physical injury and following pathogen-driven cuticular degradation (Hillyer, 2010).

Although *Culex* and *Anopheles* mosquitoes are effective vectors of vertebrate pathogens, exposure or resistance to infection is often the result of intricate co-evolutionary processes in which mosquitoes and pathogens engage in counter-adaptations for survival and infection (Hillyer, 2010). As a result of this evolutionary arms race, only *Aedes* spp. are capable of transmitting dengue fever virus while *Culex* spp., the sole vector of Japanese encephalitis virus (Nasci and Miller, 1996). Similarly, according to Gwadz and Collins (1996), of over 3,000 known species of mosquitoes, only a section of *Anopheles* spp. is capable of transmitting *Plasmodium* parasites. Even within the susceptible *Anopheles gambiae* s.l, some species are resistant to infection while others, are capable of drastically reducing pathogen numbers (Niare *et al.*, 2002; Riehle *et al.*, 2006). The specificity of mosquito-pathogen relationship also differs among species of parasites (Hillyer, 2010). Beernsten *et al.* (1989) reported that the mosquito *Armigeres subalbatus* effectively transmits *Brugia pahangi* but is

resistant to *Brugia malayi*. This means that physiological compatibility and innate immune responses of mosquitoes are factors that account for the ability of pathogens to survive inside these vectors (Hillyer, 2010).

In summary, pathogen destruction by mosquitoes is accomplished by three primary mechanisms: cell-mediated phagocytosis, melanization and lysis. Each of these mechanisms is initiated by pattern recognition receptors and the factors leading to destruction can be subdivided into cellular and humoral components (Hillyer, 2010). The cellular response includes phagocytosis and encapsulation by hemocytes and pericardial cells (Hillyer *et al.,* 2003; Castillo *et al.,* 2006). The humoral response includes pattern recognition receptors, inducible antimicrobial peptides, the phenoloxidase cascade system of melanization and wound healing, and reactive oxygen and reactive nitrogen intermediates (Christensen *et al.,* 2005; Hillyer and Estévez-Lao, 2010).

2.5.2. Cibarial armature

Cibarial armatures are essential determining factor of the transmission of *W. bancrofti*. The ability of the mosquito vector to ingest and support the development of mf determines its competence. This ability is reduced when the mosquitoes possess cibarial armatures or teeth-like structures in the foregut that lacerate ingested mf (McGreevy *et al.,* 1978). Ingested parasites enter the body of the mosquito through the proboscis and enter the gut. In the foregut, parasites are presented with the first line of defence from the mosquito host (Osei-Poku, 2012). Cibarial teeth presented in the foregut lacerate mf, thus, decreasing the number of ingested parasites that enter the midgut (Bryan and Southgate, 1988; McGreevy *et al*., 1978). However the extent of reduction will depend on the number of cibarial teeth; the higher the number the more effective the armature should be at reducing mf numbers (Amuzu *et al*., 2010). *Anopheles gambiae* and *An. funestus* complexes are vector species

that possess cibarial teeth (Southgate and Bryan, 1992). Amuzu *et al*. (2010) investigated the vector capabilities of *An. gambiae* s.l after six rounds of MDA and revealed that *An. gambiae* s.s has more cibarial teeth than *An. melas* which may be inducing the exhibition of 'facilitation' by *An. gambiae* s.s and 'limitation' by *An melas*.

2.5.3. Midgut

The midgut is an important route for the filarial parasites since the ingested mfs pass through the single cell layer of the midgut epithelium to enter the haemolymph to their developmental sites in the mosquito. According to Chen and Shih (1988), mfs can exsheath in the midgut of the mosquito host or during the migration across the midgut, the sheath is damaged, facilitating exsheathment in the hemocoel (Christensen and Sutherland, 1984). Nevertheless, of the mfs in the midgut, it is only those that traverse to the thoracic muscles that develop successfully. The midgut has been confirmed to present a selective barrier to parasites and a decline in the number of parasites occur here (Al-Olayan *et al.*, 2002; Michalski *et al*., 2010). In their comparative studies, Michalski *et al.* (2010) demonstrated the differential susceptibility of *Culex pipiens pipiens* mosquitoes for *W. bancrofti* but not *Brugia* species as a result of the mosquito midgut environment. Thus, *W. bancrofti* larvae ingested with a bloodmeal can penetrate the *Culex* midgut, however *Brugia* larvae ingested by *Cx. p. pipiens* are unable to penetrate the midgut epithelium and die within the lumen. They further suggested that toxic factor(s) exist within the lumen of the *Cx. p. pipiens* midgut that physically and lethally damage *Brugia* parasites.

2.6. Diagnostic techniques of lymphatic filariasis

Several tools exist for the diagnosis of LF. Current existing techniques employed in the LF control programmes in various endemic regions include detection of mf, detection of circulating filarial antigen, detection of anti-filarial antibodies and detection of parasite genomic DNA in mosquito and blood samples.

2.6.1. Detection of microfilariae

This technique involves direct demonstration of mf in peripheral blood. Though this method is traditional, it is still relevant to the success of GPELF. The mfs of *W. bancrofti* have nocturnal periodicity and therefore the blood samples for diagnosis are collected at night.

2.6.1.1. Thick blood smear method

The Giemsa stained thick smear is the most frequently used method for diagnosis of LF. Twenty to sixty microlitres of blood sample is applied on a clean microscopic glass slide and dried overnight before dehaemoglobinization and subsequent fixation and staining (Wamae and Njenga, 2008). Microscopy is later employed for the morphological identification of mf. McMahon *et al.* (1979) identified two limitations of this method; low sensitivity due to small volume of blood sample used and loss of mf during the dehaemoglobinization step.

2.6.1.2. Knott's concentration method

This method is very sensitive in detecting mf (Knott, 1939). Here 1 ml of venous blood collected in a tube containing anticoagulant is mixed with 10 ml of 2% formalin. The mixture is left for at least 15 minutes before centrifugation. The supernatant is discarded and

the remaining sediment examined for mf as a wet smear on a microscope glass slide. A drop of 1% methylene blue may be added to the sediment to aid examination. Individuals with elevated levels of gamma globulin may have a large amount of sediments, thus making the Knott's concentration method difficult to perform because of the longer time required in examining the specimen (Wamae and Njenga, 2008).

2.6.1.3. The membrane filtration technique

In this method 1- 5 ml of venous blood collected in a tube containing anticoagulant is first mixed with a solution to lyse the erythrocytes and then filtered through a 5 µM pore size Nucleopore filter. The filter is carefully held between plastic supports within a leak – proof reusable filter holder. After filtration, the filter is removed using forceps and placed on a glass slide for examination and counting of mf under a microscope (Wamae and Njenga, 2008). One limitation of the membrane filtration technique is that the blood sample must be processed immediately after collection otherwise the filters may get clogged, thus preventing the blood specimen from passing through with the potential of even contaminating the user (Dickerson *et al.,* 1990; Wamae and Njenga, 2008). The difficulty of getting venous blood as most endemic communities are reluctant to give is another drawback to this technique. Also, trained phlebotomist is required for the blood collection process (Wamae and Njenga, 2008).

2.6.1.4. The counting chamber method

This diagnostic technique also detects mf directly in peripheral blood. For this technique, 100 µl of finger-prick or venous blood is collected and transferred to a tube containing 900 µl of 3% acetic acid (Simonsen *et al.*, 2014). The acetic acid serves as a preservative as well as a lysing solution for the erythrocytes (Wamae and Njenga, 2008). In the laboratory, the

blood specimen is transferred to a Sedgewick-Rafter chamber and examined directly for mf under a compound light microscope. The Sedgewick-Rafter counting chambers are available in plastic and glass versions. The glass chambers are durable but expensive (Wamae and Njenga, 2008). A major drawback of the plastic chamber identified by Wamae and Njenga (2008) is that, it easily gets scratches which under the microscope may appear as mf. They also identified two advantages of the counting chamber technique in the quantification of mf. These include the relative convenience of the counting chamber technique because the acetic acid preserves the blood samples which can be stored for later examination in the laboratory. Also, there is little chance of losing mf during processing because the blood specimen is transferred directly into the counting chamber for examination. Studies conducted by Agbolade and Akinboye (2005) disclosed a higher microfilaraemic sensitivity of the counting chamber technique than the thick blood smear technique. This finding is in consonance with earlier reports by Denham *et al.* (1971) and McMahon *et al*. (1979).

2.6.2. Detection of circulating filarial antigen

This method is conveniently used to detect molecules shed by the adult worms or mf (Wamae and Njenga, 2008). The immunochromatographic test (ICT) and the Og4C3 enzyme linked immunosorbent assay (ELISA) are the two commercially existing techniques that detect circulating filarial antigen (CFA). These diagnostic tests have been confirmed by Weil *et al.* (1997) that they are very convenient for daytime diagnostic examination and also offer higher sensitivity and specificity. An ELISA test kit uses, Og4C3, a monoclonal antibody, to identify this LF parasite (Itoh *et. al.,* 1998; Pani *et. al.,* 2000). With this test, as little as 100 µl plasma samples from the test individual is required. Pani *et al.* (2004) in their study revealed the sensitivity of the Og4C3 ELISA assay to be 91.2% as compared to the conventional night blood smear technique.

The ICT rapid card test is specific for *W. bancrofti* CFA. One hundred microliters of finger-pricked blood is added to the sample pad of the card. The pad contains a gold-labelled polyclonal anti-filarial antibody that binds antigen from the blood. When the card is closed, the pad touches a nitrocellulose strip. The antibody-antigen complexes move along the strip and are trapped by an immobilized anti-filarial monoclonal antibody (AD12.1) in the strip's coating. The result is read after 10 minutes, and appears as pink test line (in case of a positive test) next to a control line that appears in all valid cards (Plate 6). Thus, blood samples from antigen negative individuals show one pink line, whereas those from antigen positive individuals show two pink lines (Wamae and Njenga, 2008). Studies have revealed that the ICT has a specificity of 100% and a sensitivity of 95% (Simonsen and Dunyo, 1999; Pani *et al.*, 2000) in the detection of *W. bancrofti* CFA. Contrarily, Phantana *et al*. (1999) disclosed 100% sensitivity and 96.37% specificity of the ICT card. In assessing the sensitivity of the new format ICT card, Pani *et al.* (2004) revealed that the ICT card test is 100% in detecting mf carriers.

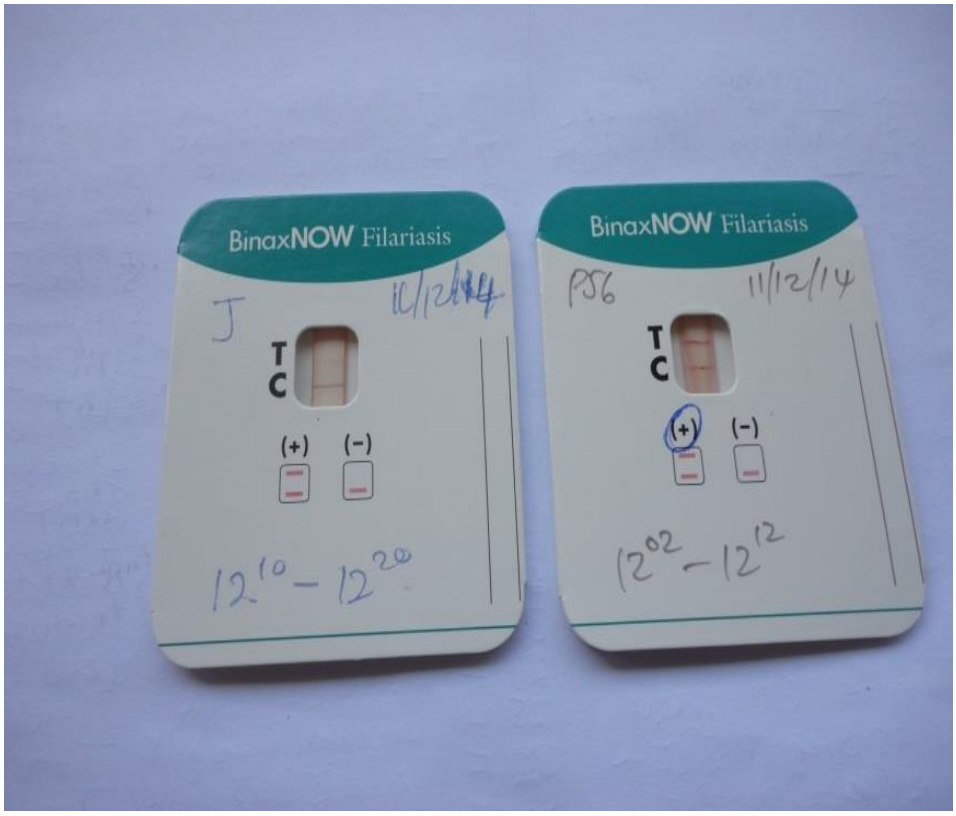

Plate 6: Immunochromatographic test cards used for LF detection. The left ICT card shows negative result for LF infection, while the right card shows positive result. **T=** Test line, **C=** Control line. Source: Picture taken during a field work in Agona Princess.

2.6.3. Detection of anti-filarial antibodies

Early research on LF diagnosis proposed that detection of anti-filarial IgG4 antibody has higher specificity for filarial infection (Lal and Ottesen, 1998). According to Wamae and Njenga (2008), selected recombinant proteins developed from filarial cDNA libraries have been identified for further research. This is as a result to get more specific antigens for antibody-detection based diagnostic tests (Lammie *et al.*, 2004). Itoh *et al.* (2001) also confirmed that an ELISA that detects filarial-specific IgG4 antibodies in urine has been designed. Wamae and Njenga (2008), report that the assay has high sensitivity (96%) and specificity (99%). Also urine samples are easier to collect than blood samples.

2.6.4. Detection of *Wuchereria bancrofti* DNA

This is a molecular identification technique that involves the use of Polymerase Chain Reaction (PCR), Real-time quantitative PCR and Loop-Mediated Isothermal Amplification (LAMP). The identification of a *W. bancrofti* repeated DNA sequence (Zhong *et al.*, 1996) has facilitated the design of a PCR based assay capable of detecting *W. bancrofti* genomic DNA in human blood (Zhong *et al*., 1996) and in mosquito vectors (Chanteau, 1994). The PCR is an enzyme-catalysed biochemical reaction in which a small quantity of a specific DNA segments are amplified into large quantities, using two oligonucleotide primers, and a DNA polymerase (Mullis, 1990).

Laney *et al*. (2010) have successfully developed an assay that specifically detects the infective stage of *W. bancrofti* in mosquitoes. The assay detects an L3-activated mRNA transcript by reverse-transcriptase PCR (RT-PCR). The assay can also be used to detect any of the larval stages of *W. bancrofti* in pooled vector mosquitoes.

An alternative to PCR in detecting *W. bancrofti* DNA is the Loop-mediated isothermal amplification (LAMP) [Takagi *et al.*, 2011]. The LAMP method amplifies DNA with high specificity, sensitivity and rapidity under isothermal conditions (Poole *et al.*, 2012; de Souza *et al*., 2014). Amplification and detection of gene can be completed in a single step, by incubating the mixture of samples, primers, DNA polymerase with strand displacement activity and substrates at a constant temperature. It provides high amplification efficiency, with DNA being amplified 10^9–10^{10} times in about 1 hour. The resulting product is a turbid solution (Figure 5), indicative of product amplification. Sample confirmation can therefore be done visually (de Souza *et al.*, 2014).

Unlike PCR technology in which the reaction is carried out with a series of alternating temperature steps or cycles, LAMP is carried out at a constant temperature, and does not

require a thermal cycler. Takagi *et al.* (2011) highlighted that the LAMP method is superior to PCR in terms of running cost and demonstration time.

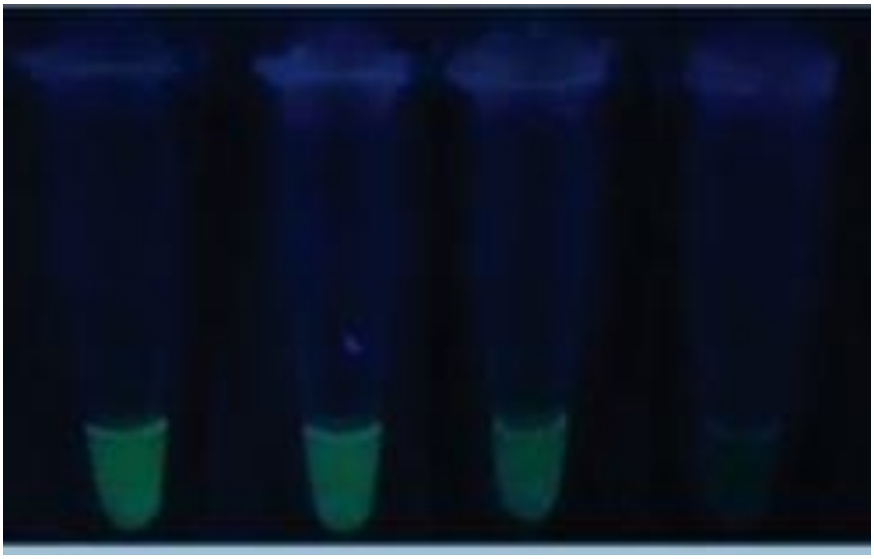

Figure 5: Products of samples after Loop-Mediated Isothermal Amplification. Positive samples fluoresce green while negative sample remain dark. Source: Poole *et al.* (2012)

2.7. Control of Lymphatic filariasis

The control of LF is mainly based on twin pillars; targeting the transmission of the disease and the management of the morbidity associated with the disease. The approach to disease transmission can either target vector control or the use of anti-filarial drugs to break the transmission cycle.

2.7.1. Use of Anti-filarial Drugs

Lymphatic filariasis elimination programme involves annual mass administration of a combination of two anti-filarial drugs to entire populations at risk. The purpose of the chemotherapy is to reduce circulating mf to a minimum threshold in the human population

such that the vectors exhibiting 'facilitation' may not be able to ingest and enhance the development of mf and therefore transmission would be halted. This is achieved through the administration of single doses of two drug-regimens; 6 mg/kg of body weight diethylcarbamazine citrate (DEC) + 400 mg albendazole or 150 µg/kg of body weight ivermectin + 400 mg albendazole (Ottesen 2000; WHO, 2014). The treatment combination of ivermectin and albendazole is used in areas where onchocerciasis is co-endemic. Though the use of DEC remains the main treatment drug in most endemic countries, its use is limited in areas where onchocerciasis and loaisis are co-endemic due to the severe side effects in people infected with *Lao lao* (WHO, 2000).

These anti-filarial drugs are usually given by mass administration for 4-6 years, until adult filarial worms have reached the end of their reproductive lifespan. In endemic areas where MDA coverage is poor or where transmission is very severe, MDA may have to be longer in order to ensure interruption of transmission (WHO, 2011).

2.7.2. Vector control

The World Health Organization has recommended vector control to other strategies appropriate for the prevention, control, elimination and eradication of neglected tropical diseases (WHO, 2012). The World Health Organization strategy for LF elimination was based primarily on chemotherapy until vector control was recommended in 2012. The effect of vector control on LF transmission is now being appreciated (Bockarie *et al*, 2009).

There are new improved techniques for heightening the efficacy of vector control which include the use of insecticide-treated materials (ITMs), residual spraying and the use of floating layers of expanded polystyrene beads (EPBs).The use of ITMs for malaria control also serves as a control measure for LF transmission since both diseases are transmitted by the same vector (WHO, 2002). The use of EPBs creates a physical barrier to egg-laying adult

Culex while suffocating larvae and pupae (Maxwell *et al*., 1990; Sunish *et al*., 2002). These and other techniques have enabled the elimination of the disease in Japan, Taiwan, Solomon Islands, South Korea and some parts of China (Webber 1991).

There are documented evidences in the Pacific which report how LF was eliminated using vector control alone. Bockarie (1994) reports how filariasis was eliminated using indoor residual spraying (IRS) with DDT in certain areas of Papua New Guinea. Webber (1977) also gave a similar report on how LF was eliminated from certains areas in Solomon Islands using the same technique.

2.7.3. Morbidity management

To manage morbidity and decrease the disability caused by LF, the principal strategy focuses on decreasing secondary bacterial and fungal infection of individuals whose limbs and genitals have already been compromised by filarial infection. Secondary infections caused by bacteria or fungi are the main pathogenic determinant of worsening the conditions of lymphoedema and elephantiasis (Ottesen, 2000).

It has been recommended that the maintenance of personal hygiene by regular washing of elephantoid limbs with anti-bacterial soap and the application of antibiotic topical ointments reduce the incidence of acute attacks and the advancement of elephantiasis (Shenoy *et al*., 1998, Dreyer *et al*., 1999). The wearing of suitable foot wears, keeping the nails and toes clean, treating of wounds with medicated creams, elevating the limbs at night and regular physical exercise with low-intensity movement of the joints are the recognized methods proposed by the GPELF in managing lymphoedema and elephantiasis (WHO, 2013). Surgical operation for men with hydrocoele is also an element of morbidity control recommended by the GPELF (WHO, 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study sites

The study was conducted in two sites, Agona Princess (N 04.79288º W 002.13464º) and Akonu (N 04.83456º W 002.20485º). Agona Princess is located in the Ahanta West District and Akonu in the Nzema East Municipality, both in the Western Region of Ghana. Dunyo *et al*. (1996) identified these areas as LF endemic with overall mf prevalence of 9-25%. The main occupations of the inhabitants of Agona Princess are farming, and other small-scale businesses. Indigenes of Akonu are primarily fisherfolks; but a few are petty traders and subsistence farmers. Houses found in these study communities were mostly mud houses roofed with dried coconut leaves and bamboo sticks. In addition, few of the houses were also constructed with cement blocks with most of the rooms having no ceilings. The eaves of the houses also had gaps. Unlike Akonu which is located along the sea, Agona Princess is located along various rivers, lagoons and mangroves which serve as breeding sites for *Anopheles* mosquito, the main vector of the parasite.

Agona Princess is one of the sub-districts of the Ahanta West District alongside Agona-Nkwanta, Apowa and Dixcove. These sub-districts were created for effective health administration. Currently the population size of the district is 114,971 with a projected growth rate of 2.0% (District Health Administration, Agona Nkwanta). Females make up 52.0 % of the population, whilst the males comprise 48.0 %. Agona Princess represents 12% (13,796) of the total population and made up of nine communities namely Adalazo, Aketekye, Asuboi, Awonakrom, Silmowu, Princess Town, Nkwantanan, Animakrom and Bokazo (District Health Administration, Agona Nkwanta). Ahanta West covers a total land area of 591 square kilometres and lies between latitude 4.45”N and longitude 1.58”W. The

district is predominantly rural and found within South-Western Equatorial climate zone of Ghana.

The highest mean temperature is 34ºC which is recorded between March and April, while the lowest temperature of 20ºC is experienced in August. Relative humidity is very high averaging between 75%, to 80% in the dry season. It experiences double maxima rainfall of over 1700 mm.

The District is bounded on the East by the Sekondi Takoradi Metropolitan Assembly (STMA), on the West by the Nzema East Minicipal, and the North by Mpohor Wassa East and Tarkwa Nsuem Districts and the Gulf of Guinea to the South (http://ahantawest.ghanadistricts.gov.gh). Agona Princess was among the sub-districts in Ahanta West which commenced MDA with albendazole and ivermectin in 2000 for the Ghana filariasis elimination programme. However, after more than 5 rounds of MDA, mf prevalence was reduced to 0.4% (Ghana Filariasis Elimination Programme, 2008). The programme has now completed more than ten rounds of MDA in the area and it is one of the sentinel sites where the programme is evaluated annually for microfilaria prevalence and density as part of exit plan implementation for the programme.

The Nzema East municipality is made up of five sub-municipals namely; Axim/Nsein, Bamiankor, Kutukrom, Gwira Eshiem and Gwira Banso. Akonu is located in the Axim/Nsein sub-municipality. The population size of the municipality is 60,828. This is made up of 29,947 males and 30,881females. (GSS, PHC 2010). The area is located at the southern end of the Western Region and lie between the Wet Semi-Equatorial Climate zones of the West African Sub-region. Rain falls throughout the year with the highest monthly mean occurring around May and June. The average temperature is about 29.4ºC. It occupies a land area of 2,194 square kilometres (http://nzemaeast.ghanadistricts.gov.gh). It is bordered on the West by Ellembelle, North by Wassa Amenfi, and the East by Wassa West Districts.

On the south, it is bounded by Ahanta West District (Municipal Health Directorate, Axim). MDA with ivermectin and albendazole began in the municipality in 2001.

3.2. Identification and selection of study participants

This was a cross-sectional survey conducted in two LF endemic communities in the Western Region of Ghana. The survey involves recruiting 370 study participants living in the endemic communities.

3.2.1. Inclusion criteria

Children and adults between the ages of 6-90 years, individuals willing to participate in the study and individuals who are residents in the study communities were enrolled into the study.

3.2.2. Exclusion criteria

Non-residents, children below the age of six, sick individuals and those unwilling to participate in the study were excluded.

3.3. Parasitological surveys

To determine the prevalence of the disease in the study communities, the immunochromatographic test (ICT) card was used to determine the prevalence of circulating filarial antigens (CFA). The thick blood smears (TS) and the counting chamber (CC) techniques were used to determine the prevalence of mirofilaraemia. Microfilaria examination was done on only CFA positive individuals due to 100% sensitivity of the ICT card in detecting mf carriers (Phantana *et al.*, 1999; Pani *et al.*, 2004). A pre-treatment

survey of the human population for filarial infection was carried out in December 2014. Subsequent post-treatment surveys were carried out in March and June 2015.

3.3.1. Determination of circulating filarial antigens (CFA)

To determine the prevalence of antigenaemia in the study communities, the immunochromatographic (ICT) card test was used. The ICT card test was performed at the study sites as per the instructions of the manufacturer. Each card was carefully removed from the pouch, labeled by indicating the ID number of the participant and date of the test. The ICT card was then placed flat on a leveled bench. One hundred microlitres of finger pricked blood was drawn into a calibrated capillary tube and used for the ICT test. The blood drawn into the capillary tube was applied to the white portion of the pink and white pad after opening. This was done carefully in order not to apply the blood directly to the pink portion of the sample pad. The blood sample was allowed to migrate to the pink portion for about 30 seconds before the card was closed and pressed along the edges to seal it. The starting time was then recorded. After 10 minutes, the appearance of the diagnostic line was observed and recorded. The test was considered positive when both lines (test and control) could be read through the visualisation window and negative when only the control line could be seen stable and permanent. All ICT positive individuals were treated with the recommended treatment dosage of ivermectin and albendazole after the survey.

3.3.2. Determination and quantification of microfilaraemia

After testing for the CFA, individuals who tested positive were made to converge at the market place where 1 ml of venous blood was drawn from each study subject between 21.00 and 24.00 hours into heparinised blood collection tubes containing EDTA and kept in an ice chest with ice packs. This was later transported to the laboratory for processing. In the

laboratory, the counting chamber (CC) and thick blood smear (TS) techniques were employed for the detection of mf.

For the counting chamber technique, 100 µl of the venous blood from each of the research subjects was diluted in 900 µl of 3% acetic acid in an eppendorf tube. The blood specimens were later transferred into a Sedgewick-Rafter counting chamber and examined for mf load under a compound microscope set at × 100 magnification. The quantification was expressed as mf/ml of blood.

For the thick smear method, 60 µl of blood sample was taken and for each sample three smears (20 µl each) were prepared on a clean microscopic glass slide. The blood smears were dried overnight at room temperature and dehaemoglobinized by washing them with water. The slides were labelled and stored in a dust-free environment until staining. Later the blood smears were fixed in methanol and stained with Giemsa. The compound microscope was employed for the morphological identification of mf set at × 100 magnification.

3.3.3. Morphological identification of microfilariae using microscopy

In this study, the mf of the *W. bancrofti* parasite was observed using the low power (× 10) compound microscope. Morphological characteristics of the mf were the criteria used to identify them. In identifying mf of *W. bancrofti* the features taken into account included the presence of discrete nuclei running through almost the entire body length but does not extend to the end of the tail; the presence of sheath especially at the cephalic and tail ends (WHO, 1997); and the morphological dimensions (260 x 8µm) (Simonsen, 2008).

3.4. Mosquito collection

Each of the two study communities was divided into four sections. Twenty houses were randomly selected in each section. A total of 80 houses were therefore selected in each site.

The sampling was done for five consecutive days in the month of December 2014. A second sampling was done in March 2015 for another five consecutive days.

Residents of selected houses were informed of the collection and asked not to leave windows and doors open in the morning. Pyrethrum Spray Catch (PSC) was used in the sampling of adult indoor resting mosquitoes between 0500 hours and 0800 hours each morning. White clothes were laid on the entire floor of the selected room for easy recognition of mosquitoes. The spray guns were filled with Raid® insecticide (Pynamin Forte - 0.05%, Neopynamin - 0.05%, Deltamethrin - 0.015%, Solvent, Fragrance - 99.885%) and the rooms of the houses sampled were then sprayed. 10-15 minutes after spraying, the white clothes were gently removed from the room by folding them from the edges. The knock-down mosquitoes were sorted with forceps outside the room in the open. This was done to separate all genera of mosquitoes caught from the other blood feeding insects. Knocked down mosquitoes were then transferred into a well labelled eppendorf tubes and placed in a cool box and transported to the laboratory for further processing. The label information included the house number, number of individuals that slept in the room, and the number of mosquitoes caught in the room.

Plate 7: Housing structure and adult mosquito sampling at Agona Princess during the December 2014 mosquito collection. **A** is a clay house with bamboo roofing with gaps at the eaves. **B** and **C** shows rooms being sprayed with pyrethrum spray. **D** showing how the light-coloured sheet is laid. Source: Picture taken during a field work in Agona Princess.

3.5. Morphological identification of mosquitoes

The mosquitoes brought from the field were sorted out into the different species of *Aedes, Anopheles, Culex and Mansonia* using the morphological keys described by Gillies & de Meillon (1968) and Gillies & Coetzee (1987). Each mosquito was picked with forceps into a petri dish and examined under a dissection (stereo) microscope. All *Anopheles* mosquitoes were identified by the dark spots on the upper margins of the wings, and maxillary palps which are about the same length as their proboscis. Culicines were characterized based on the absence of the pale and dark band patterns on the coastal margins of their wings and their maxillary palps shorter than their proboscis. The other genera were also characterized using the morphological identification keys. In all genera of mosquitoes, the males were distinguished from the females by the presence of their bushy antenna. The different species that had been morphologically identified were recorded.

3.6. Dissection of mosquitoes and morphological identification of *W. bancrofti*

On a well-labelled glass slide, each mosquito was divided into head, thorax and abdomen. The legs were detached from the body and placed in 1.5 ml eppendorf tubes for species identification, using molecular methods. Each of the body parts (head, thorax and abdomen) was teased (using dissecting pins) in a drop of water, under a dissecting microscope.

The larval stages of the *W. bancrofti* parasite were observed after dissection of the mosquitoes using a compound microscope. Morphological characteristics of the parasites were the principles used to identify and differentiate between mf and the three larval stages. In identifying mf of *W. bancrofti* the features taken into account included the presence of discrete nuclei running through almost the entire body length but does not extend to the end of the tail; the presence of sheath especially at the cephalic and tail ends (WHO, 1997); and the morphological dimensions (260 x 8 μm) (Simonsen,2008). The three larval stages were

also identified by examining the presence of nuclei along the length of the parasitic worm. Using morphological features and dimensions, the first larval stage (L_1) which is normally found in the thoracic muscles is about the size of the mf, the second larval stage (L_2) which also harbours the thoracic muscles is thicker and sausage-shaped and the third larval stage (L_3) usually found in the head tissues and proboscis is longer and thin with well-developed anterior and posterior ends.

3.7. Molecular analysis

Genomic DNA was extracted from the body and legs of the mosquitoes and used for *W. bancrofti* and mosquito species identification respectively, using the conventional PCR technique.

3.7.1. DNA extraction

The dried carcasses of dissected mosquitoes (December 2014 samples) together with any *W. bancrofti* were scraped off from the glass slides in pools of 20 or less (where the numbers in the groups of species were not up to 20) into a 1.5 ml eppendorf tube. March 2015 mosquito samples were also scraped singly into their respective eppendorf tubes. *Wuchereria bancrofti* genomic DNA of each specimen was extracted using the E.Z.N.A. Tissue DNA Kit following the manufacturer's protocol for DNA extraction from animal tissues (OMEGA bio-tek, Norcross, USA).

Two hundred microlitres of TL buffer was added to each specimen of scrapped carcasses. A volume of 25 µl OB Protease Solution (Omega bio-tek, Norcross, USA) was then added to each of the samples, vortexed thoroughly and incubated at 55 ºC. During incubation, the samples were vortexed every 30 minutes for 3 hours to speed up lysis of the tissues. After incubation, each of the samples in the eppendorf tubes were centrifuged at maximum speed

(≥10,000 x g) for 5 minutes for the debris to settle. The supernatant was then transferred into a new sterile 1.5 ml eppendorf tube, 220 µl of BL Buffer (Omega bio-tek, Norcross, USA) added and vortexed thoroughly. This was incubated at 70°C for 10 minutes. Two hundred and twenty microlitres of absolute ethanol was pipetted into the samples and vortexed thoroughly after incubation. HiBind® DNA Mini Column was later inserted into a 2 ml collection tube. The entire sample was then transferred into the HiBind® DNA Mini Column and centrifuged at maximum speed for 1 minute. After centrifuging, the filtrate was discarded and the collection tube was reused. A volume of 500 µl HBC buffer was pipetted into the sample in the HiBind® DNA Mini Column and centrifuged at maximum speed for 30 seconds. The filtrate and collection tube were discarded after centrifuging and the HiBind® DNA Mini Column inserted into a new 2 ml collection tube. Seven hundred microlitres of DNA wash buffer was added to the sample and centrifuged at maximum speed for 30 seconds. The filtrate was discarded and the collection tube was reused. The empty HiBind® DNA Mini Column was centifuged at maximum speed for 2 minutes to dry the column and transferred into a nuclease-free 1.5 ml eppendorf tube. A volume of 100 µl Elution Buffer heated to 70°C was pipetted into the HiBind® DNA Mini Column and allowed to sit at room temperature for 2 minutes. This was centrifuged at maximum speed for 1 minute. The elution step was then repeated to increase DNA yield. The eluted DNA was finally stored in a freezer at a temperature of -20 ºC.

3.7.2. Identification of *Wuchereria bancrofti* using PCR

In the identification of *W. bancrofti*, conventional PCR technique was used for the detection of the LF parasite given a band size of 188 bp (Derua *et al*., 2012). The PCR-mix contained ddH_2O, Go-Taq and 0.2 µM each of primers NV-1 and NV-2. The primer sequence details and their expected band sizes of the PCR products are given in Table 1. Five microliters of

DNA extracts from mosquito carcasses were used as templates for the PCR reaction giving a final volume of 20 µl. The cycling conditions used were 95 °C for 10 minutes followed by 40 cycles of denaturation at 94 °C for 1 minute, annealing at 55 °C for 1 minute and extention at 72 °C for 1 minute then a final extension at 72 °C for 10 minutes.

After DNA amplification, the PCR products were electrophoresed on 2% agarose gel. The gel was prepared by dissolving 4.1 g of agarose powder in 205 ml 1×TAE buffer in a conical flask. The mixture was heated to completely dissolve the powder in a microwave oven for 2 minutes and later cooled until vapour stops rising. A volume of 2.6 µl Ethidium Bromide was added to the gel, well mixed and poured into the mould. For the electrophoresis, 5 µl of each PCR product sample was loaded into the wells after placing the solidified gel in 1XTAE buffer in a mini gel system (BIORAD, USA). Samples were run alongside a molecular ladder to determine the size of amplicons. 100 volts of electric current was passed through it for 45 minutes, after which the gel was visualized over a UV transilluminator (UPC, USA) at short wavelength.

Table 1: Oligonucleotide primer sequences details and their expected band sizes of the PCR products

Wuchereria bancrofti	Primer sequences 5'→ 3'	Product size (band size)
NV-1(forward)	CGTGATGGCATCAAAGTAGCG	188
NV-2 (reverse)	CCCTCACTTACCATAAGACAAC	188

3.7.3. Extraction of DNA from *An. gambiae* complex

The boiled preparation DNA extraction method was used for the extraction of genomic DNA from each *An. gambiae* mosquito. One to three legs from each mosquito was placed in a 1.5 ml eppendorf tube, containing 50 µl ddH_2O, and homogenized with a sterile plastic pestle. The homogenates were incubated in a water bath at 90°C for 15 minutes and later centrifuged briefly (5-10 seconds) and supernatant stored at -20°C until ready to use.

3.7.4. Identification of sibling species of *An. gambiae* s.l

The extracted DNA from a single mosquito was used for species identification (Fanello *et al*., 2002). Five sets of oligonucleotide primers were used in PCR for identification of members of the species complex (Scott *et al*., 1993). The primer sequence details and their expected band sizes of the PCR products are shown in Table 2. The PCR reaction was performed in a total volume of 20 µl, containing 0.15 µM of each of the five oligonucleotide primers and 1× GO-Taq buffer. Three microlitres of the extracted DNA was used as template for the amplification reaction. Sterile distilled water was used to make up the volume to 20 µl. The amplification was carried out using a thermal cycler. The cycling conditions for the reactions were as follows: 94°C for 3 min (initial denaturation), followed by 35 cycles at 94°C for 30 sec (denaturation), 50°C for 30 sec (annealing), 72°C for 1 min (extension) and ended with a final extension cycle at 72°C for 5 min. Positive and negative controls were added to the reaction. After amplification, 5 µl of each of the amplicons was loaded into wells of a 2% agarose gel for electrophoresis. The gel was observed under a UV illuminator (TOYOBO Transilluminator Model TM-20 connected to a TOYOBO FAS-III monitor for printing pictures of electrophoregrams). The result was read from the electrophoregram.

Table 2: DNA sequence details of the oligonucleotide primers used for the PCR-based methods for the identification of the siblings of the *An. gambiae* s.l (Scott *et al*., 1993).

An. gambiae s.l	Primer sequences 5'→ 3'	Product size (band size)
Universal (UN)	GTGTGCCCCTTCCTCGATGT	468
An. gambiae (GA)	CTGGTTTGGTCGGCACGTTT	390
An. melas (ME)	TGACCAACCCACTCCCTTGA	464
An. arabiensis (AR)	AAGTGTCCTTCTCCATCCTA	315
An. quadriannulatus (QD)	CAGACCAAGATGGTTAGTAT	153

3.8. Data analysis

This study involves both parasitological and entomological investigations of LF in the study areas. Parasitological and entomological indices were therefore calculated to ascertain the level of human filarial infection and transmission in the study sites. Data entry and validation on parasitological surveys were done in Microsoft excel 2010 version. The data was analysed into tables with the aid of SPSS version 16 statistical software. Because the data does not follow a normal distribution, the non-parametric equivalent of t-test, known as Mann-Whitney U test was used to test for the statistical significance of the sensitivities of the CC and TS in microfilarial detection. The test of significance was analysed using the Minitab (fifteenth edition) statistical software.

3.8.1. Parasitological indices

The parasitological indices determined include CFA prevalence, microfilaria prevalence and geometric mean intensity of mf (GMI).

$$\textbf{CFA prevalence} = \frac{\text{Number of individuals found positive for CFA}}{\text{Total number of individuals examined}} \times 100$$

$$\textbf{Mf prevalence} = \frac{\text{Number of individuals positive for microfilariae}}{\text{Total number of individuals examined for microfilariae.}} \times 100$$

Mf prevalence was calculated as: $\left(\frac{b}{a}\right) \times \left(\frac{d}{c}\right) \times 100$, where a = number of individuals in the community examined for CFA, b = number of those examined for CFA being positive, c = number of CFA positives examined for mf, and d = number of those examined for mf being positive (Simonsen *et al.*, 2014).

Geometric mean intensities (GMIs) of mf counts was also determined as antilog [(Σ log x +1)/n], with x, being the number of mf/ml of blood in mf positive individuals and n, the number of individuals examined (Boakye *et al.*, 2004).

3.8.2. Entomological indices

The entomological parameters calculated include infection rate, infectivity rate, annual biting rate, annual infective biting rate, worm load and annual transmission potential. These parameters help in determining the transmission status of an endemic area. The formulae for the estimation are shown in Appendix 3.

3.9. Ethical approval for the study

Ethical clearance was obtained from the Institutional Review Board (IRB) of Noguchi Memorial Institute for Medical Research. Permission to conduct the studies in the selected communities was sought from the Ahanta West and Nzema East Municipal Health Administrations. Prior to the study, there was a community entry where the study was explained to the communities and consent sought at both community and individual levels. Participation was strictly voluntary and the right to withdraw from the study without any retribution was ensured. All procedures required for use of human subjects (in mosquito sampling and blood collection) were strictly followed.

CHAPTER FOUR

RESULTS

4.1. Demographic data of participants

A total of three hundred and seventy (370) study participants were enlisted into the study after signing the informed consent form. Out of the 370 study participants, 231 (62.4%) were females, 139 (37.6%) were males.

The age range of the study participants was 6 to 85 years. The age group with the highest study participants was 11-15 years consisting of 89 (24.1%) participants and the age group with the least study participants was 55-60 years with 9 (2.4%) persons (Figure 6).

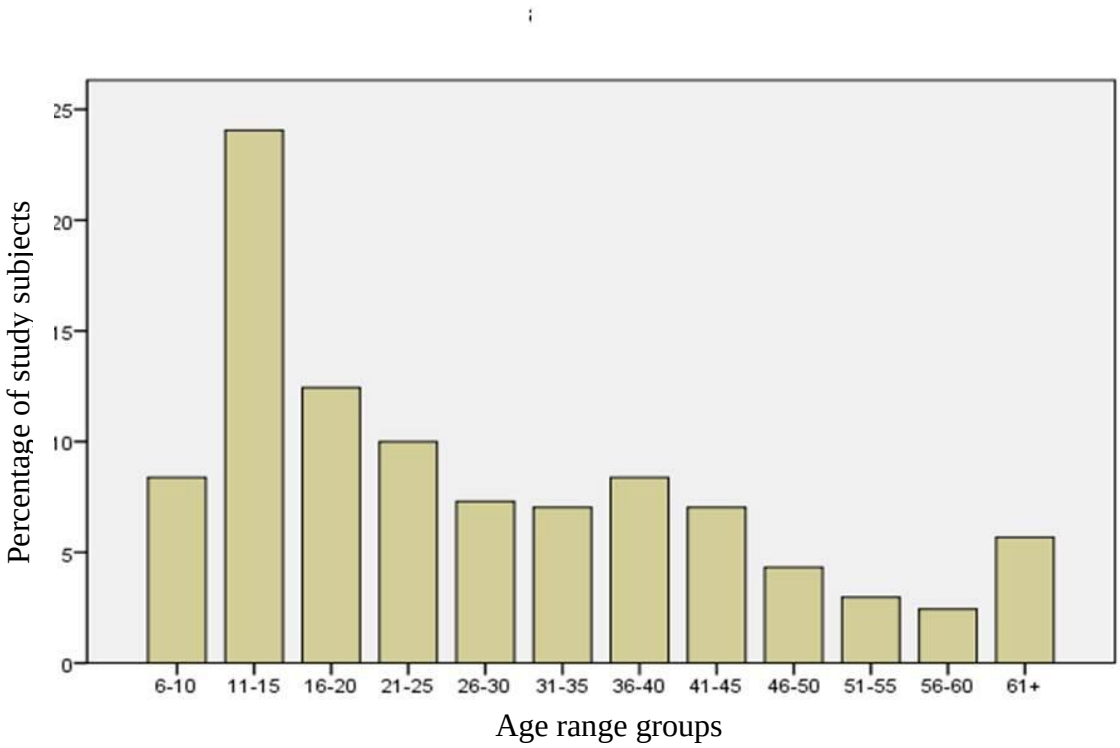

Figure 6: Age range group distribution among study participants recruited in both study communities.

4.2. Prevalence of antigenaemia during December 2014 parasitological survey

The overall prevalence of antigenaemia determined was 13%. This represented forty eight (48) individuals who tested positive for the circulating filarial antigen (CFA) by the immunochromatographic test. Eighty-seven percent [87% (n=322)] tested negative (Figure 7). Twenty-nine [29 (21%)] of the individuals who tested positive were males while 19 (8.2%) were females (Figure 8). In Akonu, 27 (23.5%) participants tested positive for CFA, whereas, 21 (8.2%) in Agona Princess tested positive (Figure 9).

The study subjects who were investigated for antigenaemia were categorized into twelve age groups (Figure 6). The highest prevalence was seen in age group 51-55 years with 3 (27.3%) participants being positive. The age group 6-10 years recorded the lowest antigenaemia prevalence of 3.2% (Figure 10).

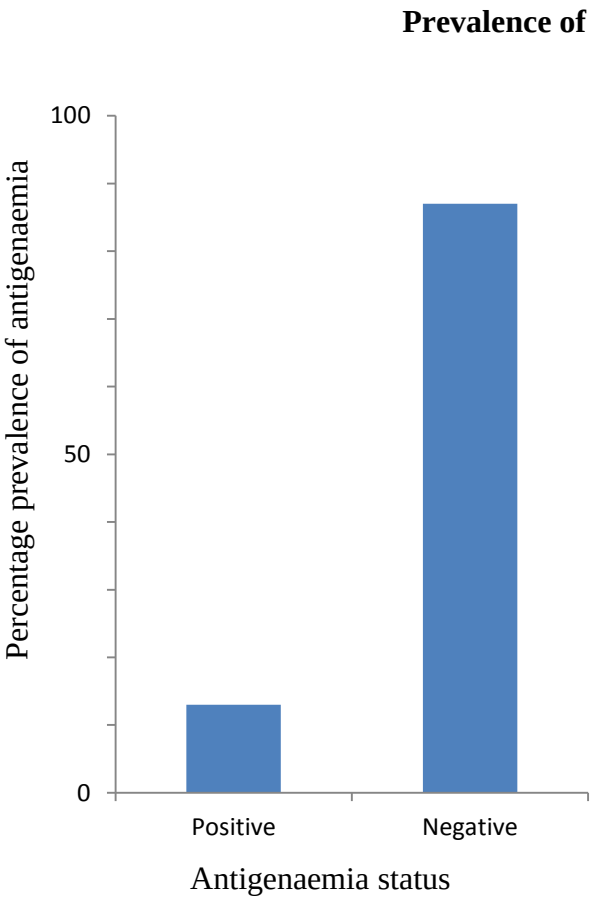

Figure 7

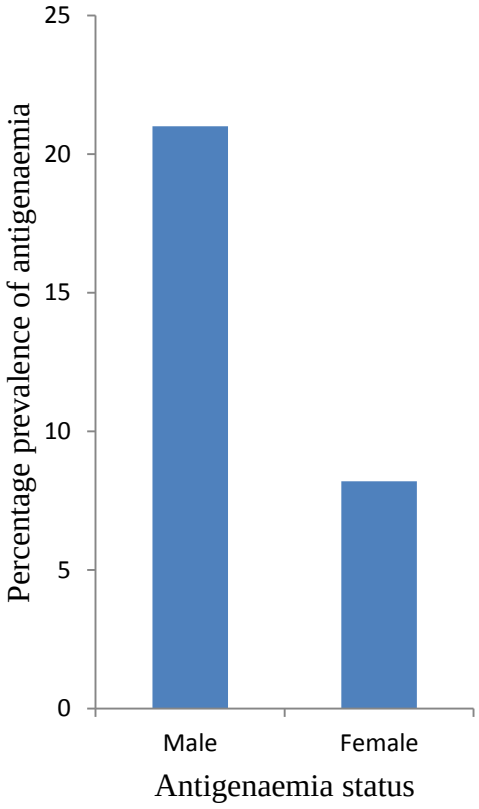

Figure 8

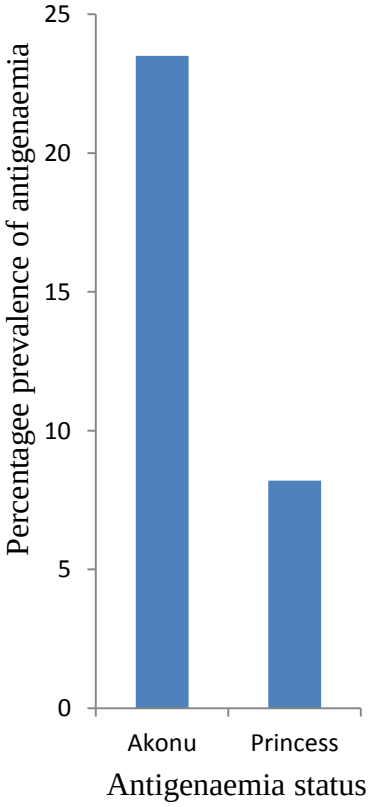

Figure 9

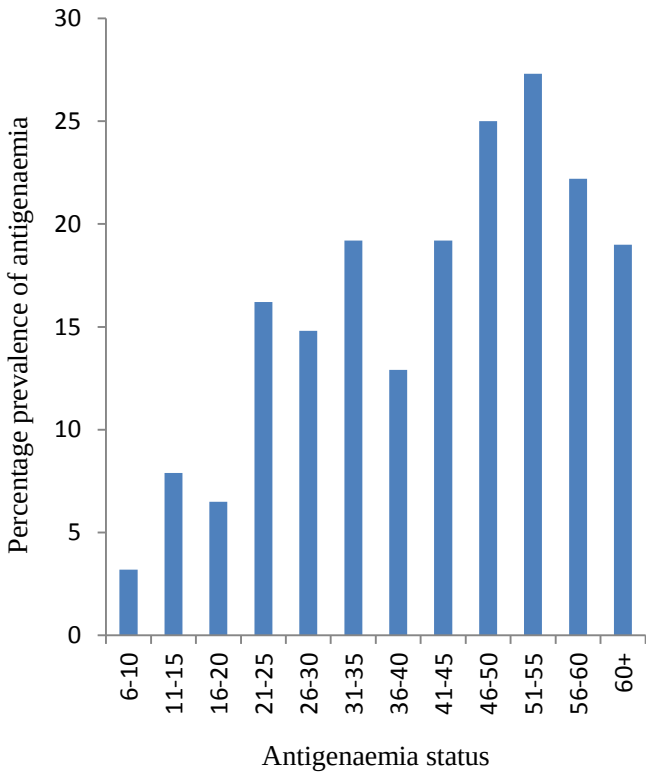

Figure 10

4.3. Prevalence of microfilaraemia during December 2014 parasitological survey

Of a total of 370 study participants, 15 (4%) individuals tested positive for mf using both counting chamber (CC) and thick smear (TS) techniques. These mf positive individuals were among the 48 persons who tested positive for CFA. Of the 48 CFA positives examined using CC and TS, 33 tested negative. The overall mf intensity (range and GMI) estimated based on CC and TS is presented in Table 3.

4.3.1. Prevalence and intensity of microfilaraemia in the study communities.

Out of 115 study participants examined from Akonu, 9 tested positive for mf, resulting in prevalence of 7.8%. A total of 255 subjects were examined from Princess, an mf prevalence of 2.4% (n=6) was observed. These prevalences were estimated based on the counting chamber technique. For the TS technique, 8 individuals tested positive for mf in Akonu representing 7%. Seven (7) positive cases were recorded at Princess, representing 2.7% of those examined. The mf intensity (range and GMI) for the respective communities estimated based on CC and TS are shown in Table 3.

Table 3: Microfilaria prevalence and mean intensity of infection among study participants from the study sites

Community	Mf detection technique	No. positive for mf	Mf Prevalence (%)	Mf Intensity for positives (mf/ml)	
				Range	GMI
Akonu	CC	9	7.8	1-226	12.5
	TS	8	7.0	1-50	6.2
Princess	CC	6	2.4	1-22	6.6
	TS	7	2.7	3-34	8.5
Mean prevalence/intensity	CC	15	4.0	1-226	9.7
	TS	15	4.0	1-50	7.2

4.3.2. Prevalence and intensity of microfilaraemia among gender

For both techniques employed for mf detection, more males tested positive than females. For the CC technique, out of the 15 mf positive study participants, 11 (7.9%) were males whilst 4 (1.7%) were females. The TS technique on the other hand detected mf positives in 10 males and 5 females, representing 7.2% and 2.2% respectively. The respective mf intensity range and the corresponding GMIs estimated based on CC and TS are shown in Table 4.

Table 4: Prevalence and intensity of microfilaraemia by gender among all study participants from the two study sites.

Gender	Mf detection technique	No. positive for mf	Mf Prevalence (%)	Mf Intensity for positives (mf/ml)	
				Range	GMI
Male	CC	11	7.9	1-226	10.2
	TS	10	7.2	1 - 50	8.3
Female	CC	4	1.7	4 - 13	8.6
	TS	5	2.2	2 - 10	5.4

4.3.3. Prevalence and intensity of microfilaraemia among different age groups

Microfilariae prevalence was high among study subject with age groups 46-50 years. Prevalence of 18.8% was recorded for this group using both mf detection techniques. For the age groups 6-10 years, 16-20 years and 51-55 years, no positive cases were recorded using both counting chamber and thick smear methods. The age group 41-45 years recorded the highest mf GMI (Table 5).

Table 5: Prevalence and intensity of microfilaraemia among different age groups of study participants from both study communities.

Age group	Mf detection technique	No. positive for mf	Mf Prevalence (%)	Mf Intensity for positives (mf/ml)	
				Range	GMI
6-10	CC	0	0.0	0	0.0
	TS	0	0.0	0	0.0
11-15	CC	3	3.4	1 – 6	4.1
	TS	1	1.1	2	3.0
16-20	CC	0	0.0	0	0.0
	TS	0	0.0	0	0.0
21-25	CC	1	2.7	4	5.0
	TS	1	2.7	3	4.0
26-30	CC	3	11.1	2 – 16	9.0
	TS	3	11.1	1 - 14	6.5
31-35	CC	1	3.8	1	2.0
	TS	2	7.7	3 - 4	4.5
36-40	CC	1	3.2	26	27
	TS	1	3.2	9	10
41-45	CC	2	7.7	10-226	50.0
	TS	2	7.7	10-19	14.9
46-50	CC	3	18.8	2-139	21.3
	TS	3	18.8	1 - 50	9.0
51-55	CC	0	0.0	0	0.0
	TS	0	0.0	0	0.0
56-60	CC	1	11.1	1	2.0
	TS	1	11.1	1	2.0
61+	CC	0	0.0	0	0.0
	TS	1	4.8	34	35.0

4.4. Prevalence of antigenaemia three months Post- MDA (March 2015 parasitological Survey)

Three months after treating CFA positive individuals with ivermectin and albendazole, 36 antigenaemia positive individuals were re-examined for CFA and mf in the month of March, 2015 out of the 48 who tested positive during the parasitological survey conducted in December 2014. The remaining 12 of the CFA positive individuals were absent during the second parasitological survey. Of the 36 individuals, 17 (47.2%) tested positive while 19 (52.8%) tested negative for CFA. Thus, the overall antigenaemia prevalence reduced by 52.8%, resulting in a prevalence of 5% after three months of treatment.

4.4.1. Prevalence of antigenaemia in the study communities.

Nineteen (19) individuals were re-examined in Akonu for antigenaemia prevalence. Ten [10 (52.6%)] individuals tested positive. In Princess, 17 individuals were re-examined and 7 (41.2%) tested positive. Thus, antigenaemia reduced by 47.4% and 58.8% in Akonu and Princess respectively, to 9.5% and 2.8%.

4.4.2. Prevalence of antigenaemia among gender.

Of the 36 study participants who were re-examined for *W. bancrofti* filarial antigen, 70.6 % (n=12) of the positive individuals were males while 29.4 % (n=5) were females. For the 19 CFA negative participants, 9, representing 47.4 % were males and 10, representing 52.6 %, were females. Antigenaemia prevalence therefore translated to 9.2% and 2.2% for males and females respectively.

4.4.3. Prevalence of antigenaemia among age groups

The age group 11-15 years had the highest number of participants (n=6) while the age groups 6-10 and 36-40 years had the least number of study participants (n=1). Treatment of study subjects reduced the prevalence rate by 100% in the age groups 6-10 and 36-40 years. There was no change in the CFA prevalence of the age group 46-50 after treatment (Table 6).

Table 6: Prevalence of antigenaemia among different age groups of study participants from both study communities.

Age range (Years)	No. of participants	Antigenaemia positive (%)	Antigenaemia negative (%)
6-10	1	0 (0)	1 (100)
11-15	6	4 (66.7)	2 (33.3)
16-20	3	1 (33.3)	2 (66.7)
21-25	5	1 (20)	4 (80)
26-30	3	1 (33.3)	2 (66.7)
31-35	3	1 (33.3)	2 (66.7)
36-40	1	0 (0)	1 (100)
41-45	3	2 (66.7)	1 (33.3)
46-50	3	3 (100)	0 (0)
51-55	2	1 (50)	1(50)
56-60	2	1 (50)	1 (50)
61+	4	2 (50)	2 (50)
Total	36	17 (47.2)	19 (52.8)

4.5. Prevalence of microfilaraemia three months Post-MDA (March 2015 Parasitological survey)

Among the 36 study participants re-examined for mf, the CC technique detected 4 (11.1%) individuals as being positive while the TS detected 1 (2.8%) individual positive. This translated to 32 (88.8%) individuals testing negative for the CC and 35 (97.2%) negative for the TS. The overall mf prevalence three months after treatment translated to 1% (CC) and 0.3% (TS). The overall mf intensity range and the corresponding GMI estimated based on CC and TS were as presented in Table 7.

4.5.1. Prevalence and intensity of microfilaraemia in the study communities

Nineteen (19) study participants from Akonu were re-examined, as a result, 3 (15.8%) tested positive for mf. At the same study area, the TS technique revealed zero positives. For Princess, a total of 17 subjects were tested, and for both techniques, 1 (5.9%) individual tested positive. Ninety-four percent [94.1% (n=16)] tested negative for both techniques. The prevalence translated to 2.8% (CC) and 0.4% (CC and TS) respectively for Akonu and Princess. The mf intensities range and the corresponding GMIs estimated in the study communities are shown in Table 7.

Table 7: Microfilaria prevalence and mean intensity of infection among study participants from the study sites.

Community	No. examined for mf	Mf detection technique	No. positive for mf	Mf prevalence (%)	Mf Intensity for positives (mf/ml)	
					Range	GMI
Akonu	19	CC	3	2.8	1-11	4.6
		TS	0	0	0	0.0
Princess	17	CC	1	0.4	1	2.0
		TS	1	0.4	1	2.0
Mean prevalence/intensity	36	CC	4	1	1-11	3.7
		TS	1	0.3	1	2.0

4.5.2. Prevalence and intensity of microfilaraemia among gender

For both techniques employed for mf detection, only males tested positive. The CC technique detected 4 (19%) of the males as being mf positive while the TS detected 1 (4.8%) positive. In females, mf prevalence decreased from 1.7% (CC) and 2.2% (TS) to 0% three months after the MDA. The mf prevalence estimated for the males corresponded to 3.1% (CC) and 0.8% (TS). The respective mf intensities and their corresponding GMIs estimated based on CC and TS techniques are shown in Table 8.

Table 8: Prevalence and intensity of microfilaraemia by gender among all study participants from the two study sites

Gender	No. examined for mf	Mf detection technique	No. positive for mf	Mf prevalence (%)	Mf Intensity for positives (mf/ml)	
					Range	GMI
Male	21	CC	4	3.1	1-11	3.7
		TS	1	0.8	1	2.0
Female	15	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0

4.5.3. Prevalence and intensity of microfilaraemia among the different age groups.

For all the age groups, mf prevalence reduced to zero except the age group 61+ where the TS technique detected 1 individual positive with mf density of 1mf/ml and a GMI of 2mf/ml. The CC technique detected 1 individual each among the age groups 11-15, 16-20, 41-45 and 61+ years as being positive for mf. The mf intensity ranges and the corresponding GMIs were as presented in Table 9.

Table 9: Prevalence and intensity of microfilaraemia among different age groups of study participants from both study communities.

Age group	No. examined for mf	Mf detection technique	No. positive for mf	Mf prevalence (%)	Mf Intensity for positives (mf/ml)	
					Range	GMI
6-10	1	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
11-15	6	CC	1	1.1	1	2.0
		TS	0	0.0	0	0.0
16-20	3	CC	1	2.2	3	4.0
		TS	0	0.0	0	0.0
21-25	5	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
26-30	3	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
31-35	3	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
36-40	1	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
41-45	3	CC	1	4.2	11	12.0
		TS	0	0.0	0	0.0
46-50	3	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
51-55	2	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
56-60	2	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
61+	4	CC	1	4.8	1	2.0
		TS	1	4.8	1	2.0

4.6. Prevalence of antigenaemia six month Post-MDA (June 2015 Parasitological survey)

Thirty-five (35) CFA positive individuals recorded during the December 2014 parasitological survey were re-examined for CFA and mf in the month of June, 2015. Of these 35 individuals, 29 were the same persons examined in March 2015 while 6 were absent but resurfaced for the third survey. Thirteen persons (13) were therefore absent for the third parasitological survey. Twenty-seven [27 (77.1%)] tested positive while 8 (22.9%) tested negative (Figure 11). The overall CFA prevalence determined was 7.6%.

In the study communities, Nineteen (19) individuals were re-examined in Akonu, 16 (84.2%) tested positive while 3 (15.8%) tested negative. In Princess, 16 individuals were screened, 11 (68.8%) tested positive while 5 (31.2%) tested negative. The community antigenaemia prevalence translated to 15% and 4.4% for Akonu and Princess respectively.

In terms of gender, in total 19 males were re-examined. Fifteen [15 (79%)] tested positive and 4 (21%) tested negative. Sixteen (16) females were also screened for CFA, 12 (75%) tested positive and 4 (25%) tested negative. The CFA prevalence for the males and females was 11.6% and 5.3% respectively.

For the age groups, the highest number of positive cases was recorded in the age group 11-15 years [5 persons (18.5%)] [Figure 12].

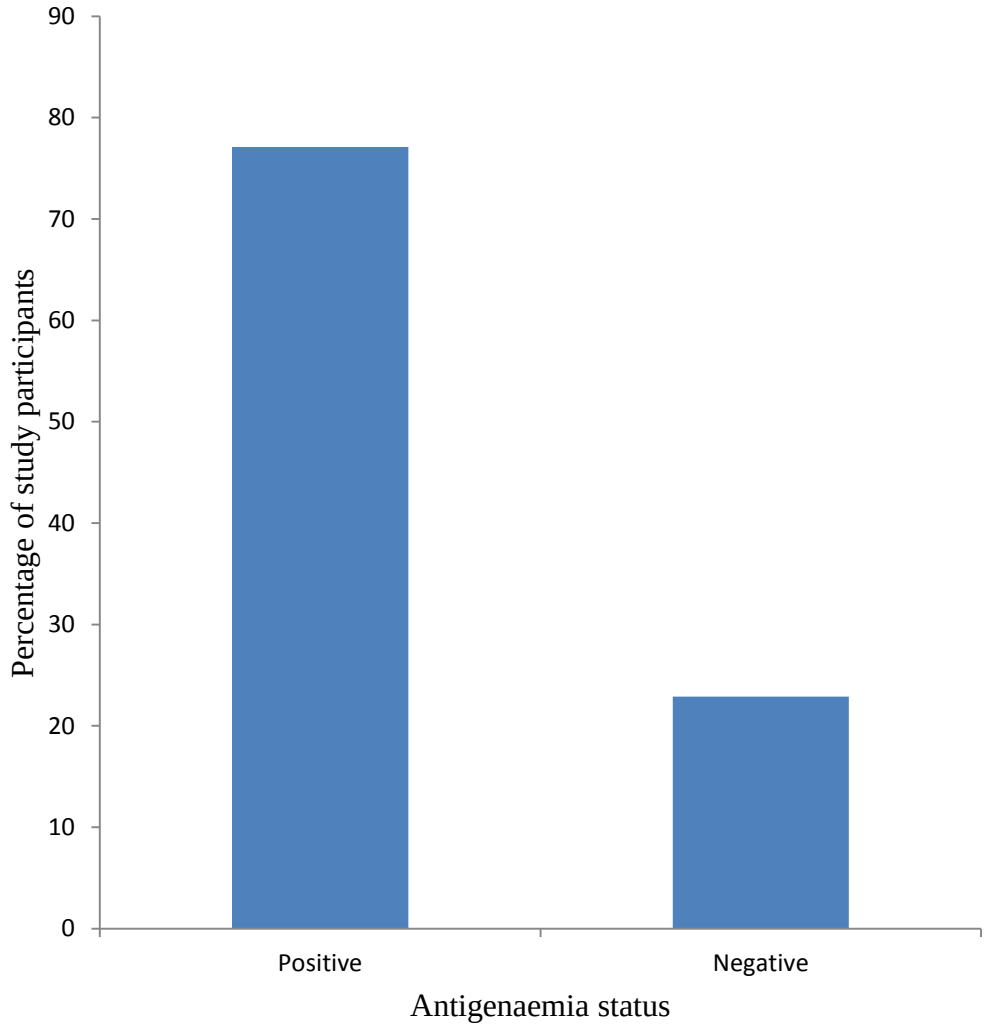

Figure 11: Antigenaemia status among all the study participants six months after MDA with ivermectin and albendazole.

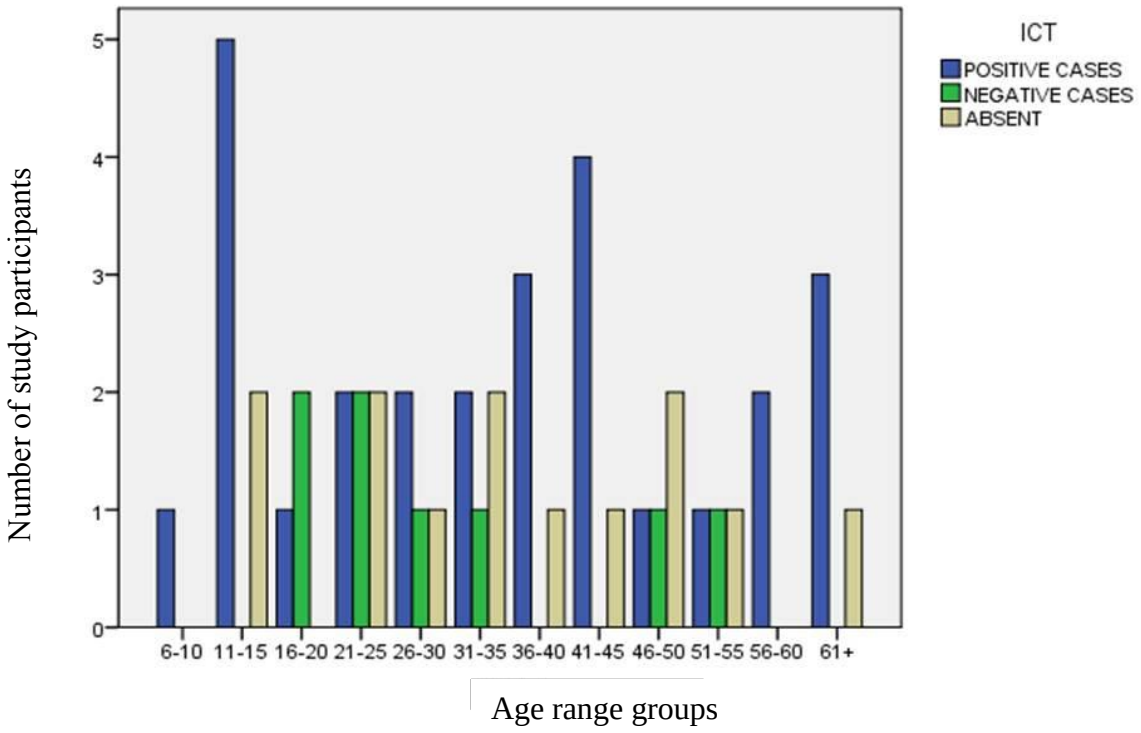

Figure 12: Overall antigenaemia status in the different age range groups from both communities six months after MDA.

4.7. Prevalence of microfilaraemia six months Post-MDA (June 2015 parasitological survey)

The CC technique detected 4 (11.4%) individuals positive for mf while the TS detected 1 (2.9%) positive individual. The overall mf prevalence estimated based on the CC and TS were 1% and 0.3% respectively. The overall mf intensity range and the corresponding GMIs estimated were as presented in Table 10.

4.7.1. Prevalence and intensity of microfilaraemia in the study communities

For the third parasitological survey, 19 persons were screened from Akonu, with the CC detecting 2 (10.5%) persons as mf positives. The TS identified 1 (5.3%) person as being mf positive. At Princess, 16 subjects were tested; the CC detected 2 (12.5%) individuals who were mf positive. The prevalence and intensity of mf in the study communities were as shown in Table 10.

Table 10: Microfilaria prevalence and mean intensity of infection among study participants from the study sites.

Community	No. examined for mf	Mf detection technique	No. positive for mf	Mf prevalence (%)	Mf Intensity for positives (mf/ml)	
					Range	GMI
Akonu	19	CC	2	2	9-10	10.5
		TS	1	0.9	6	7.0
Princess	16	CC	2	0.8	2	3.0
		TS	0	0.0	0	0.0
Mean prevalence/GMI	35	CC	4	1	2-10	5.6
		TS	1	0.3	6	7.0

4.7.2. Prevalence and intensity of microfilaraemia by gender

All the mf positive cases detected by both techniques were males. The CC detected 4 (21%) males mf positive cases whilst the TS detected a single (5.3%) male positive case. The 16 females examined for mf were all mf negatives. The prevalence and intensity of mf according to gender is shown in Table 11.

Table 11: Prevalence and intensity of microfilaraemia by gender among all study participants from the two study sites.

Gender	No. examined for mf	Mf detection technique	No. positive for mf	Mf prevalence (%)	Mf Intensity for positives (mf/ml)	
					Range	GMI
Male	19	CC	4	3.0	2-10	5.6
		TS	1	0.8	6	7.0
Female	16	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0

4.7.3. Prevalence and intensity of microfilaraemia among age groups

For the age groups, the CC revealed a person each from the age groups 16-20, 31-35, 41-45 and 61+ as mf positive. The TS on the other hand, showed only 1 person from the age group 16-20 to be mf positive. The prevalence and intensity of mf by age groups was as displayed in Table 12.

Table 12: Prevalence and intensity of microfilaraemia among different age groups of study participants from both study communities.

Age group	No. examined for mf	Mf detection technique	No. positive for mf	Mf prevalence (%)	Mf Intensity for positives (mf/ml)	
					Range	GMI
6-10	1	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
11-15	5	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
16-20	3	CC	1	2.2	9	10.0
		TS	1	2.2	6	7.0
21-25	4	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
26-30	3	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
31-35	3	CC	1	4.2	2	3.0
		TS	0	0.0	0	0.0
36-40	3	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
41-45	4	CC	1	4.0	10	11
		TS	0	0.0	0	0.0
46-50	2	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
51-55	2	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
56-60	2	CC	0	0.0	0	0.0
		TS	0	0.0	0	0.0
61+	3	CC	1	5.0	2	3.0
		TS	0	0.0	0	0.0

4.8. Microfilariae sensitivities and intensities using counting chamber (CC) and thick smear (TS) techniques

At the first (December 2014) parasitological survey, 15 microfilaraemia cases were simultaneously detected with both diagnostic techniques. Three months later, the CC detected 4 persons to be mf positive while the TS detected 1 person as being mf positive. Similar results were obtained six months (June 2015) after the first parasitological survey. The sensitivity and intensities of mf infection in relation to CC and TS techniques were as shown in Table 13 and 14 respectively.

Table 13: Sensitivity of counting chamber (CC) in comparison to thick smear (TS) technique in the detection of mf.

Parasitological surveys	No. positive for CFA	Mf detection technique	No. positive for mf (%)	No. negative for mf (%)
Pre-treatment (Dec. 2014)	48	CC	15 (31.2)	33 (68.8)
		TS	15 (31.2)	33 (68.8)
3-months Post-MDA (March 2015)	17	CC	4 (23.5)	13 (76.5)
		TS	1 (6.0)	16 (94.0)
6-months Post-MDA (June 2015)	27	CC	4 (15.0)	23 (85.0)
		TS	1 (3.7)	26 (96.3)

The Mann-Whitney statistic determined was 12.5 and the associated p-value corresponded to 0.5127. The p-value > 0.05, suggesting the CC and the TS were not significantly different in terms of their sensitivity in mf detection

Table 14: The prevalence and intensity of microfilaria estimated using counting chamber (CC) and thick smear (TS) techniques among all study participants at various stages.

Parasitological survey	Mf detection technique	Mf prevalence (%)	Intensity (mf/ml)	
			Range	GMI
Pre-treatment (Dec. 2014)	CC	4	1-226	9.7
	TS	4	1-50	7.2
3-months Post-MDA (March 2015)	CC	1	1-11	3.7
	TS	0.3	1	2.0
6-months Post-MDA (June 2015)	CC	1	2-10	5.6
	TS	0.3	6	7.0

Using the Mann-Whitney test to test for the statistical significance of the CC and TS in terms of mf prevalence; the test statistic determined was 12.5 with the associated p-value translating to 0.5127. The P-value > 0.05 suggesting the mf prevalence estimated based on CC technique in the three parasitological surveys were statistically similar with that estimated based on the TS.

In terms of GMI, the Mann- Whitney statistic was 11.0 with an associated p-value of 1.0. The p-value > 0.05 suggesting there were statistically no significant differences between the GMIs observed with both techniques.

4.9. Entomological survey(s)

Mosquitoes collected from the study communities were sorted into the different species of *Aedes, Anopheles* and *Culex* and investigated for *W. bancrofti* infection through dissection. Molecular analysis was carried out on selected *An. gambiae* s.l for molecular identification. Entomological indices were also estimated to determine the transmission status in the study communities.

4.9.1. Species composition of collected mosquitoes

In the December 2014 collection, a total of 98 mosquitoes were collected in the study communities. After morphological examination, 96 (98%) were identified as *Anopheles gambiae* and 2 (2%) as *Culex* species.

For the March 2015 collection, 34 mosquitoes were collected. Morphologically, 29 (85%) were identified as *An. gambiae*, 1 (3%) as *Aedes* spp., and 4 (12%) as *Culex* spp. In all a total of 132 mosquitoes were collected for the study.

There was no mosquito collection during the June 2015 survey. This was due to the demanding nature of work whenever mosquito collection was done alongside with parasitological survey.

4.9.2. Molecular identification of *Anopheles gambiae* complexes

Of the *An. gambiae* s.l collected, a total of 22 (18%) were randomly selected for sibling species identification using molecular methods. Molecular studies identified all of them as *An. melas*.

4.9.3. Estimation of entomological indices for *An. gambiae* s.l

Each of the 132 mosquitoes caught in the study communities were teased and observed for the presence of mf or any of the larval stages (L_1-L_3) of *W. bancrofti* using microscopy. Three L_2 stages of *W. bancrofti* were recovered from one *An. melas* (Plate 8).

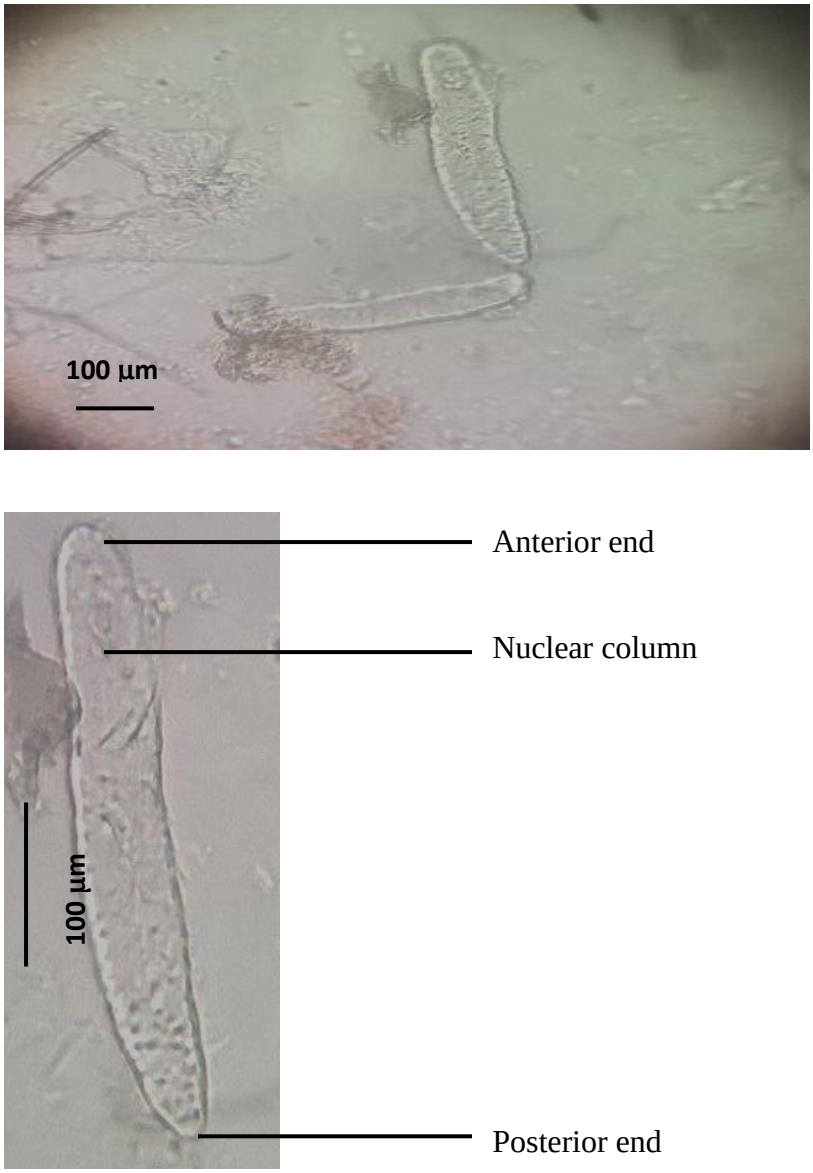

Plate 8: The second stage (L_2) larvae of *W. bancrofti* found in the thoracic muscles of a dissected *An. melas* collected at Agona Princess during the March 2015 mosquito collections.

4.9.3.1. Biting rate

Six (5 blood fed and 1 unfed) and 119 (108 blood fed and 11 unfed) *An. gambiae* s.l were dissected in Akonu and Princess respectively. The respective biting rates were 0.13 and 0.33 bites/person/night. The MBR for the two study communities therefore were 3.9 and 9.9 bites/person/month respectively. The ABR was estimated to the respective 47.5 and 120.5 bites/person/year for Akonu and Princess.

4.9.3.2. Infection and Infectivity prevalence of collected mosquitoes

The infection prevalence of mosquitoes for Akonu and Agona Princess were 0 and 0.84% respectively. As examined by microscopy, no mosquito was found to be infected with the L_3 stage of *W. bancrofti* therefore, the infectivity prevalence was zero. All the other entomological indices calculated was zero.

4.10. Molecular identification of *Wuchereria bancrofti*

Genomic DNA was extracted from the dried carcasses of all the 132 mosquitoes for molecular identification of *W. bancrofti*. The extracted DNA from the dried carcasses were used for the molecular amplification of *W. bancrofti*. Electrophoresis of the PCR products showed DNA amplification for the only positive sample examined for *W. bancrofti* with a diagnostic band size of 188bp as shown in plate 9 below. There were no DNA amplifications for the other 131 negative samples examined for *W. bancrofti*.

Plate 9: Gel electrophoregram for the identification of *W. bancrofti* from mosquito samples. Lane M = 100bp molecular weight marker, Lane 1 = *W. bancrofti* genomic DNA, Lane 2 - 4 = Negative samples, Lane 5 = negative control and Lane 6 = positive control.

CHAPTER FIVE

DISCUSSION

Following the launching of GPELF in the year 2000 with the goal of global elimination of LF as a public health problem, national control programmes have been established in most endemic countries where annual MDAs with the recommended two-drug regimens have been implemented (WHO, 2010). Regular annual monitoring of the effect of the MDA is important to evaluate the progress of the programme and to look for evidence to help identify areas where MDAs can be discontinued, or where more efforts and alternative control tools need to be implemented. Systematic evaluations and assessments of the effect of MDAs on transmission and human infection in sentinel sites have been documented in countries using the DEC/albendazole combination such as Egypt (Ramzy *et al.*,2006), Papua New Guinea (Weil *et al.*, 2008), American Samoa (Liang *et al.*, 2008), India (Ramaiah *et al.*, 2011) and Samoa (Joseph *et al.*, 2011).

Evaluation of the effect of ivermectin and albendazole drug combinations on mf prevalence and density was carried out in 2007 in 15 sentinel sites in Ghana and the results have been documented (Ghana Filariasis Elimination Programme, 2008). The goal of this study was to evaluate and analyse the impact of MDAs on LF infection and transmission in two endemic coastal communities in the Western Region of Ghana. The west coast of Ghana is well known to be endemic for LF, and has been a focus of comprehensive research in the past. Prior to the commencement of Ghana Filariasis Elimination Programme, Dunyo *et al.* (1996) identified the west coast of the country as LF endemic with overall mf prevalence of 9-25% and intensities of 321-1172 mf/ml of blood.

The antigen prevalence of LF reported by the Ghana Neglected Tropical Diseases Programme, 2013-2017 strategic plan, was between 20-40% in the northern and 10-20% in the southern part of the country. However, this study revealed an overall antigenaemia of

13% after ten rounds of MDA. The overall mf prevalence detected by both CC and TS techniques in this study corresponded to 4%. Though the antigen and mf prevalence is within the range of figures reported by Neglected Tropical Diseases Control Programme (NTDCP) and Dunyo *et al.* (1996) respectively, the figures are high since the target set by WHO for LF not to be considered a public health problem is a prevalence of below 2% for antigenaemia and 1% for microfilaraemia (WHO, 2012).

Three months after treatment of individuals positive for *W. bancrofti* infections, the overall antigen prevalence reduced by 52.8% leading to a prevalence rate of 5%. Microfilaria prevalence on the other hand reduced by 88.9% (CC) and 97.2% (TS) indicating mf prevalence of 1% (CC) and 0.3% (TS). This result is in consonance with a similar study conducted by Simonsen *et al.* (2014) where CFA and mf prevalence of 15.5% and 3.5% respectively decreased by 75.5% and 89.6% after treatment with ivermectin and albendazole. The third parasitological survey recorded an increase in antigenaemia prevalence (7.6%) while the mf prevalence remained the same. The increase in antigen prevalence was partly due to 25% CFA positive individuals being absent for the second parasitological survey but resurfaced for the third survey. Also 23% CFA negative individuals who were recorded during the second parasitological survey absented themselves during the third parasitological survey. Since these individuals were mf-negatives, no change in the mf prevalence was observed in the third parasitological survey.

In the study communities, initially antigenaemia was 23.5% for Akonu and 8.2% for Agona Princess. Three months after treatment, the rate reduced to 9.5% and 2.8% respectively. The third parasitological survey recorded an increase in the prevalence rate (15% for Akonu and 4.4% for Princess).

Microfilariae prevalence rates of 7.8% (CC), 7.0% (TS) and 2.4% (CC), 2.7% (TS) were initially detected in Akonu and Princess respectively. Three months after the MDA

intervention, the mf prevalence reduced by 84.2% (CC), 100% (TS) and 94.1% (both CC and TS) for Akonu and Princess respectively. The mf prevalence rates detected therefore corresponded to 2.8% (CC) and 0.4% (CC and TS) respectively for Akonu and Princess. The third parasitological survey also recorded similar mf prevalence to that of the second [2% (CC), 0.9% (TS) and 0.8% (CC) for Akonu and Princess respectively]. Data obtained from the administrative districts of Akonu and Agona Princess, reveals mf prevalence of 2.8% and 0.7% for the Axim municipal and Ahanta West District respectively (NTDCP, 2014). With these mf prevalence rates, Agona Princess can be considered eligible for transmission assessment surveys (TAS) to move to the post-MDA surveillance phase for discontinuation of MDA, if only this level of prevalence can be sustained, considering that the area has had at least 10 rounds of MDA. According to WHO (2012), an implementation unit is considered eligible for TAS when at least 5 rounds of MDA have been implemented, MDA coverage exceeds 65% in the total population of the unit and the prevalence of infection in sentinel and spot-check sites is below 1% for the presence of mf.

On the screening tools used for *W. bancrofti* detection, this study revealed that the ICT card test is more sensitive in detecting mf carriers in comparison with the CC and TS techniques, as all the mf-positive samples detected by both techniques were also positive for CFA by the ICT card. In addition to the mf carriers, the ICT card test detected 33 (68.8%) mf-negative persons (estimated by CC and TS techniques) as CFA positive during the December 2014 parasitological survey. During the March 2015 survey, 76.5% and 94.0% mf-negative individuals estimated based on CC and TS respectively were CFA positives. The third parasitological survey also recorded similar result. This could be due to differences between the screening tools used. While the CC and TS techniques detect only mfs, the ICT card test detects CFA shed by both adult worms and mfs (Weil *et al.*, 1997). *Wuchereria bancrofti* diagnostic techniques based on direct detection of mf can be insensitive because mf may be

absent from the circulation despite infection with other stages of the parasite or may be present in very low numbers (Wamae and Njenga, 2008). Some infected individuals may also be harbouring growing worms or non-fecund adults or adults of a single sex and may be CFA positives (Pani and Dhanda, 1994; Pani and Lall, 1998). Therefore, it is not startling that a large number of CC and TS mf-negative individuals were positive for the ICT card test. Njenga and Wamae (2001) reported that the sensitivity of the ICT card test was greater than that of microfilarial diagnostic techniques during a study conducted in Kenya to evaluate the ICT card test.

In terms of the sensitivity of the CC and TS in microfilarial determination, Agbolade and Akinboye (2005) reported a higher sensitivity of the CC technique (79.3%) than the TS technique (39.1%). The study done by Agbolade and Akinboye (2005) was in conformity with previous findings by Denham *et al*. (1971) and McMahon *et al*. (1979). This study has also shown a relatively high microfilaraemic positivity sensitivity of the CC technique than TS technique but statistically there was no significant difference between the two diagnostic techniques.

According to Southgate (1992), low-density mf is the density of circulating mf that cannot be detected in a significant number of instances when standard survey techniques are employed. Quantitatively, it is defined as a count of less than 4 mf/20µl (200 mf/ml) of capillary blood or less than 30 mf/ml of venous blood. In this study, 100µl and 60µl of venous blood was used in the counting chamber and thick smear techniques respectively for the detection of mf. As such it could be deduced that the overall mean mf intensity of 9.7 and 7.2 mf per ml of blood for CC and TS respectively in the entire study for mf positive individuals were really low. Three months after intervention, the overall GMIs for positive individuals reduced drastically to 3.7 and 2.0 mf per ml of blood using the respective CC and TS techniques. The third parasitological survey also recorded overall GMIs of 5.6 and 7.0 mf

per ml for CC and TS respectively. Statistically, there were no significant differences between the GMIs observed with both techniques. This might have been due to low microfilarial intensities of *W. bancrofti* in the study areas. Agbolade and Akinboye (2005) also reported a similar result when they used both techniques in detection of *Loa loa* and *Mansonella perstans* in three Nigerian rural communities. Though mf GMIs calculated on the basis of mf positives were low in this study, these individuals remain an important source of mf reservoirs for the continued transmission of LF.

Microfilariae prevalence decreased progressively in this study after treatment. This observation therefore seems to emphasize the importance of regular treatment of individuals having *W. bancrofti* infections.

The mosquito species composition remained considerably the same as has been reported for previous studies, that is, predominantly Anopheline. This observation has been documented by Appawu *et al.* (2001), Dunyo *et al.* (1996) and Dzodzomenyo *et al.* (1999) where they implicated *An. gambiae* s.l as the main vector of LF in Ghana. Molecular studies for species identification revealed the sibling *An. gambiae* s.l vectors as predominately *An. melas*. There was an apparent decrease in vector densities and biting rates between the December 2014 and March 2015 collections.

The ability of mosquito vectors to transmit *W. bancrofti*, particularly at low mf intensity vary and three density-dependent processes; limitation, facilitation and proportionality have been described to explain them (Southgate and Bryan, 1992). “Limitation” describes a process where the vectors are competent and transmission is stable at very low parasitaemia levels and “facilitation” for the vectors that are competent mainly at high parasitaemia levels. For “proportionality”, there is a constant percentage (linear relationship) of mf ingested by the vector during a blood meal developing to the infective stage (Southgate and Bryan, 1992). Southgate & Bryan (1992) in their studies identified *Anopheles* species as exhibiting the

process of facilitation, and therefore it is presumed that low level circulating mfs in the human population resulting from MDA would lead to interruption of transmission and elimination of lymphatic filariasis in Anopheline transmission areas. However, other studies (Amuzu *et al.*, 2010) seem to suggest that other *An. gambiae* s.l sibling members may not necessarily exhibit facilitation. For example, they suggested that *An. melas* exhibits limitation and hence in areas where the major vector is the above mentioned species, other control tools such as vector control may be required to augment the MDAs in order to reach set targets for disease control and elimination.

The extent of current transmission could be defined through entomological indicators. The approach involves dissection and examination of mosquitoes for the presence of *W. bancrofti* larvae. Molecular xenomonitoring has also become a relevant tool especially in areas where transmission is very low and large numbers of mosquitoes have to be dissected to accurately determine the level of transmission. For this study, transmission was assessed by dissection of mosquito vectors and molecular analysis using the PCR. Dissection result showed the recovery of three L_2 stages in the thoracic muscles of one *An. melas*, the principal vector at Agona Princess. Molecular analysis also confirmed the presence of *W. bancrofti* DNA in the infected mosquito. The low infection rate may be due to the decrease in *W. bancrofti* mf prevalence over the course of the study. This could also suggest that the reduction in human mf infection was primarily due to the treatment of CFA and mf positive individuals with ivermectin and albendazole. Evidence from Tanzania revealed that MDAs has succeeded in reducing population levels of *W. bancrofti* (Simonsen *et al*, 2010), and a combination of ivermectin and albendazole appears to give a faster but shorter-lived reduction in microfilaria rates than DEC and albendazole (Fernando *et al.*, 2011). This may account in part for the bounce back of CFA prevalence six months after the MDA. In Kenya, a study has proven that a reduction in mf levels can be maintained even when some rounds of MDA have been

missed (Njenga, 2011). Contrarily, there is evidence for a declining effect of MDA after multiple rounds of treatment, resulting in residual transmission (Simonsen *et al*, 2010). This waning effect of MDA after multiple rounds may be a result of incomplete coverage of the population, or the inadequate macrofilaricidal effect of drug combinations used (Ashton *et al*., 2011). Further, drug resistance to both albendazole and ivermectin has been reported in parasitic nematodes of veterinary importance (Prichard, 1990; Wolstenholme *et al*. 2004), suggesting that the possible development of drug resistance in humans cannot be underestimated. Schwab *et al*. (2007) in their studies reported that under albendazole and ivermectin combination for treatment, selection for resistance to one drug is enhanced if resistance to the other drug is already present. Moreover, the issue of non-compliance to MDA by LF endemic populations may be a factor affecting the waning effect of the program in reducing mf prevalence and density. Offei and Anto (2014) established this fact when they reported only 43.8% of eligible community members in Ahanta West District of Ghana complying with MDA.

In this study, no infective stage larva was detected in any of the dissected mosquitoes. This could due to the fact that very few mosquitoes were caught and examined for infective larvae. The mosquito collections were done during the dry season, hence the low number. The method used in the collection of the mosquitoes, that is, spray catches could also have contributed to the low mosquito numbers obtained. This is a major challenge in the study. In LF transmission assessment, collection of numerous mosquitoes is necessary for detection of infective ones especially when transmission level is low.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

In 2008, the Ghana Filariasis Elimination Programme reported a downward trend in *W. bancrofti* transmission and human infection in 15 sentinel sites in Ghana after five annual rounds of MDA. Meanwhile, after at least 5 rounds of MDA, it is expected that mf should reach a minimum threshold in the human population such that transmission would be interrupted. However, LF was still widespread in many parts of the country after 10 rounds of MDA. This called for a heightened control programme in order to reach the targeted infection levels below which transmission could no longer be sustained.

This study has documented a decrease in *W. bancrofti* infection in humans in previously highly endemic areas of the west coast of Ghana. The study areas had received multiple rounds of MDA with albendazole and ivermectin. Ten annual rounds of MDA, has brought parasitaemia to low levels in the study communities. Low levels of circulating mf in the human population might have contributed to the zero recovery of infective stage larvae of *W. bancrofti* in the *Anopheles* mosquitoes. The very low numbers of collected mosquitoes could also have contributed to the zero recovery of the infective stage larvae of *W. bancrofti*.

6.2. RECOMMENDATIONS

In this study, very few mosquitoes were caught and examined for infective larvae. As such no infective larva was recovered in the dissected mosquitoes. Individuals willing to take similar projects would have to collect large number of mosquitoes for dissection to accurately determine the level of transmission in endemic areas and or use a different mosquito

collection methods that yields more mosquitoes, such as human landing catches (HLC) instead of the spray catches used in this study.

Because of the low levels of mf intensities in the study areas, subsequent study would have to employ larger sample sizes during parasitological surveys to precisely determine the mf prevalence rate.

Though human infections and transmission had declined, extra efforts have to be made in order to achieve the recommended target cut-off level of 2% CFA (for both Akonu and Princess) and 1% mf (for Akonu) for interrupting transmission and stopping MDA is reached. The extra effort could specifically include intense public education and engagement of the target populations in control activities, to ensure higher treatment coverages.

It is also recommended that vector control be added to the programme to include the distribution of insecticide treated bed nets to every household in the endemic areas to speed up the attainment of the set target for elimination. Environmental management to limit breeding of the vectors should also be encouraged. Monitoring and evaluation could also continue to play a principal role to guide the programme and to ensure that the current major achievements will ultimately lead towards the elimination of the programme.

Finally, there is the need for intensive research to monitor parasite repopulation rates and establish the possible development of drug resistance to ivermectin and albendazole in LF endemic areas in the country following several years of MDA.

REFERENCES

Aboagye-Antwi, F. (2003). Studies on the roles of *Culex* and *Anopheles* species in the transmission of *Wuchereria bancrofti* (Spirurida: Filariidae) in the Gomoa District of Ghana. M Phil Thesis, Zoology Department, University of Ghana.

Addis, D. G. and Brady, M. A. (2007). Morbidity management in the Global Programme to Eliminate Lymphatic Filariasis: a review of the scientific literature. *Filaria Journal*, 6 (2), 1-19.

Agbolade, M. A. and Akinboye, D. O. (2005). Detection of microfilariae with counting chamber technique in some Nigerian rural communities. *African Journal of Biotechnology*, 4 (4), 367-370.

Ahorlu, C. K, Dunyo, S. K, Koram, K. A, krumah, F. K, Aagaard-Hansen, J., Simonsen, P. E. (1999). Lymphatic filariasis related perceptions and practices on the coast of Ghana. Implication for prevention and control. *Acta Tropica*, 73 (3), 251-264.

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. *Parasite & Vectors*, 3, 1-6.

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. *Tropical Medicine and International Health*, 6 (7), 511-516.

Al-Olayan, E. M., Williams, G. T., and Hurd, H. (2002). Apoptosis in the malaria protozoan, *Plasmodium berghei*: a possible mechanism for limiting intensity of infection in the mosquito. *International Journal for Parasitology 32*, 1133-1143.

Ashton *et al.* (2011). The impact of mass drug administration and long-lasting insecticidal net distribution on *Wuchereria bancrofti* infection in humans and mosquitoes: an observational study in northern Uganda. *Parasites & Vectors*, 4, 134.

Bain, O. (1971). Transmission des filarioses. Limitation des passages de microfilaires ingérées vers l'hémocèle des vecteurs; interprétation. *Ann. Parasitol. Hum. Comp*., 46.

Beckett, E. B. (1971). Histological changes in mosquito flight muscle fibres associated with parasitization by filarial larvae. *Parasitology*, 63, 365-372.

Beerntsen, B. T., Luckhart, S. and Christensen, B. M. (1989) *Brugia malayi* and *Brugia pahangi*: inherent difference in immune activation in the mosquitoes *Armigeres subalbatus* and *Aedes aegypti. Journal of Parasitology,* 75 (1), 76-81.

Bernhard, P., Makunde, R.W., Magnussen, P., and Lemnge, M.M. (2000). Genital manifestations and reproductive health in female residents of a *Wuchereria bancrofti*-endemic area in Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 94 (4), 409- 412.

Black IV, W. C., Bennett, K. E., Gorrochotegui-Escalante, N., Barillas-Mury, C. V., Fernandez-Salas, I., de Lourdes Munoz, M., Farfan-Ale, J. A., Olson, K. E., and Beaty, B. J. (2002). Flavivirus susceptibility in *Aedes aegypti*. *Archives of Medical Research* 33, 379-388.

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. *Annals of Tropical Medicine and Parasitology,* 98 (5), 501-508.

Bockarie, M. (1994). Can lymphatic filariasis be eradicated in Papua New Guinea? *Papua New Guinea Medical Journal*, 37, 61-64.

Bockarie, M., Pedersen, E., White, G., Michael, E. (2009). Role of Vector Control in the Global Programmme to Eliminate Lymphatic Filariasis. *Annual Review of Entomology*, 54, 469-487.

Brengues, J., and Bain, O. (1972). Passage des microfilaires de l'estomac vers l'hemocele du vecteur, dans les couples *Wuchereria bancrofti - Anopheles gambiae* A, *Wuchereria bancrofti - Aedes aegypti et Setaria labiatopapillosa - Aedes aegypti*. *Cah. O. R. S. T. O. M. Ser Entomol Med Parasitol.* 10, 235-249.

Brito, A. C., Williams, P., Fontes, G. and Rocha, E. M. M. (1997). A comparison of two Brazilian populations of *Culex quinquefasciatus* (Say, 1823) from endemic and non- endemic areas to infection with *Wuchereria bancrofti* (Cobbold, 1877). *Memórias do Instituto Oswaldo Cruz*, 92 (1), 33-36.

Bryan, J. H., McMahon, P. and Barnes, A. (1990). Factors affecting transmission of *Wuchereria bancrofti* by Anopheline mosquitoes. 3. Uptake and damage to ingested microfilariae by *An. gambiae, An. arabiensis, An. merus* and *An. funestus* in East Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 84, 265-268.

Bryan, J. H., and Southgate, B. A. (1988). Factors affecting transmission of *Wuchereria bancrofti by* Anopheline mosquitoes. 2. Damage to ingested microfilariae by mosquito foregut armatures and development of filarial larvae in mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 82 (1), 138–145.

Calheiros, C. M. L., Fontes, G., Williams, P. and Rocha, E. M. M. (1998). Experimental infection of *Culex (Culex) quinquefasciatus* and *Aedes (Stegomyia) aegypti* with *Wuchereria bancrofti*. *Memorias do Instituto Oswaldo Cruz*, 93 (6), 855-860.

Cano, J., Rebollo, M. P., Golding, N., Pullan R. L., Crellen, T., Soler, A., Kelly- Hope, L., Lindsay, S. W., Hay, S. I, Bockarie, M.J. and Brooker, S.J. (2014). The global distribution and transmission limits of lymphatic filariasis: past and present. *Parasites & Vectors,* 7, 1-19.

Castillo, J. C., Robertson, A. E. and Strand, M. R. (2006). Characterization of hemocytes from the mosquitoes *Anopheles gambiae* and *Aedes aegypti*. *Insect Biochemistry and Molecular Biology,* 36 (12), 891-903.

CDC (1993). Recommendations of the International Task Force for Disease Eradication. Morbidity and Mortality Weekly Report. Centers for Disease Control and Prevention, Atlanta.

CDC (2013). Global Health, Division of Parasitic Diseases and Malaria

CDC (2014).Global Health, Division of Parasitic Diseases and Malaria.

Chanteau, S., Luquiaud, P., Failloux, A. B. and Williams, S. A. (1994). Detection of *Wuchereria bancrofti* larvae in pools of mosquitoes by the polymerase chain reaction. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 88 (6), 665-666.

Cheng, J. T., Mohan, S., Nasr, S. H., and D'Agati, V. D. (2006). Chyluria presenting a milky urine and nephrotic-range proteinuria. *Kidney International,* 70 (8), 1518-1522.

Chen, C. C. and Shih, C. M. (1988). Exsheathment of microfilariae of *Brugia pahangi* in the susceptible and refractory strains of *Aedes aegypti*. *Annals of Tropical Medicine and Parasitology,* 82**,** 201-206.

Christensen, B. M., Sutherland, D. R., (1984). *Brugia pahangi*: Exsheathment and midgut penetration in *Aedes aegypti*. *Transactions of the American Microscopical Society,* 103, 423-433.

Christensen, B. M., Li, J, Chen, C-C. *et al*. (2005). Melanization immune responses in mosquito vectors. *Trends in Parasitology*, 21 (4), 192-199.

Crans, W.J. (1973). Experimental infection of *Anopheles gambiae* and *Culex quinquefasciatus pipiens fatigans* with *Wuchereria bancrofti* in coastal East Africa. *Journal of Medical Entomology*, 10, 189-193.

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. *Journal of Vector Ecology*, 21, 1-5.

de Souza, D.K. (2010). Diversity in *Anopheles gambiae* s.s and *Wuchereria bancrofti*, and the Distribution of Lymphatic Filariasis in Ghana. PhD Thesis, Kwame Nkrumah University of Science and Technology.

de Souza, D. K., Koudou, B., Kelly-Hope, L. A., Wilson, M. D., Bockarie, J. M. and Boakye, D. A. (2012). Diversity and transmission competence in lymphatic filariasis vectors in West Africa and the implications for accelerated elimination of Anopheles- transmitted filariasis. *Parasites & Vectors*, 5, 259.

de Souza, D. K., Sesay, S., Moore, M. G. *et al* (2014). No Evidence for Lymphatic Filariasis Transmission in Big Cities Affected by Conflict Related Rural-Urban Migration in Sierra Leone and Liberia. *PLOS Neglected Tropical Diseases*, 8 (2), 1-6.

De Vries, C.R. (2002). The role of the urologist in the treatment and elimination of lymphatic filariasis worldwide. *BJU International*, 89(suppl. 1), 37-43.

Denham, D. A., Dennis, D. T., Ponnudurai, T., Nelson, G. S. and Guy, F. (1971). Comparison of a counting chamber and thick smear methods of counting microfilariae. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 65, 521-526

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. *Malaria Journal*, 11, 188.

Dickerson, J. W., Eberhard, M. L. and Lammie, P. J. (1990). A technique for microfilarial detection in preserved blood using nuclepore filters. *Journal for Parasitology*, 76, 829-833.

Dreyer, G., Medeiros, Z., Netto, M.J., Leal, N.C., de Castro, L.G. and Piessens, W.F. (1999). Acute attacks in the extremities of persons living in an area endemic for bancroftian filariasis: Differentiation of two syndromes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 93(4), 413-417.

Dreyer, G. *et al.* (2002). *Basic lymphedema management*. Hollis, New Hampshire, Hollis Publishing.

Duerr, H. P., Dietz, K. and Eichner, M. (2005). Determinants of the eradicability of filarial infections: a conceptual approach. *Trends Parasitol*ogy, 21(2), 88-96.

Dunyo, S. K., Appawu, M. A., Nkrumah, F. K., Baffoe-Wilmot, A., Pedersen, E. M. and Simonsen, P. E. (1996). Lymphatic filariasis on the coast of Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 90 (6), 634-638.

Dunyo, S. K., Nkrumah, F. K., Ahorlu, C. K, and Simonsen, P. E. (1998). Exfoliative skin manifestations in acute lymphatic filariasis. *Transactions of Royal Society of Tropical Medicine and Hygiene,* 92 (5), 539-540.

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. *Tropical Medicine and International Health*. TM IH 413-418.

Ellis, R. (2004). *Municipal mosquito control guidelines*, Canada.

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. *PLoS Neglected Tropical Diseases,* 3 (10), e529.

Evans, D.B., Gelband, H. and Vlassoff, C. (1993). Social and economic factors and the control of lymphatic filariasis: A review. *Acta Tropica*, 53 (1), 1-26.

Failloux, A. B., Raymond, M., Ung, A., Glaziou, P., Martin, P. M. V. and Pasteur, N. (1995). Variation in the vector competence of *Aedes aegypti polynesiensis* for *Wuchereria bancrofti*. *Parasitology*, 111 (1), 19-29.

Fanello, C., Santolamazza, F. and Della Torres, A. (2002). Simultaneous identification of species and molecular forms of the Anopheles gambiae complex by PCR-RFLP. *Med Vet* Entomol, 16**,** 461- 464.

Fernando, S.D., Rodrigo, C. and Rajapakse, S. (2011). Current evidence on the use of antifilarial agents in the management of bancroftian filariasis. *Journal of Tropical Medicine* 2011 (2011), 1-12.

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. *Tropical Medicine and International Health*, 10 (4), 387–392.

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.

Gillies, M. T. (1955). The pre-gravid phase of ovarian development in *Anopheles funestus*. *Annals of Tropical Medicine and Parasitology,* 49**,** 320-325.

Gillies, M. T. and De Meillion, B. (1968). The Anophelinae of Africa South of the Sahara (Ethiopian Zoographical Region). *South African Institute for Medical Research, Johannesburg,* 54**,** 343.

Gilles, H. M. (1999). Epidemiology of malaria. In Gilles, H. M. and Warrel, D. A. (Eds.) *Essential Malariology.* 3rd ed.

Gillies, M. T. and Coetzee, M. (1987). A supplement to the Anophelenae of Africa South of the Sahara. *South African Institute for Medical Research, Johannesburg,* 55**,** 105-126.

Gimnig, J. E., Ombok, M., Kamau, L. & Hawley, W. A. (2001). Characteristics of larval anopheline (Diptera: Culicidae) habitats in western Kenya. *Journal of Medical Entomology,* 38**,** 282-288.

Gwadz, R., Collins, F. H. (1996). *Anopheline mosquitoes and the pathogens they transmit*. In: Beaty, B. J., Marquardt, W. C. (Eds.), The Biology of Disease Vectors. Niwot: University Press of Colorado, 1996, 73-84.

Gyapong, J. O., Adjei, S. and Sackey, S. O. (1996a). Descriptive epidemiology of lymphaticfilariasis in Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 90, 26-30.

Gyapong, M., Gyapong, J. O., Adjei, S. and Vlassof, C. (1996b). Filariasis in Northern Ghana: Some cultural beliefs and practices and their implications for disease control. *Social Science and Medicine,* 43, 235-242.

Gyapong, J. O., Kumaraswami, V., Biswas, G., Ottesen, E. A. (2005). Treatment strategies underpinning the global programme to eliminate lymphatic filariasis. *Expert Opinion on Pharmacotherapy,* 69, 179-200.

Haddix, A. C. and Kestler, A. (2000). Lymphatic filariasis: economic aspects of the disease and programmes for its elimination. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 94**,** 592-593.

Hillyer, J. F., Schmidt, S. L., Christensen, B. M. (2003). Rapid phagocytosis and melanization of bacteria and *Plasmodium* sporozoites by hemocytes of the mosquito *Aedes aegypti*. *Journal of Parasitology,* 89 (1), 62-69.

Hillyer, J. F. (2010). *Mosquito immunity*. Landes Bioscience and Springer Science.

Hillyer, J. F and Estévez-Lao, T. Y. (2010). Nitric oxide is an essential component of the hemocyte-mediated mosquito immune response against bacteria. *Developmental and Comparative Immunology* 34(2), 141-149.

Hotez, P. J., Remme, J.H.F. *et al*. (2004). Combating tropical infectious diseases: report of the disease control priorities in developing countries project. *Clin infect Dis* 38, 871-8.

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. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 92, 513- 515.

Joseph, H., Maiava, F., Naseri, T., Silva, U., Lammie, P. and Melrose, W. (2011). Epidemiological assessment of continuing transmission of lymphatic filariasis in Samoa. *Annals of Tropical Medicine and Parasitology*, 105, 567-578.

Kan, S. P. and Ho, B. C. (1973). Development of *Brugia pahangi* in the flight muscles of *Aedes togo*: Ultrastructural changes in the infected muscle fibers and the infecting filarial larvae. *American Journal of Tropical Medicine and Hygiene*, 22, 179-188.

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. *Journal of Medical Entomology*, 46, 380-390.

Knott, J. (1939). A method for making microfilarial surveys on day blood. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 33, 191-196.

Kuhlow, K. and Zielke, E. (1978). Dynamics and intensity of *Wuchereria bancrofti* transmission in the savanna and forest regions of Liberia. *Tropenmedizin und Parasitologie*, 29, 371-381.

Kwansa-Bentum, B., Aboagye-Antwi, F.,Otchere, J., Wilson, M. D. and Boakye, D. A. (2014). Implications of low-density microfilariae carriers in *Anopheles* transmission areas: Molecular forms of *Anopheles gambiae* and *Anopheles funestus* populations in perspective. *Parasites & Vectors*, 7, 157.

Lal, R. B., Ottesen, E. A. (1988). Enhanced diagnostic specificity in human filariasis by IgG4 antibody assessment. *Journal of Infectious Diseases* 158, 1034-1037.

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. *PLoS Neglected Tropical Diseases,* 4 (2), 1-10, e602 Online: http://www.pubmedcentral.nih.gov/articlerender

Lammie, P. J., Weil, G., Noordin, R., Kaliraj, P., Steel, C., Goodman, D., Lakshmikanthan, V.B., Ottesen, E. (2004). Recombinant antigen-based antibody assays for the diagnosis and surveillance of lymphatic filariasis- a multicentre trial. *Filarial Journal*, 3, 9.

Lehane, M. J., Laurence, B. R. (1977). Flight muscle ultrastructure of susceptible and refractory mosquitoes parasitized by larval *Brugia pahangi*. *Parasitology*, 74: 87- 92.

Liang, J. L., King, J. D., Ichimori, K., Handzel, T., Pa'au, M., Lammie, P. J. (2008). Impact of five annual rounds of mass drug administration with diethylcarbamazine and albendazole on *Wuchereria bancrofti* infection in American Samoa. *American Journal of Tropical Medicine and Hygiene*, 78, 924–928.

Lymphatic filariasis: progress of disability prevention activities. *Weekly Epidemiological Record*, 2004, 79:417–424.

Mackenzie, C. D., Mwakitalu, E. M., Mandara, W. L., Mwingira, U. and Malecela, M. N. (2008). *The morbidity of lymphatic filariasis in Eastern and Southern Africa*, pp 60-71. In: Simonsen, P. E., Malecala, M. N., Michael, E and Mackenzie, C. D. (Eds), Lymphatic filariasis research and control in Eastern and Southern Africa. DBL- Centre for Health.

Magayuka, S. A. and White, G. B. (1972). Hybrid compatibilities and susceptibility of *Culex pipiens fatigans Wied,* to *Wuchereria bancrofti* (Cobbold) in East Africa. *Bulletin World Health Organization,* 46, 801-805.

Manguin, S., Bangs, M. J., Pothikasikorn, J. and Chareonviriyaphap, T. (2010). Review on global co-transmission of human *Plasmodium* species and *Wuchereria bancrofti* by *Anopheles* mosquitoes. *Infection, Genetics and Evolution,* 10 (2), 159-177.

Maxwell, C. A., Curtis, C. F., Haji, H., Kisumku, S., Thalib, A. I. and Yahya, S. (1990). Control of bancroftian filariasis by integrating therapy with vector control using polystyrene beads in wet pit latrines. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 74**,** 709-714.

McGreevy, P. B., Bryan, J. H., Oothuman, P., and Kolstrup, N. (1978). The lethal effects of the cibarial and pharyngeal armatures of mosquitoes on microfilariae. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 72, 361-368.

McGreevy, P. B., Kolstrup, N., Tao, J., McGreevy, M. M. and Marshall, T. F. (1982). Ingestion and development of *Wuchereria bancrofti* in *Culex quinquefasciatus, Anopheles gambiae* and *Aedes aegypti* after feeding on humans with varying densities of microfilariae in Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 76, 288-296.

McMahon, J. E., Marshall, T. F., Vaughan, J. P. and Abaru, D. E. (1979). Bancroftian filariasis: A comparison of microfilariae counting techniques using counting chamber, standard slide and membrane (nuclepore) filtration. *Annals of Tropical Medicine and Parasitology.* 73, 457-464.

McMahon, J. E., Magayuku, S. A., Kolstrup, N., Mosha, F. W., Bushrod, F., Abaru, D. E. and Bryan, J. H. (1981). Studies on the transmission and prevalence of bancroftian filariasis

in four coastal villages of Tanzania. *Annals of Tropical Medicine and Parasitology*, 75, 415-431.

Meyrowitsch, D. W., Pedersen, E. M., Alifrangis, M., Scheike, T. H., Malecela, M. N., Magesa, S. M., Derua, Y. A., Rwegoshora, R. T., Michael, E. and Simonsen, P. E (2011). Is the current decline in malaria burden in sub-Saharan Africa due to a decrease in vector population? *Malaria Journal,* 10, 188.

Michael, E., Bundy, D.A. and Grenfell, B.T. (1996). Reassessing the global prevalence and distribution of lymphatic filariasis. *Parasitology*, 112, 409-428.

Michael, E. and Bundy, D.A.P. (1997). Global mapping of lymphatic filariasis. *Parasitology Today*, 13 (12):472–476.

Michalski, M. L., Erickson, S. M., Bartholomay, L. C., and Christensen, B. M. (2010). Midgut barrier imparts selective resistance to filarial worm infection in *Culex pipiens pipiens*. *PLoS Neglected Tropical Diseases* 4, e875.

Mullis, K. B. (1990). The unusual origin of the polymerase chain reaction. *Scientific American*, 262, 56-65.

Murthy, P. K. and Sen, A. B. (1981). Sequential development changes in microfilariae of subperiodic *Brugia malayi* to infective larvae in susceptible strain of *Aedes aegypti* (Macdonald). *Journal of Communicable Diseases*, 13, 102–109.

Mwandawiro, C. S., Fujimaki, Y., Mitsui, Y. and Katsivo, M. (1997). Mosquito vectors of bancroftian filariasis in kwale district, Kenya. *East African Medical Journal* 74, 288-293.

Nasci, R. S. and Miller, B. R. (1996). *Culicine mosquitoes and the agents they transmit*, pp 85-97. In: Beaty, B. J. and Marquardt, W. C. (Eds.), The Biology of Disease Vectors. Niwot: University Press of Colorado.

Nelson, G.S. (1979). Filariasis, *New England Journal of Medicine*, 300, 1136-1139.

Niaré, O., Markianos, K., Volz, J. *et al.* (2002). Genetic loci affecting resistance to human malaria parasites in a West African mosquito vector population. *Science*, 298 (5591), 213-216.

Offei M., Anto F. (2014). Compliance to Mass Drug Administration Programme for Lymphatic Filariasis Elimination by Community Members and Volunteers in the Ahanta West District of Ghana. *J Bacteriol Parasitol* 5: 180. doi: 10.4172/2155-9597.1000180

Osei-Poku, J. (2012). The evolution and genetics of vector competence in mosquito disease vectors, Ph.D. dissertation, Clare College, University of Cambridge.

Ottesen, E.A., Duke, B.O.L., Karam, M. and Behbehani, K. (1997).Strategies and tools for the control/elimination of lymphatic filariasis. *Bulletin of the World Health Organization*, 75 (6), 491-503.

Ottesen, E. A. (2000). Editorial: The Global Programme to Eliminate Lymphatic Filariasis. *Tropical Medicine and International Health*, 5 (9), 591-594

Pani, S. P. and Dhanda, V. (1994). *Natural history and dynamics of progression of clinical manifestation of filariasis*, pp. 1-8. In: Kumar S, Sen, A. K., Dutta, G. P., Sharma, R. N. (eds). *Tropical disease: Molecular biology and control strategies*. New Delhi:Publication and Information Directorate, Council of Scientific and Industrial Research.

Pani, S. P. and Lall, R. (1998). Clinical features, pathogenesis and management of lymphatic filariasis. *Indian Council for Medical Research Bullettin* , 28**,** 41–51

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*. *Tropical Medicine and International Health*, 5, 359-363.

Pani, S. P., Hoti, S. L., Vanamail, P., and Das, L. K. (2004). Comparison of an immunochromatographic card test with night blood smear examination for detection of *Wuchereria bancrofti* microfilaria carriers. *The National Medical Journal of India*, 17 (6), 304-306.

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 in Parasitology*, 25, 319-327.

Perrone, J. B. and Spielman, A. (1986). Microfilarial perforation of the midgut of a mosquito. *Journal for Parasitology*, 72, 723-727.

Phantana, S., Sensathein, S., Songtrus, J., Klagrathoke, S. and Phongnin, K. (1999). ICT Filariasis Test: A new screening test for bancroftian filariasis. *Southeast Asian Journal of Tropical Medicine and Public Health*, 30 (1), 47-51.

Pichon, G., Perault, G. and Laigret, J. (1974). Redement parasitaire chez les vecteurs de Filarioses. *Bulletin of the World Health Organization*. 51, 517–524.

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. *Annals of Tropical Medicine and Parasitology*, 96, S143- S152.

Poole, C. B., Nathan, Tanner, N. A., Zhang, Y., Evans Jr., T.C. and Carlow, C. K. S. (2012). Diagnosis of Brugian Filariasis by Loop-Mediated Isothermal Amplification. *PLOS Neglected Tropical Diseases*, 6 (2), 1-9.

Prichard, R. K. (1990). Anthelmintic resistance in nematodes: extent, recent understanding and future directions for control and research. *International Journal for Parasitology* 20, 515–523.

Ramaiah, K. D., Vanamail, P., Yuvaraj, J. and Das, P. K. (2011). Effect of annual mass administration of diethylbarbamazine and albendazole on bancroftian filariasis in five villages in south India. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 105, 431-437.

Ramzy, R. M. R., El Setouhy, M., Helmy, H., Ahmed, E. S., Elaziz, K. M. A., Farid, H. A., Shannon, W. D. and Weil, G. J. (2006). Effect of yearly mass drug administration with diethylcarbamazine and albendazole on bancroftian filariasis in Egypt: A comprehensive assessment. *Lancet*, 367, 992-999.

Rebollar-Tellez, E. A. (2005). Human body odor, mosquito bites and the risk of disease transmission. *Folia Entomologica Mexicana,* 44**,** 247-265.

Riehle, M. M., Markianos, K., Niaré, O. *et al* (2006). Natural malaria infection in *Anopheles gambiae* is regulated by a single genomic control region. *Science*, 312(5773), 577-579.

Rueda, L. M. (2004). *Pictoral keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue Virus Transmission.* Magnolia press, Auckland, New Zealand.

Rueda, L. M. (2007). Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in Freshwater. *Hydrobiologia*, 595, 477–487.

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. *American Journal of Tropical Medicine and Hygiene*, 49**,** 520-529.

Service, M. W. (1980). *A Guide to Medical Entomology*, London, Macmillan Press. Pp. 204-210.

Severson, D. W., Brown, S. E., and Knudson, D. L. (2001). Genetic and physical mapping in mosquitoes: molecular approaches. *Annual review of entomology*, 46, 183-219.

Shenoy, R. K., Suma, T. K., Rajan, K. and Kumaraswami, V. (1998). Prevention of acute adeno-lymphangitis in brugian filariasis: comparison of the efficacy of ivermectin and diethylcarbamazine each combined with local treatment of the affected limb. *Annals of Tropical Medicine and Parasitology*, 92**,** 587-594.

Shenoy, R. K., Suma, T.K. and Kumaraswami, V. (2003). A qualitative study on the feasibility and benefits of foot hygiene measures practiced by patients with Brugian filariasis. *Journal of Communicable Diseases*, 35, 9–16.

Simonsen, P. E. and Dunyo, S. K. (1999). Comparative evaluation of three new tools for diagnosis of bancroftian filariasis based on detection of specific circulating antigens. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 93, 278-282.

Simonsen, P.E. (2008). *Helminthic infections*. Page 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 Neglected Tropical Diseeases*, 4, e696.

Simonsen, P. E., Yahya, A. D., Stephen M. M., Erling, M. P., Anna-Sofie, S., Mwelecele, N. M. and William, N. K. (2014). Lymphatic filariasis control in Tanga Region, Tanzania: status after eight rounds of mass drug administration. *Parasites & Vectors*, 7, 507, 1-19.

Smyth, J. D. (1996). Phasmid Nematoda: Spirurida. *Animal Parasitology*. Cambridge, Cambridge University Press.

Southgate, B. A. (1992). The significance of low density microfilareamia in the transmission of lymphatic filarial parasites. *Journal of Tropical Medicine and Hygiene*, 95, 79-86.

Southgate, B. A. and Bryan, J. H. (1992). Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 4. Facilitation, limitation, proportionality and epidemiological significance. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 86, 523-530.

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, 243-255.

Suma, T. K., Shenoy, R. K., Kumaraswami, V. (2002). Efficacy and sustainability of a footcare programme in preventing acute attacks of adenolymphangitis in Brugian filariasis. *Tropical Medicine and International Health*, 2002, 7,763-766.

Sunish, I. P., Rajendran, R., Mani, T. R., Munirathinam, A., Tewari, S. C., Hiriyan, J., Gajanana, A. and Satyanarana, K. (2002). Resurgence in filarial transmission after withdrawal of mass drug administration and the relation between antigenaemia and microfilaraemia- a longitudinal study. *Tropical Medicine and International Health*, 7**,** 59-69.

Takagi, H., Itoh, M., Kasai, S., Yahathugoda, T. C., Weerasooriya, M. V., *et al.* (2011) Development of loop-mediated isothermal amplification method for detecting *Wuchereria bancrofti* DNA in human blood and vector mosquitoes. *Parasitolology International,* 60, 493-497.

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. *Mosquito News,* 23 297-298.

Toumanoff, C. (1958). Filariose humaine et sa transmission dans la Basse-Guinée (Estuaire du Rio Nunez) *Bull. Soc. Pathol. Exo.* 51, 908-912.

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.

Wamae, C. N. and Njenga, S. M. (2008). *Diagnostic techniques and their role in lymphatic filariasis control programmes in Eastern and Southern Africa,* pp. 12-23. In: Simonsen, P. E., Malecala, M. N., Michael, E and Mackenzie, C. D. (Eds), Lymphatic filariasis research and control in Eastern and Southern Africa. DBL- Centre for Health Research and Development, Denmark.

Webber, R. H. (1977). The natural decline of *Wuchereria bancrofti* infection in a vector control situation in the Solomon Islands. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 71**,** 396-400.

Webber, R. H. (1991). Can anopheline-transmitted filariasis be eradicated? *Journal of Tropical Medicine and Hygiene*, 94, 241-244.

Weil, G. J., Lammie, P. J. and Weiss, N. (1997). The ICT Filariasis Test: a rapid-format antigen test for diagnosis of bancroftian filariasis. *Parasitology Today*, 13, 401-404.

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. *PLoS Neglected Tropical Diseases*, 2(12), e344.

Wolstenholme, A. J., Fairweather, I., Prichard, R. K., von Samson-Himmelstjerna, G. and Sangster, N. C. (2004). Drug resistance in veterinary helminths. *Trends in Parasitology* 20, 469–476.

Won, K. Y., de Rochars, M. B., Kyelem, D., Streit, T. G., Lammie, P. J. (2009). Assessing the impact of a missed mass drug administration in Haiti. *PLoS Neglected Tropical Diseases*, 3(8), e443.

World Health Organization (1992). *Lymphatic filariasis: the disease and its control. Fifth report of the WHO expert committee on filariasis.* WHO Technical Report Series, 821:1.

World Health Organization (1995). *The world health report 1995: bridging the gaps*. Geneva

World Health Organization (1997). *Bench Aids for the diagnosis of filarial infections*, Geneva.

World Health Organization (1997). *Vector control. Methods for use by individuals and communities*. Geneva.

World Health Organization (2002). *Surgical approaches to the urogenital manifestations of lymphatic filariasis: report of an informal consultation, 15-16 April 2002*. Geneva.

World Health Organization (2010). *Lymphatic filariasis. Progress report 2000-2009 and strategic plan 2010-2020.* Geneva, Switzerland.

World Health Organization (2011). *Monitoring and epidemiological assessment of mass drug administration in the Global Programme to Eliminate Lymphatic Filariasis: a manual for national elimination programmes,* Geneva

World Health Organization (2012). *Accelerating work to overcome the global impact of neglected tropical diseases - A roadmap for implementation.* Geneva- Switzerland.

World Health Organization (2013). *Global Programme to Eliminate Lymphatic Filariasis: Practical entomology*. Geneva.

World Health Organization (2013). *Global Programme to Eliminate Lymphatic Filariasis. Lymphatic filariasis: Managing morbidity and preventing disability*. Geneva.

World Health Organization: Lymphatic Filariasis, Fact Sheet No. 102. 2014.

Wynd, S., Melrose, W. D., Durrheim, D. N., Carron, J. and Gyapong, M. (2007). Understanding the community impact of lymphatic filariasis: a review of the sociocultural literature. *Bulletin of the World Health Organization*, 85, 493-498.

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 Neglected Tropical Diseases,* 5(12): e1366. doi:10.1371/journal.pntd.0001366.

Zhong, M., McCarthy, J. S., 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. *American Journal of Tropical Medicine and Hygiene*, 54, 357-363.

Zielke, E. and Kuhlow, F, (1977). On the inheritance of susceptibility for infection with *Wuchereria bancrofti* in *Culex pipiens fatigans*. *Tropenmed Parasitol*, 28, 68-78.

Zimmerman, R. H., Galardo, A. K. R., lounibos, L. P., Arruda, M. and Wirtz, R. (2009). Bloodmeal Hosts of Anopheles Species (Diptera: Culicidae) in a Malaria-Endemic Area of the Brazilian Amazon. *Journal of Medical Entomology*, 43**,** 947-956.

LIST OF APPENDICES

APPENDIX 1: Total number of mosquitoes caught in Akonu.

Mosquito species	December-14	March-15	Total/spp
An. gambiae s.l.	3	3	6
Aedes sp.	0	0	0
Culex sp.	0	2	2
Mansonia sp.	0	0	0
Total/year	3	5	8

APPENDIX 2: Total number of mosquitoes caught in Agona Princess.

Mosquito species	December-14	March-15	Total/spp
An. gambiae s.l.	93	26	119
Aedes sp.	0	1	1
Culex sp.	2	2	4
Mansonia sp.	0	0	0
Total/year	95	29	124

APPENDIX 3: Formulae for the calculation of entomological indices

· **Infection rate** = proportion of mosquitoes carrying *W. bancrofti* larvae of any stage (L_1-L_3).

· **Infectivity rate** = proportion of mosquitoes containing at least one infective (third stage) larvae.

· **Annual biting rate (ABR)** = number of blood-fed mosquitoes caught in rooms x 365/ (total number of people sleeping in rooms x number of captures)

·**Annual infective biting rate (AIBR)** = ABR x infectivity rate.

·**Worm load** = total number of L_3 /total number of mosquitoes carrying L_3.

·**Annual transmission potential (ATP)** = AIBR x worm load (Appawu *et al.*, 2001)

APPENDIX 4 : Biting rate estimation for *An. gambiae* s.l

Study community	Number of mosquitoes caught			Number of individuals sleeping in rooms	Number of capture nights	Biting rate (BR)	Monthly biting rate (MBR)	Annual biting rate (ABR)
	Blood fed	Unfed	Total					
Akonu	5	1	6	20	2	0.13	3.9	47.5
Princess	108	11	119	110	3	0.33	9.9	120.5

APPENDIX 5: Estimation of entomological indices for *An. gambiae* s.l

Study community	No. of mosquitoes	Microscopy				Infection rate	Infectivity rate	Worm load	BR	IBR	TP	ATP
		Mf	L_1	L_2	L_3							
Akonu	6	0	0	0	0	0	0	0	0.13	0	0	0
Princess	119	0	0	3	0	0.84	0	0	0.33	0	0	0

APPENDIX 6: Data of parasitological survey carried out in March 2015.

#	Community	District	Date sampled, March, 2015	ID	NAME	AGE	SEX	NIGHT BLOOD, yes=1, no= 0	ICT, pos=1, neg= 0	mf in blood film/ 60ul, no blood= 9999	mf in acetic acid/ 100ul, no blood= 9999	comment
1	AK	Nz East	28/03/2015	A 001	John Quaison	42	M	1	1	0	11	xx
2	AK	Nz East	28/03/2015	A 002	Thomas Quaw	40	M	1	0	0	0	
20	AK	Nz East		A 004	Thomas Ben Coffie	52	M	0	9999	9999	9999	travelled
3	AK	Nz East	28/03/2015	A 005	Isaac Cobbinah	47	M	1	1	0	0	
21	AK	Nz East		A 021	Gifty Quaicoe	15	F	0	9999	9999	9999	travelled
4	AK	Nz East	28/03/2015	A 024	Amos Quaison	12	M	1	1	0	1	xx
5	AK	Nz East	28/03/2015	A 027	Lydia Quayson	10	F	1	0	0	0	
6	AK	Nz East	28/03/2015	A 033	Mary Wusumeenu	30	F	1	1	0	0	
22	AK	Nz East		A 036	Akosua Donkoh	45	F	0	9999	9999	9999	travelled
7	AK	Nz East	28/03/2015	A 040	Matilda Quayson	11	F	1	0	0	0	
23	AK	Nz East		A 045	Francis Kwofie	40	M	0	9999	9999	9999	travelled
24	AK	Nz East		A 055	Thomas Kodjo	39	M	0	9999	9999	9999	travelled
8	AK	Nz East	28/03/2015	A 057	Arliba Liba	50	M	1	1	0	0	
9	AK	Nz East	28/03/2015	A 061	Emil Koffie	27	M	1	0	0	0	
10	AK	Nz East	28/03/2015	A 062	Clement Nyanzu	22	M	1	0	0	0	
11	AK	Nz East	28/03/2015	A 063	Benjamin Quaison	14	M	1	0	0	0	
12	AK	Nz East	28/03/2015	A 066	Egya Abure	72	M	1	0	0	0	
25	AK	Nz East		A 069	Daniel Lawson	34	M	0	9999	9999	9999	travelled
13	AK	Nz East	28/03/2015	A 071	Abraham Amu	23	M	1	1	0	0	
14	AK	Nz East	28/03/2015	A 073	John Kokyere	57	M	1	1	0	0	
15	AK	Nz East	28/03/2015	A 074	Hamilton Quayson	16	M	1	1	0	3	xx
16	AK	Nz East	28/03/2015	A 077	Amuko Eduku	55	F	1	1	0	0	
26	AK	Nz East		A 079	Suzzy Duku	40	F	0	9999	9999	9999	unknown
17	AK	Nz East	28/03/2015	A 080	Caroline Yankey	16	F	1	0	0	0	
18	AK	Nz East	28/03/2015	A 083	Anthony K. Sam	57	M	1	0	0	0	
19	AK	Nz East	28/03/2015	A 093	Samuel Quayson	44	M	1	1	0	0	
27	AK	Nz East		A 097	Emmanuel Awotwe	29	M	0	9999	9999	9999	travelled
1	P-Town	Ah West	30/03/2015	P 002	Cecilia Mensah	71	F	1	1	0	0	
2	P-Town	Ah West	30/03/2015	P 006	Patrick Acquah	23	M	0	9999	9999	9999	travelled
3	P-Town	Ah West	30/03/2015	P 027	Dorothy Annor	21	F	1	0	0	0	
4	P-Town	Ah West	30/03/2015	P 056	Sister Betty	32	F	0	9999	9999	9999	refused
5	P-Town	Ah West	30/03/2015	P 093	Mary Appiah	16	F	1	0	0	0	
6	P-Town	Ah West	30/03/2015	P 116	Mathew Livingstone	47	M	0	9999	9999	9999	refused
7	P-Town	Ah West	31/03/2015	P 120	Joseph Siaw	35	M	1	1	0	0	
8	P-Town	Ah West	30/03/2015	P 124	Joseph Kwebi Asmah	83	M	1	1	1	1	xx
9	P-Town	Ah West	30/03/2015	P 125	Joseph Ackah	22	M	1	0	0	0	
10	P-Town	Ah West	30/03/2015	P 127	Mienza Borbae	35	M	1	0	0	0	
11	P-Town	Ah West	30/03/2015	P 129	Priscilla Essien	25	F	1	0	0	0	
12	P-Town	Ah West	30/03/2015	P 135	J.T. Darko	46	M	1	1	0	0	
13	P-Town	Ah West	30/03/2015	P 153	Emmanuel Assilidwo	15	M	1	1	0	0	
14	P-Town	Ah West	30/03/2015	P 173	Lydia Ackah	12	F	1	1	0	0	
15	P-Town	Ah West	30/03/2015	P 193	Comfort Mensah	35	F	1	0	0	0	
16	P-Town	Ah West	30/03/2015	P 194	Sarah Nkrumah	41	F	1	0	0	0	
17	P-Town	Ah West	30/03/2015	P 199	Auntie Ahu	55	F	1	0	0	0	
18	P-Town	Ah West	30/03/2015	P 219	Bridget Owu	12	F	1	1	0	0	
19	P-Town	Ah West	30/03/2015	P 243	Comfort Aselefi	70	F	1	0	0	0	
20	P-Town	Ah West	30/03/2015	P 253	Stephen Oppong	27	M	1	0	0	0	
21	P-Town	Ah West	30/03/2015	P 254	John Dadzie	41	M	0	9999	9999	9999	missed

Appendix 7: Data of parasitological survey carried out in June 2015

#	Community	District	Date sampled, June, 2015	ID	NAME	AGE	SEX	NIGHT BLOOD, yes=1, no= 0	ICT, pos=1, neg= 0	mf in blood film/ 60ul, no blood= 9999	mf in acetic acid/ 100ul, no blood= 9999	comment
1	AK	Nz East	10/06/2015	A 001	John Quaison	42	M	1	1	0	10	
2	AK	Nz East	10/06/2015	A 002	Thomas Quaw	40	M	1	1	0	0	
20	AK	Nz East		A 004	Thomas Ben Coffie	52	M	9999	9999	9999	9999	travelled
3	AK	Nz East	10/06/2015	A 005	Isaac Cobbinah	47	M	1	1	0	0	
21	AK	Nz East		A 021	Gifty Quaicoe	15	F	9999	9999	9999	9999	travelled
4	AK	Nz East	10/06/2015	A 024	Amos Quaison	12	M	1	1	0	0	
5	AK	Nz East	10/06/2015	A 027	Lydia Quayson	10	F	1	1	0	0	
6	AK	Nz East	10/06/2015	A 033	Mary Wusumeenu	30	F	1	1	0	0	
22	AK	Nz East	11/06/2015	A 036	Akosua Donkoh	45	F	1	1	0	0	
7	AK	Nz East	10/06/2015	A 040	Matilda Quayson	11	F	1	1	0	0	
23	AK	Nz East	10/06/2015	A 045	Francis Kwofie	40	M	1	1	0	0	
24	AK	Nz East	10/06/2015	A 055	Thomas Kodjo	39	M	1	1	0	0	
8	AK	Nz East		A 057	Arliba Liba	50	M	9999	9999	9999	9999	Not available
9	AK	Nz East	10/06/2015	A 061	Emil Koffie	27	M	1	0	0	0	
10	AK	Nz East	10/06/2015	A 062	Clement Nyanzu	22	M	1	0	0	0	
11	AK	Nz East		A 063	Benjamin Quaison	14	M	999	9999	9999	9999	Schooling at Axim
12	AK	Nz East		A 066	Egya Abure	72	M	9999	9999	9999	9999	Indisposed
25	AK	Nz East		A 069	Daniel Lawson	34	M	9999	9999	9999	9999	travelled
13	AK	Nz East	10/06/2015	A 071	Abraham Amu	23	M	1	1	0	0	
14	AK	Nz East	10/06/2015	A 073	John Kokyere	57	M	1	1	0	0	
15	AK	Nz East	11/06/2015	A 074	Hamilton Quayson	16	M	1	1	6	9	
16	AK	Nz East	10/06/2015	A 077	Amuko Eduku	55	F	1	1	0	0	
26	AK	Nz East		A 079	Suzzy Duku	40	F	9999	9999	9999	9999	travelled
17	AK	Nz East	10/06/2015	A 080	Caroline Yankey	16	F	1	0	0	0	
18	AK	Nz East	11/06/2015	A 083	Anthony K. Sam	57	M	1	1	0	0	
19	AK	Nz East	10/06/2015	A 093	Samuel Quayson	44	M	1	1	0	0	
27	AK	Nz East		A 097	Emmanuel Awotwe	29	M	9999	9999	9999	9999	travelled
1	P-Town	Ah West	08/06/2015	P 002	Cecilia Mensah	71	F	1	1	0	0	
2	P-Town	Ah West		P 006	Patrick Acquah	23	M	9999	9999	9999	9999	travelled
3	P-Town	Ah West	09/06/2015	P 027	Dorothy Annor	21	F	1	1	0	0	
4	P-Town	Ah West		P 056	Sister Betty	32	F	9999	9999	9999	9999	refused
5	P-Town	Ah West	09/06/2015	P 093	Mary Appiah	16	F	1	0	0	0	
6	P-Town	Ah West	09/06/2015	P 116	Mathew Livingstone	47	M	1	0	0	0	
7	P-Town	Ah West	09/06/2015	P 120	Joseph Siaw	35	M	1	1	0	2	
8	P-Town	Ah West		P 124	Joseph Kwebi Asmah	83	M	9999	9999	9999	2	Only enough blood for acetic
9	P-Town	Ah West		P 125	Joseph Ackah	22	M	9999	9999	9999	9999	travelled
10	P-Town	Ah West	08/06/2015	P 127	Mienza Borbae	35	M	1	0	0	0	
11	P-Town	Ah West	08/06/2015	P 129	Priscilla Essien	25	F	1	0	0	0	
12	P-Town	Ah West		P 135	J.T. Darko	46	M	9999	9999	9999	9999	Not available
13	P-Town	Ah West	08/06/2015	P 153	Emmanuel Assilidwo	15	M	1	1	0	0	
14	P-Town	Ah West	08/06/2015	P 173	Lydia Ackah	12	F	1	1	0	0	
15	P-Town	Ah West	08/06/2015	P 193	Comfort Mensah	35	F	1	1	0	0	
16	P-Town	Ah West	08/06/2015	P 194	Sarah Nkrumah	41	F	1	1	0	0	
17	P-Town	Ah West	09/06/2015	P 199	Auntie Ahu	55	F	1	0	0	0	
18	P-Town	Ah West	08/06/2015	P 219	Bridget Owu	12	F	1	1	0	0	
19	P-Town	Ah West	08/06/2015	P 243	Comfort Aselefi	70	F	1	1	0		
20	P-Town	Ah West	09/06/2015	P 253	Stephen Oppong	27	M	1	1	0	0	
21	P-Town	Ah West		P 254	John Dadzie	41	M	9999	9999	9999	9999	Not available